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Forensic Chemistry

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Introduction

OVERVIEW AND ORIENTATION

Forensic chemistry exists where science and the law overlap. You might expect the marriage of science and the law to be an easy and natural one, but frequently it is not. The widespread perception is that science and the judicial system both exist to seek the truth, but that is an incomplete description. Although tackling the definitions of scientific and legal truth is beyond the scope of this book, their intersection is at the heart of it, even when hidden behind chemical equations and reaction mechanisms. The term *forensic* refers to law enforcement, the judicial system, and the courts, and without *forensic*, there is no forensic chemistry. Accordingly, this brief chapter will provide you with the minimum legal context needed to explore forensic chemistry and the larger world of forensic science.

1 WHAT IS FORENSIC CHEMISTRY?

Forensic chemistry is applied analytical chemistry. If that were the extent of it, however, there would be no need for a separate course or textbook on the subject. What then makes forensic chemistry unique? Arguably, it is the same consideration that defines forensic science as a distinct discipline: the skill, art, and science of comparison. Analytical chemistry encompasses qualitative and quantitative analysis, but forensic chemistry adds comparative analysis to the task list. For example, spectroscopic analysis can quickly determine whether a fiber is made of nylon or whether a piece of plastic is polyethylene. These are analytical descriptors that answer analytical questions such as, What is it? and How much of it is there? Analytical chemistry provides qualitative and quantitative data that are required to answer **forensic questions** such as the following:

- Where could this fiber have come from?
- Could this piece of plastic have come from this plastic trash bag?
- Was weathered gasoline used to start this fire?
- Did this paint chip come from that car?

- Does this white powder contain a controlled substance?
- Do the quantities of drugs and metabolites found in these postmortem samples allow for determination of a cause of death?

The forensic question is often not the same as the **legal question**. For example, if a drug analysis section receives a white powder as evidence, the forensic question is probably, Does this white powder contain a controlled substance? If so, what and how much? The chemist performs the analysis and provides data such as "The sample was found to contain cocaine as the hydrochloride salt. The net weight of the powder was 6.234 g ± 0.012 g." This statement provides a concise answer to the forensic question but not the legal question, which is probably, Is the defendant guilty of felony possession of a controlled substance? The forensic chemist has supplied data that can contribute to answering this question, but only a piece of the total answer. It is good practice to keep this distinction in mind.

EXHIBIT A

The Origins of Science and Chemistry

Ancient chemistry was likely related to medicines and materials. Knowledge was based on experiment and experience and was passed on to a select few. Early humans used plant and animal products as treatments and learned from experience what worked and what did not, but there was no understanding of natural laws (i.e., science) to guide them. The Greeks were the first to set forth the idea of science as a system or method of looking at the world, and this system began to take shape 2500 years ago. By that time, chemistry was already well established in certain areas, including natural dyes, simple metallurgy, soapmaking, cosmetics, fermented beverages, and ceramics. The Greeks created a philosophy that allowed knowledge derived from experiment to be studied systematically and then extended logically to new situations.

Source: Salzberg, H. W. "Ancient Technology: The Roots of Chemistry," in *From Caveman to Chemist: Circumstances and Achievements.* Washington, DC: American Chemical Society, 1991, 1–15.

When a forensic scientist works with an exhibit of evidence, generally there are three tasks to be accomplished. First is **identification**. In drug analysis, this task incorporates qualitative identification and, sometimes, quantitative analysis. In other cases,

EXHIBIT B

Live by the poison, die by the poison

The Greeks may have formulated the idea of science, but it was the practical Romans who formulated the first essential elements of forensic science. One of the most common, most feared, and most difficult crimes to detect in the ancient world was poisoning. A very early law outlawing this crime was set forth by Rome in 82 B.C. Nearly 250 years prior to that, the Romans had executed a number of women convicted of poisoning husbands, fathers, other relatives, and significant others. The women were executed by being forced to drink their own concoctions, leading to various versions of the title quotation. The word *forensic* is tied to the Latin word *forum,* a place where the Romans conducted business and legal proceedings. To speak in the forum was to speak the truth (or so it was hoped or assumed), leading to the link between forensic and modern debate teams. However, the word also refers to speaking the truth in public, a good job description for forensic chemists.

such as fiber analysis, identification is the easy part. The next step is **classification** of the evidence. Is the fiber nylon 6 or nylon 66? Is it red, yellow, or blue? Has it aged? What is its cross section? The answers to these questions reduce the size of the class to which the fiber belongs. The smaller the class membership, the more meaningful is the evidence. Taken to its logical conclusion, classification results in placing the fiber in a class with only one member. This process is referred to as **individualization** or establishing a **common source**, although this terminology seems to be losing favor in the forensic community. Regardless, the concept is a useful one, even if reducing the number of possible sources to a single entity is rarely possible.

Continuing with this example, assume that a fiber is found at a crime scene. The forensic analyst determines that it is a red nylon fiber with a circular cross section. A suspect wearing a red nylon windbreaker is arrested. Nylon fibers from the windbreaker (often labeled *K* for "known") are subjected to the same tests as was the fiber in question (*Q*) from the scene, with similar results. The analysis demonstrates that *Q* and *K* belong to the same class, but this is not proof of a common source. Rather, this is an example of inclusive evidence (described below). In other words, the test fibers from the jacket and the fiber from the crime scene have not been individualized, and the analyst cannot assign a common source (the jacket) to *Q* and *K*. This does not mean that the evidence is useless, but it does limit what can be said with confidence. The jacket is not excluded as a possible source.

Drug analysis, both of physical and biological evidence, falls outside the traditional forensic framework of identification–classification–individualization. Analytical instrumentation, properly applied, nearly always allows for the unambiguous identification of a chemical compound, be it a drug or metabolite. Classification involves presumptive testing and screening tests, but identification follows classification rather than preceding it, as in the case of our hypothetical fiber. Identification, classification, and individualization are all involved in forensic chemistry, even if the order varies.

2 PRECEDENT IN CHEMISTRY AND THE LAW

Science exists to uncover a deeper understanding of the universe, guided by the principles of the scientific method. The tools used are experimentation and observation. Courts exist to settle disputes between individuals and the state (**criminal law**) or among individuals or entities (**civil law**). Courts are guided by the law, precedent, and function using an **adversarial system**. It would be a mistake to assume that the courts use a model similar to the scientific method or that science works on the basis of argument. There are elements of each in both systems, but to the forensic chemist, the differences are as important as the similarities (Figure 1).

Both science and the courts are tasked with deriving information from evidence pertinent to the issue at hand. Science employs the scientific method to do so, whereas the courts employ the adversarial system, in which two opposing parties present arguments before the **trier of fact**. Scientific evidence and testimony may support or refute either argument. The relative strengths of the arguments guide the court in settling the issue. Scientific knowledge and findings are part of that process, but only part. All scientists can and should do is produce the best science possible, followed by making the clearest presentation possible. How the data are used is for the courts to decide. Many such decisions are based on **precedent**, or that which has gone before. When a precedent is created, new rules or new applications of rules to decide a case or issue are developed and used.[1] Precedent is a guide for decisions and is based on past lessons. In that sense, precedent is knowledge gained previously in similar settings. Science also invokes the concept of precedent, since new ideas are derived from previous observations and experiments.

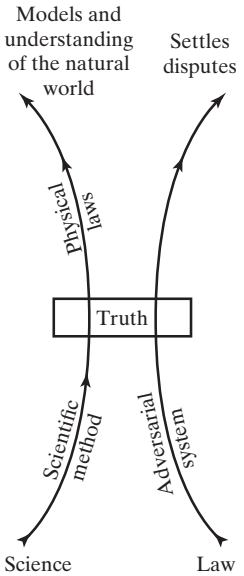

FIGURE 1 Different paths toward similar, but not identical, destinations.

EXHIBIT C

The Origin of Law

The Greeks and the Romans could not have made their contributions to forensic science had it not been for a much earlier invention: the law. The first-known codified laws were put forth by the peoples who occupied the Tigris and Euphrates River valley areas in what were the earliest-known cities and civilization. The earliest-known laws and legal systems appeared around 2000 B.C. Arguably, the most famous was Hammurabi's code, named for the Babylonian king in power around 1700 B.C.

3 KEY FORENSIC AND LEGAL CONCEPTS

This is a chemistry text first and foremost, but because it is a *forensic* chemistry text, brief mention of the discipline's legal foundation is in order. The central precepts applicable to forensic chemistry are summarized in the paragraphs that follow, and the "Further Reading" and "References" sections at the end of the chapter list additional resources.

3.1 Criminal and Civil Cases

Forensic chemists working in local, state, and federal laboratories are usually involved in criminal cases. Criminal law deals with crimes by a person or persons against the state, which can be any level of government, including cities, counties, states, and the federal government.[1] Civil cases arise from disputes that involve private rights or from disputes such as those between two individuals or two corporations. Cases referred to informally as lawsuits, wherein the complainant is said to be "filing suit," involve civil law.

3.2 Admission of Evidence

The history of the admissibility of scientific evidence in the United States is surprisingly short, less than a century old.[2] To date, standards of admissibility are founded on four court rulings, and their application varies with the jurisdiction.

The Frye Rule (**general acceptance**): This standard of admission was established in a 1923 case heard in the District of Columbia Circuit Court: *Frye v. United States*, 293 F. 1013, 1014 (D.C. Cir. 1923). Distilled to its essence, the court's ruling held that evidence produced by scientific analysis is admissible as long as the techniques are accepted as valid by the relevant scientific community.[3] In effect, the court said that if the test has survived the rigor of the scientific method and peer review to reach the status of general acceptance, then it has already been tested and validated. For example, if a new technique was developed for the chemical characterization of dyes in ink, the results of tests performed in accordance with that technique would not be admitted under the ***Frye* rule** unless the court determined that analytical and forensic chemists generally recognized the technique as useful and reliable. The *Frye* standard was predominant into the early 1990s and is still used in some jurisdictions.

*The **Daubert** Decision:* This ruling, handed down by the U.S. Supreme Court (*Daubert v. Merrell Dow Pharmaceuticals* (113 S.Ct. 2786 (1993)), was based on the Federal Rules of Evidence enacted in 1975. The case focused particularly on Federal Rule 702. The decision in *Daubert* gave judges what is referred to as a **gatekeeper** in determining admissibility.

Although this decision applied only to federal cases, several states have adopted the same approach to admissibility.

Daubert has had a significant impact on forensic science in the past decade, particularly in the realm of DNA evidence, which came of age under this decision. The *Daubert* decision provided judges with a list of tools and tests that could be used to determine the admissibility of evidence. General acceptance by the relevant scientific community is one of these, but a host of others were put forward including testability of the method, peer review (e.g., publication in peer-reviewed journals), existence of standards that can be used to test the method, and the existence of known error rates. In cases where admissibility is an issue, judges can convene **admissibility hearings** (also called ***Daubert* hearings**) to determine the merit of the method. The rigor required for acceptance under *Daubert* and the role of *Daubert* in hearings that determine admissibility are forcing a reexamination of forensic mainstays such as fingerprint evidence. No doubt forensic chemistry will be affected as this situation evolves.

General Electric v. Joiner (522 U.S. 136 (1997)): This case was the second of what is now called the ***Daubert* trilogy**. In this case, the issue was related to workplace exposure to hazardous chemicals (PCBs) and the outcome was clearly going to depend heavily on which scientific studies were admitted. In terms of admissibility, the ruling in the case stressed the need to weigh the **relevancy** of the data to the question at hand. For a method or technique to be considered admissible, it must be clearly and unambiguously pertinent to the question at hand.

Kumho: *Daubert* was extended by the 1999 decision in *Kumho Tire Co., Ltd. v. Carmichael* (119 S. Ct. 1167 (1999)). This ruling, which completes the trilogy, extended the scope of *Daubert* and the judge's gatekeeper role to *all* expert testimony, not just scientific. The decision also acknowledged that standards for determining admissibility would differ, depending on the discipline in question.[3]

3.3 Inclusive versus Exclusive Evidence

Often, forensic chemists produce scientific evidence that can be described as either **inclusive** or **exclusive**. Recall the red fiber example mentioned earlier in the chapter. In that example, successive classification based on analytical data demonstrated that the red fiber from a crime scene belonged to the same class as fibers from a suspect's red nylon jacket. This is an example of inclusive evidence: the jacket is included in the population of items that could have been the source of the fiber in question. Had the fibers from the jacket been found to have a cross section different from that of the fiber found at the scene, they would have been exclusionary evidence: The jacket could *not* have been the source.

3.4 Direct and Circumstantial Evidence

Direct evidence is that which is known to a person by personal knowledge, such as eyewitness testimony. Such evidence, if found to be true, would prove a point in contention without requiring any additional analysis or inference.[3] Forensic scientists, by contrast, produce **circumstantial evidence**, or evidence that requires inference to move logically from the information provided to the answer to a question. For example, if blood is found on a knife, and DNA typing showed that the blood matched that of a suspect, with a probability of 1 in 6 trillion, the trier of fact must still infer that the blood came from the suspect, since the deposition of the blood was not directly witnessed. Contrary to popular belief, circumstantial evidence is not, by definition, weak evidence.

3.5 Chain of Custody

The "chain" as it is called, is a paper form that tracks evidence from its creation or collection to its final disposal. A "cradle-to-grave" document that completely describes the history of a sample or an exhibit constituting evidence, the chain is initiated when the sample is collected or created and is updated each time the sample is transferred from one person to another. The chain ensures that the sample's history has no gaps and that the sample was in the direct control of one person at all times, though not always the same person. When a sample is in the laboratory, it either is stored in a secure, locked storage area or is being analyzed. Any break in the chain, no matter how innocent or inadvertent, raises the possibility that the sample could have been tampered with. Accordingly, painstaking steps are taken to ensure the integrity of all evidence. Among these steps are establishing security measures, guaranteeing controlled access to storage areas, and implementing specific protocols for opening, marking, sealing, and transporting evidence. Maintenance of the chain is a fundamental responsibility of any forensic analyst.

3.6 Destructive Testing

If an exhibit of evidence is consumed in testing, the tests performed on it can never be repeated or verified. Although this is not a limitation when the case consists of several milliliters of blood or a large bindle of white powder, other cases are not so simple. If the exhibit is a single fiber or one tiny paint chip, analytical options are limited. Solubility tests would be a poor choice for a single paint chip, but microspectrophotometry (nondestructive) would be ideal.

Applying the Science 1 The Power of a Common Source and Circumstantial Evidence

The Wayne Williams case was made without eyewitnesses, without DNA, and without fingerprints. In 1982, Wayne Williams was convicted of murdering 2 young boys in Atlanta, but he was likely responsible for the killings of at least 10 others. The key evidence in the case was fibers and dog hair that represented an accumulation of circumstantial evidence the jury could not ignore. In 11 of 12 fiber correlations, fibers found on the victims and in Williams's home or car were determined to be members of the same small class. Any one of these 11 correlations was inclusive evidence, but when they were considered together, the chances that 11 different fiber or hair types would be found both on the victims and in Williams's environment were too small for the jury to consider as coincidence.

Source: Deadman, H. A. "Case Reading: Fiber Evidence and the Wayne Williams Trial." In *Criminalistics: An Introduction to Forensic Science*, ed. R. Saferstein. Upper Saddle River, NJ: Prentice Hall, 2004.

4 THE FLOW OF A FORENSIC ANALYSIS

It is important to understand that forensic chemists work on the basis of a finite universe of substances that are of interest. The method selected must answer the forensic question, such as, Does this fire debris sample contain an accelerant? The forensic question dictates the method, which must be fit for the purpose of detecting accelerants. The chemist (and the legal system) is not interested in determining what kind of wood was in the fire or what other materials are present—only whether there was an accelerant. Similarly, in forensic toxicology, the universe of analytes consists of drugs, poisons, and their biotransformation products. If a postmortem blood sample arrives at the

laboratory, the toxicologist will not apply a method to determine the cholesterol level in the blood, because that information is not relevant to the case at hand (as per the *Joiner* decision). Such a method would not be fit for the purpose of detecting drugs, poisons, and metabolites. You should keep this in mind as we begin to delve deeper into the specifics of different analyses. A forensic analysis is usually a narrowing-down process, as shown in Figure 2. Although the sample matrix may be known (blood, urine, fire debris, etc.), many samples are complex mixtures that require a systematic approach to characterize them. Generically, a forensic chemist relies on three groups of techniques: visual examination and inspection (both macroscopic and microscopic), organic chemical analysis, and inorganic chemical analysis. The sample or exhibit of evidence is often referred to as a **general unknown**, even though the analyst usually has some idea of what is or might be present in the sample.

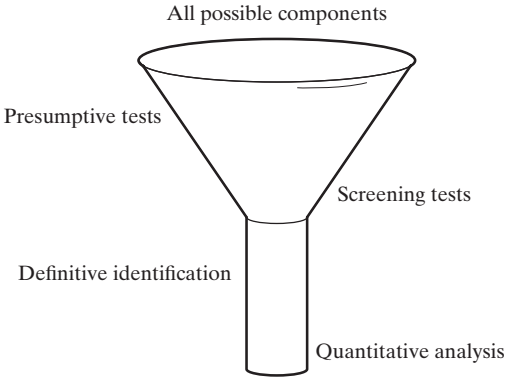

FIGURE 2 The flow of a forensic analysis.

Analysis starts with qualitative presumptive tests that narrow down the list of potential analytes and direct subsequent analysis. In some cases, visual or microscopic examination suffices as a first step. Presumptive tests utilizing chemical reagents belong to a family of analytical techniques referred to as **wet chemical methods**. Most are based on observing results when specific reagents are added to small portions of the samples. Color and crystal tests, as they are commonly called, are used in analyzing drugs, gunshot residues, and explosives. These tests will be discussed in detail in subsequent chapters. It is worth noting that as instrumentation improves and becomes more affordable, there is decreasing reliance on wet chemical methods; however they will continue to play a role for the forseeable future.

Once presumptive tests have focused the analysis on a small set of potential components, the next step usually involves the separation and isolation of target components, either for screening tests or for definitive identification. Typically, such steps involve extraction and chromatography. Forensic chemists often employ thin-layer chromatography (TLC) in the analysis of inks and drugs as a follow-up to presumptive tests, whereas toxicologists may use immunoassay to narrow the field of potential analytes. Chromatography can also be employed for sample cleanup and analyte isolation, as can solvent extractions, headspace methods, and solid-phase extractions. Confirmation of tentative identification follows, using instrumental techniques such as infrared spectrophotometry and gas chromatography–mass spectrometry (GC-MS). The latter is particularly valued because of its capabilities in separating, identifying, and quantitating analytes, although the degree of quantitative analysis required varies. In seized drug analyses, GC-MS is often the primary analytical instrument used, whereas for other purposes, such as analyzing fibers, it may be less important or even inapplicable.

As we discussed previously, the majority of forensic chemical analyses do not require the complete characterization of a sample. For example, a small resinous cube of brown material may be found to contain heroin along with many other materials; however, analytical interest usually ends with the identification of the illegal drug or substances. Occasionally, the materials used as cutting agents (diluents) are identified, but that is usually the extent of the testing. Although a complete characterization could be invaluable for linking the resin to others and for determining origins, testing for such purposes is not routinely performed, given the time and expense required. Like all analytical chemists, forensic chemists balance the need for accuracy, precision, and completeness against the reality of limited time, money, and resources. The overriding

consideration is always how well the method answers the forensic questions. The next chapter describes the acceptable compromises that yield useful, reliable, and legally defensible data.

5 THE FORENSIC MIND-SET

There is a core set of skills that any forensic scientist should cultivate as part of a forensic mind-set. The importance of comparison in forensic analyses imposes conditions on methods selected, how they are applied, and how the results are interpreted. Consider a case in which the forensic chemist is provided a tiny fragment of a thick, fibrous, silvery material with one adhesive surface. The evidence is the only remaining trace of the material that was used to bind a homicide victim. A suspect has been identified and a search of his house reveals three different rolls of duct tape. The forensic question is, From which roll, if any, could the fragment of tape have come? The identification part of analysis is simple: A quick look through a stereomicroscope shows the material to be duct tape. The challenge is how to proceed, given that the evidence cannot be destroyed.

If the analyst is lucky, it may be possible to physically match the fragment to one of the rolls (identifying a common source, or individualization). If not, the tape can be examined microscopically and with microspectrophotometry. Careful study of the fiber pattern in the tape, combined with some database searching and phone calls, may narrow the possible manufacturers of the tape (classification). A trip to the library (a building or an electronic repository) may uncover an article in a forensic, analytical, or industrial journal (e.g., *Journal of Forensic Sciences*, *Forensic Science International*, *Canadian Journal of Forensic Science*, *Adhesives Age*, or *Adhesives and Sealants Industry*) that describes how others have approached similar problems. Experimentation with tapes unrelated to the case can further refine the approach.

Although this kind of case is not routine, it highlights the skills that constitute the forensic mind-set. Forensic scientists and forensic chemists should

- assume nothing.
- be resourceful. Finding at least two journals devoted to adhesives would be of significant help in the case and would take only minutes via an electronic search. Browsing articles could produce names of experts in adhesives to contact for assistance.
- think outside the discipline. Forensic science integrates many areas, chemistry being only one, but the core skills and principals of science are always the same. The analyst in this case would probably find reading adhesives journals easier than expected.
- be creative. Often, creativity is attributed to the arts (painting, music, etc.), but successful scientists and researchers must be creative as well. Creativity involves applying a novel approach to a problem or finding a novel application of existing tools and skills. All painters use paint, but there are an infinite number of ways to assemble the colors on a canvas. Similarly, all analytical chemists have access to the same set of tools; it is how they are applied that makes an approach creative. Challenging cases require creativity.
- build a big toolbox that never stops growing.
- know their limitations and never speak beyond what their data and expertise support.
- be flexible. Just because something works in this case does not necessarily mean that it will work in the next one. The more knowledgeable and resourceful a scientist is, the more flexible he or she is.
- be persistent. The case described in this section might at first glance have seemed hopeless to the analyst, but it was not. *Difficult* is not the same as *impossible*; a good forensic scientist recognizes the difference.

6 FORENSIC CHEMISTRY TODAY

Analytical chemistry in the forensic field is generally divided into two areas: forensic toxicology and forensic chemistry. The divisions are somewhat artificial, but an understanding of them is important. Forensic toxicologists work with biological evidence and follow the trail of drugs and poisons ingested by humans or other organisms. Forensic toxicology is often associated with death investigation and the medical examiner's or coroner's office, depending on the jurisdiction. Certainly, forensic toxicologists are also forensic chemists; the division between forensic toxicology and forensic chemistry and the use of those job descriptors are rooted in history and tradition.

Forensic chemists work with physical evidence and are often employed in what are often called "crime labs," although this term seems to be falling out of favor. In general, qualifications for employment in either type of laboratory (crime lab or toxicology lab) are a B.S. in a natural science (preferably chemistry) with an emphasis on analytical and instrumental methods. Entry-level toxicology positions may require additional training or experience in toxicology or pharmacology. The moniker "drug chemist" is sometimes used if the person works exclusively in that area; some forensic chemists work in trace evidence and other forensic specialties. Forensic chemists also work with materials such as inks, dyes, fire debris, gunshot residues, dusts, explosives, polymers, paints, and glass. If there is physical evidence and it is amenable to, and benefits from, chemical analysis, a forensic chemist or someone trained in that area can be involved in analyzing that evidence.

EXHIBIT D

The First Forensic Testimony

In truth, the first instance may never be known. It has been reported that a surgeon who examined Julius Caesar's body was asked to testify as to which wound was fatal to the emperor. However, one of the first instances of modern forensic testimony was given by a chemist, M. J. B. Orfila (1787–1853), in a poisoning case. Orfila, held by many to be the father of forensic toxicology, was a prominent toxicologist and skilled chemist when the case of Marie LaFarge crossed his path in 1840. Marie LaFarge was a young French widow who, at 24, remarried. Her second marriage, to Charles LaFarge (age 30), was reportedly not a happy one. In 1839, Charles died after eating cake made by his wife; the symptoms were consistent with arsenic poisoning. Marie was charged and chemical tests were performed on the body, but the results were inconclusive. The court was unsatisfied and commissioned Orfila to journey from Italy to France to conduct a review of the scientific work in the investigation. Orfila eventually had Charles's body exhumed. A skilled analytical chemist, Orfila was able to detect arsenic in the tissues. He also showed an appreciation for the need for control samples, testing the soil in which Charles had been buried and demonstrating that the arsenic did not originate from it. Marie was convicted and sentenced to involuntary servitude, during which time she wrote a book.

National Library of Medicine

M. J. B. Orfila

Mary Evans Picture Library/Alamy

Marie Lafarge

7 BECOMING AND BEING A FORENSIC PROFESSIONAL

7.1 What Is a Profession?

Forensic science has evolved from an adjunct to medicine to a recognized profession—which raises the question, What defines something as a profession? A reasonable definition is that a profession is the practice of similar skills by a group of people who are paid to apply those skills in a more-or-less specified manner.[4] A profession is differentiated from a job in that there is a governing body that oversees, regulates, and ensures that practitioners of the profession adhere to standard guidelines and meet certain minimum requirements. In a true profession, this regulation is derived internally, not imposed by an outside entity that does not practice the profession. For example, the American Medical Association (AMA) dictates how medicine is taught and practiced. The AMA works in concert with medical schools, hospitals, and government agencies, but, fundamentally, doctors dictate how doctors work and what the public can expect from physicians. Thus, a profession requires the existence of professional associations that have recognized and accepted authority within the profession.

7.2 Certification and Professional Organizations

A profession defines what prerequisites one must have and what minimal educational standards one must adhere to in order to participate in it. In forensic science, there are a number of professional organizations. The largest is the American Academy of Forensic Sciences (AAFS, www.aafs.org), founded in 1947. The AAFS is organized into sections such as jurisprudence, toxicology, criminalistics, and engineering. Of most interest to chemists are the criminalistics and toxicology sections. The AAFS specifies membership requirements and three levels of membership but does not certify individuals (although it is a partner in the process). The primary national professional organization for forensic toxicology is the Society of Forensic Toxicologists (SOFT, www.soft-tox.org). Certification of forensic toxicologists and accreditation of forensic toxicology laboratories is handled by the American Board of Forensic Toxicology (ABFT, www.abft.org).

There is no single organization or association that deals exclusively with forensic chemists in the same way that SOFT and ABFT do with toxicologists. The American Chemical Society (ACS, www.chemistry.org) is the largest scientific society in the world and has sections devoted to analytical chemistry and just about every other chemical discipline imaginable. There is a section called "Chemistry and the Law," but its scope includes things such as patent law—important, to be sure, but not directly forensic. The American Board of Criminalistics (ABC, www.criminalistics.com/abc/) offers certification of forensic scientists in several disciplines. General-service forensic science laboratories are accredited through the American Society of Crime Laboratory Directors LAB program (ASCLD, www.ascld.org).

Summary

Forensic science and forensic chemistry are young professions distinguished from other sciences by their relationship to the legal system and by the importance of comparison in laboratory analysis. Forensic chemists need an understanding of the legal foundation of their chosen field, as well as a forensic mind-set, which relies on the fundamental precept of always being open to learning more and always adding tools to the toolbox. In that spirit, our journey into forensic chemistry can now begin.

Key Terms and Concepts

Admissibility hearing
Adversarial system
Chain of custody
Circumstantial evidence
Civil law
Classifications
Common source
Criminal law
Daubert decision
Daubert hearing
Daubert trilogy
Direct evidence
Exclusive evidence
Forensic question
Frye rule
Gatekeeper
General acceptance
General Electric v. Joiner
General unknown
Identification
Inclusive evidence
Individualization
Kumho
Legal question
Precedent
Relevancy
Trier of fact
Wet chemical methods

Problems

FROM THE CHAPTER

1. Compare and contrast the adversarial system and the scientific method. List the strengths and weaknesses of both in the context of criminal and civil law.
2. During a *Daubert* hearing, what entity ultimately decides on admissibility?
3. What role does peer review play in science and in the law? Compare and contrast.
4. Describe how a preponderance of inclusive circumstantial evidence can become conclusive in the eyes of a jury.

INTEGRATIVE

1. A great scientist can still be a terrible forensic scientist; a person who gives wonderful testimony can be a terrible forensic scientist. Comment on these observations and their implications for forensic chemistry.
2. Can jurors ask questions of expert witnesses? Comment on your findings regarding this issue.

FOOD FOR THOUGHT

1. Is the analysis of drugs with instruments such as mass spectrometers and infrared spectrometry based on comparison?
2. How important is the way in which scientific evidence is presented? Comment on the relative importance of content versus presentation. Why is learning how to testify such an important skill?

Further Reading

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Foundations

OVERVIEW AND ORIENTATION

Metrology is based on an understanding of making measurements and characterizing them using the appropriate tools and techniques. Among the most important of these tools is the appropriate application of significant figures and statistics. In this chapter, we will examine both from the perspective of forensic chemistry. With this background, we will introduce terms such as *error* and other closely associated terms vital to metrology. We will also begin our discussion of uncertainty with simple examples. Finally, we will examine and apply some simple hypothesis tests that are used in forensic chemistry.

1 METROLOGY AND MEASUREMENT

It is difficult to encompass the depth and breadth of metrology given that it spans many disciplines, trades, and industries. The topic can seem daunting even to experienced forensic and analytical chemists, but fear not. As we move through this discussion, you will find that most of the metrological principles we will discuss are familiar. What is likely new is the way in which they are integrated under the umbrella of metrology. All these principles are applied in the service of making good measurements and producing useful and reliable data.

To focus attention on metrology in forensic chemistry, we will utilize a concept introduced about 20 years ago called the **NUSAP** system for quantitative data presentation.[1,2] While not used explicitly in forensic chemistry, its principles are, making it an ideal platform for evaluating the reliability of results. NUSAP stands for Number-Units-Spread-Assessment-Pedigree and contains qualitative and quantitative criteria associated with a numerical result such as the weight of a powder or the concentration of alcohol in blood. The NUSAP system has been used for data that are used for policy decisions, such as environmental modeling and risk analysis,[1] all areas that, like forensic science, create data upon which critical decisions are made.

Consider a net weight of a white powder reported as follows:

77.56 g ± 0.31 g at the 95% confidence level

As shown in Figure 1, this expression can be broken down into individual components. The **measurand** is the quantity being measured or determined; here the weight of a powder. The N is 77.56; the units are grams (g) and the spread is ± 0.31 g. These are the quantitative elements of the reported value. The spread (or estimated uncertainty) of the result could have been obtained in several ways; many will be discussed later in this chapter. The Student's *t*-value was used here to obtain a confidence interval, a common approach in analytical chemistry, but hardly the only one. This descriptor (95% confidence interval, or CI) is the assessment of the spread A.

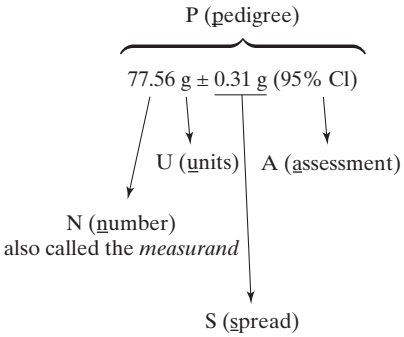


FIGURE 1 An example of the NUSAP notation applied to a forensic chemistry measurement of a mass.

The N and S are quantitative values, and U is a descriptor, but even this expression is incomplete without one additional and critical descriptor: the pedigree (P). The pedigree of a reported result refers to the history or precedent used to gather the data; it encompasses everything done to stand behind the reliability of that data. Pedigree includes quality assurance and quality control (QA/QC) and many other factors. Examples of elements of a pedigree include traceability of weights and standards, laboratory protocols and methods, analyst training, laboratory accreditation, and analyst certification—all of which support the reliability of the reported value.

An important element of NUSAP is a clear presentation estimate of uncertainty. Whenever a quantitative measurement is taken, uncertainty accompanies this measurement. In this context, uncertainty is not a synonym for doubt, vagueness, or lack of knowledge. Rather, the uncertainty is the spread or range in which a true value is expected to lie based on knowledge of the measurement process. Because this spread has an assessment and a pedigree associated with it, having uncertainty stated imparts *greater* credibility and trust in a result, not less. In other words, uncertainty is directly related to ensuring the reliability of the data, one of our primary goals. Forensic reports may not include all the components incorporated in a NUSAP approach, but this information and data should be available. For example, it may be laboratory policy to not report uncertainties in weights for exhibits having weights that fall below a legal threshold limit. This practice is reasonable and understandable. However, that uncertainty should be known and producible should it be needed by the courts, law enforcement, or other users of the data.

Before we delve too deeply into the topic of uncertainty, two important points must be emphasized. **Uncertainty** here is defined as a range that characterizes the expected spread or dispersion of a measured result.[3] There are many ways to characterize this range, and we examine several in this portion of the text. Uncertainty in this context *does not* imply doubt or lack of trust in the measured result. In fact, just the opposite is true. Reporting a reliable and defensible uncertainty adds to the validity and utility of

EXHIBIT A

Why Significant Figures Matter

In most states, a blood alcohol level of 0.08% is the cutoff for intoxication. How would a value of 0.0815 be interpreted? What about 0.07999? 0.0751? Should these values rounded off or are they truncated? If they are rounded, to how many significant digits? This is an artificial, but telling example. How numerical data are rounded depends on the instrumentation and devices used to obtain the data. Incorrect rounding might have consequences.

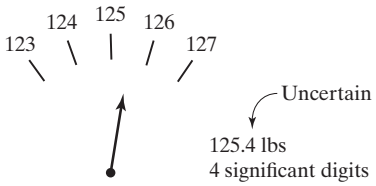


FIGURE 2 Reading the scale results in four significant digits, the last being an educated guess or an approximation that, by definition, will have uncertainty associated with it.

the data. The second point is to clearly distinguish between uncertainty and error. In the metrological context, **error** is defined as the difference between an individual measured result and the true value (i.e., accuracy).[3] Thus, error and uncertainty are *not* the same and should not be treated the same, although *both* are important to making and reporting valid and reliable results. In this chapter, we will examine a simplified approach to calculating uncertainty. We will integrate additional information to generate more realistic and defensible estimates of uncertainty. Finally, keep in mind that the best we can do is estimate uncertainty; like a true value, it can never be known exactly.

2 SIGNIFICANT FIGURES, ROUNDING, AND UNCERTAINTY

Significant figures become tangible in analytical chemistry. The concept of significant figures arises directly from the use of measuring devices and equipment and their associated uncertainty. The rules of how significant figures are managed in calculations are covered in many introductory classes, so we will focus on the highlights here. However, you should review these rules to get the most out of this section. How that uncertainty is accounted for dictates how to round numbers resulting from what may be a complicated series of laboratory procedures. The rules and practices of significant figures and rounding must be applied properly to ensure that the data presented are not misleading, either because there is too much precision implied by including extra unreliable digits or too little by eliminating valid ones.[3]

In any measurement, the number of significant digits is defined as the number of digits that are certain, plus one. The last digit is uncertain (Figure 2), meaning that it is an estimate, but a reasonable one. With the bathroom scale example, one person might interpret the value as 125.4 and another as 125.6, but it is certain that the value is greater than 125 pounds and less than 126. The same situation arises when you use rulers or other devices with calibrated marks. Digital readouts of many instruments may cloud the issue a bit, but unless you are given a defensible reason to know otherwise, assume that the last decimal on a digital readout is uncertain as well.

Recall that zeros have special rules and may require a contextual interpretation. As a starting point, a number may be converted to scientific notation. If the zeros can be removed by this operation, then they were placeholders representing a multiplication or division by 10. For example, suppose an instrument produces a result of 0.001023 that can be expressed as 1.023×10^{-3}. This demonstrates that the leading zeros are not significant, but the embedded zero is.

Trailing zeros can be troublesome. In analytical chemistry, the rule should be that if a zero is meant to be significant, it is listed, and conversely, if a zero is omitted, it was not significant. Thus, a value of 1.2300 grams for a weight means that the balance displayed two trailing zeros. It would be incorrect to record a balance reading of 1.23 as 1.2300. Recording that weight as 1.2300 would conjure up numbers that were useless at best and likely deceptive. If this weight were embedded in a series of calculations, the error would propagate, with potentially disastrous consequences. "Zero" does not imply "inconsequential," nor does it imply "nothing." In recording a weight of 1.23 g, no one would arbitrarily write 1.236, so why should writing 1.230 be any less wrong?

Another ambiguous situation that occasionally arises is associated with numbers with no decimals indicated. For example, how many significant figures are in 78? As with zeros, we need to know something about the context. If we are counting the number of students in a room, this is a whole, exact number. This number itself would not factor into significant figure determinations. The same is true of values such as metric

conversions. Each kilogram is comprised of 1000 grams. It's not 1000.2 rounded down; 1000 is an exact number. If used in a calculation, you would assume an infinite number of significant figures; like 78 above, the number of digits plays no role in rounding considerations. In some analytical contexts, you may see notation such as 327. with a decimal point placed at the end of the number. This is done purposely to tell you that this number has 3 significant digits; it is not meant to represent a whole number or exact conversion factor. Finally, keep in mind that while metric conversions are based on exact numbers, not all conversions are. For example, in upcoming chapters, we will routinely convert body weights in pounds to kilograms. The conversion factor for that calculation is 1 pound = 0.45359237 kilogram. It is up to you to decide how many significant figures are required for the calculation. When in doubt, keep them all and round at the end, but work on developing judgement skills that allow you to select the appropriate number. After all, the more digits kept, the more likely a transposition error. If you really do not need eight digits, do not use eight.

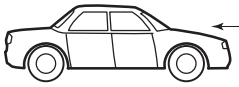
In combining numeric operations, rounding should always be done at the end of the calculation.[3] The only time that rounding intermediate values may be appropriate is in addition and subtraction operations, although caution must still be exercised. In such operations, the result is rounded to the same number of significant digits as there are in the contributing number with the fewest digits, with one extra digit included to avoid rounding error. For example, assume that a calculation requires the formula weight of $PbCl_2$:

$$\begin{array}{rr} \text{Pb} = 207.2\ \text{g/mole} & 207.2| \\ +\ \text{Cl} = 35.4527\ \text{g/mole} & 2(35.4527) = 70.9|054 \\ \hline & 278.1|054\ \text{g/mole} \end{array}$$

The correct way to round or report an intermediate value would be 278.1_1 rather than 278.1. The subscript indicates that one additional digit is retained to avoid rounding error. The additional digit does not change the count of significant digits: The value 278.1_1 still has four significant digits. The subscript notation is designed to make this clear.

By definition, the last significant digit obtained from an instrument or a calculation has an associated uncertainty. Rounding leads to a nominal value, but it does not allow for expression of the inherent uncertainty. To do this, the uncertainties of each contributing factor, device, or instrument must be known and accounted for. For measuring devices such as analytical balances, autopipets, and flasks, that value is either displayed on the device, supplied by the manufacturer, or determined empirically. Because these values are known, it is also possible to estimate the uncertainty in any combined calculation. The only caveat is that the units must be the same. On an analytical balance, the uncertainty would be listed as ± 0.0001 g, whereas the uncertainty on a volumetric flask would be reported as ± 0.12 mL. These are absolute uncertainties that cannot be combined as is, because the units do not match. To combine uncertainties, relative uncertainties must be used. These can be expressed as "1 part per . . . " or as a percentage. That way, the units cancel and a relative uncertainty results, which may then be combined with other uncertainties expressed the same way (i.e., as unitless values).

Consider the simple example in Figure 3, in which readings from two devices are utilized to obtain a measurement in miles per gallon. The **absolute uncertainty** of each device is known, so the first step in combining them is to express them as "1 part per . . . ." While not essential, such notation shows at a glance which uncertainty (if any) will dominate. It is possible to estimate the uncertainty of the mpg calculation by assuming that the odometer uncertainty of 0.11% (the **relative uncertainty**) will dominate. In many cases, one uncertainty is much larger (two or more orders of magnitude) than the other and hence will control the overall uncertainty.



Odometer (± 0.2 miles)
183.4 miles Absolute uncertainty

Fuel pump indicator 6.683 gallons
(± 0.005 gallons)
Absolute uncertainty

$$\text{mpg: } \frac{183.4 \text{ mi}}{6.683 \text{ gal}} = \boxed{27.44 \text{ mpg}}$$

Uncertainties: Relative % Uncertainty

$$\text{Odometer} = \frac{0.2 \text{ mi}}{183.4 \text{ mi}} = \frac{\frac{0.2 \text{ mi}}{0.2 \text{ mi}}}{\frac{183.4 \text{ mi}}{0.2 \text{ mi}}} = \frac{1}{917} \times 100 = 0.1_1\%$$

$$\text{Pump} = \frac{0.005 \text{ gal}}{6.683 \text{ gal}} = \frac{\frac{0.005 \text{ gal}}{0.005 \text{ gal}}}{\frac{6.683 \text{ gal}}{0.005 \text{ gal}}} = \frac{1}{1337} \times 100 = 0.07_5\%$$

Estimated: 0.11% of 27.44 mpg = 0.0302 = 0.030

Range = 27.44 ± 0.030

= $\boxed{27.41–27.47 \text{ mpg}}$

Propagated: $u_t = \sqrt{(0.001_1)^2 + (0.0007_5)^2} = 0.001_3 = 0.1_3\%$

$u_t \times \text{mpg} = 0.03_6$

Range = $\boxed{27.40–27.48 \text{ mpg}}$

FIGURE 3 Calculation of mpg (measurand) and an associated uncertainty estimate based on individual uncertainties from two measuring devices.

Better in this case is accounting for both uncertainties, because they are within an order of magnitude of each other (0.07% vs. 0.11%). Relative uncertainties are combined with the use of the formula:

$$u_t = \sqrt{(u_1^2 + u_2^2 + u_3^2 + \cdots + u_n^2)} \quad \textbf{(1)}$$

For the example provided in Figure 3, the results differ only slightly when uncertainties are combined, because both are close to 0.1%, so neither overwhelms the other.

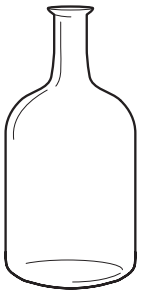
Equation 1 represents the **propagation of uncertainty** (also called propagation of error in older references). The changeover from the "error" model to the uncertainty model occurred in the 1990s. It is useful for estimating the contribution of instrumentation and measuring devices to the overall uncertainty, but this approach is too simplistic for most forensic applications. For example, suppose some amount of gasoline in the preceding example overflowed the tank and spilled on the ground. The way you do the calculation does not capture this type of problem. The resulting calculation would be correct but not reliable. Spilling gasoline is the type of procedural error that is detected and addressed by quality assurance. In turn, quality assurance requires an understanding of the mathematics of multiple measurements, or statistics.

EXAMPLE PROBLEM 1

A drug analysis is performed with gas chromatography/mass spectrometry (GC/MS) and requires the use of reliable standards. The lab purchases a 1.0-mL commercial standard that is certified to contain the drug of interest at a concentration of 1.00 mg/mL with a reported uncertainty

of ±1.0%. To prepare the stock solution for the calibration, an analyst uses a syringe with an uncertainty of ±0.5% to transfer 250.0 μL of the commercial standard to a Class-A 250-mL volumetric flask with an uncertainty of ±0.08 mL. Using the NUS portions of the NUSAP model, report the concentration of the diluted calibration solution. NOTE: As recommended, final values are rounded at the end, and the calculation is done as one operation. Here, the intermediate steps are shown for illustrative purposes only.

Answer:



Commercial standard

$\frac{1.00\ \text{mg}}{\text{mL}} \pm 1.00\%$

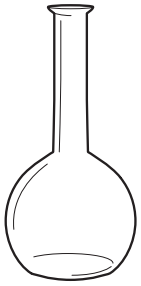
Relative uncertainty

$\underline{= 0.010}$

Syringe

Relative uncertainty

$\underline{= 0.005}$



Volumetric flask

Relative uncertainty

$\frac{0.08\ \text{mL}}{250.0\ \text{mL}} = 0.0003_2 = 0.03_2\%$

1. Calculate the final concentration:

Concentrated		Diluted	
C_1V_1	=	C_2V_2	
$\frac{1.00\ \text{mg}}{\text{mL}}$	0.250 mL	?	250.0 mL

$$C_2 = \frac{\left(\frac{1.00\ \text{mg}}{\cancel{\text{mL}}}\right)(0.250\ \cancel{\text{mL}})}{250.0\ \text{mL}} = \frac{0.00100\ \text{mg}}{\text{mL}}$$

$$C_2 = \frac{1.00\ \mu\text{g}}{\text{mL}} \times \frac{1000\ \text{mL}}{\text{L}} = 1000.\ \text{ppb}$$

2. Calculate the propagated uncertainty:

$$u_t = \sqrt{(0.010)^2 + (0.005)^2 + (0.0003)}$$

$$u_t = 0.01_1$$

3. Apply to the final concentration:

$C_2\ (u_t) = 11._0$

The calculated intermediate value is 0.0112, of which only one is significant, this significant digit is a zero.

4. Report concentration and uncertainty:

1000.0 ppb ± 11. ppb

N U S

Note the use of the decimal here; we are making it clear that 11 really does have 2 significant digits.

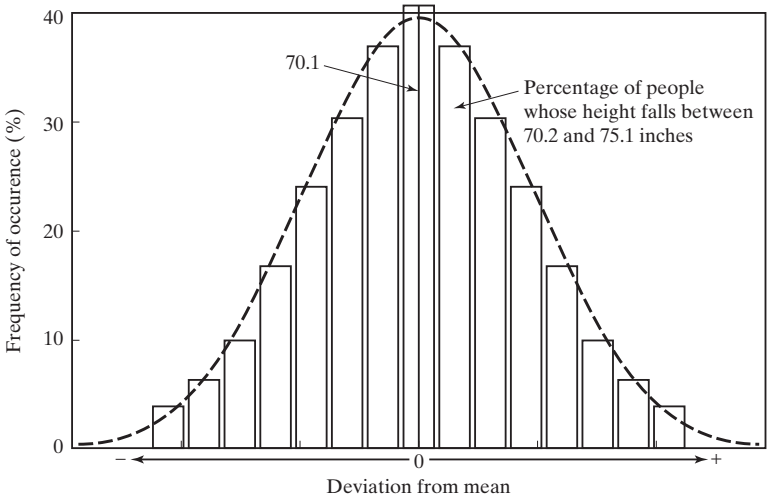


FIGURE 4 A histogram that approximates a Gaussian or normal distribution. Most measurements cluster around the central value (the mean), and the frequency of occurrence decreases as the value moves away from the mean. "Frequency" refers to how often a measurement occurs; 40% of the 5000 measurements were the mean value of 70.1 inches.

3 FUNDAMENTAL STATISTICS

The application of statistics requires replicate measurements. A replicate measurement is defined as a measurement of a criterion or value under the same experimental conditions for the same sample used for the previous measurement. That measurement may be numerical and continuous, as in determining the concentration of cocaine, or categorical (yes/no; green/orange/blue, and so on). We will focus attention on continuous numerical data.

Start with a simple example. Assume you are asked to determine the average height of people living in your town, in which 5,000 people live. You dutifully measure everyone's height (N=5000) and calculate the average, which comes out to 70.1 inches. You count all the people whose height is between 70.2 and 75.1 inches and record the number. You do the same on both sides of the average height and then create a bar chart of number of occurrences within each five inch block. The results are shown in Figure 4, a presentation called a histogram. What it tells us is that most of the heights measured were close to the mean, but there are people whose height is significantly larger than the mean and those who are notably smaller. The farther you move from the mean, the fewer the number of people that fit into a given height box. The shape of the superimposed curve approximates a Gaussian distribution or normal distribution. There are numerous types of these probability distributions, but here we will work only with normal distributions. It is important to note that the statistics to be discussed in the sections that follow assume a normal distribution and *are not valid* if this condition is not met. The absence of a normal distribution does not mean that statistics cannot be used, but it does require a different group of statistical techniques.

In a large population of measurements (or parent population), the average is defined as the population mean μ. In most measurements of that population, often (but not always) only a subset of the parent population (n) is sampled. In our height example, the entire population N was measured, but take a different example. You receive a kilogram block of cocaine and are asked to determine the percent purity, starting with

True value		*13.2% +/– 0.1%*
	Trainee	**Forensic chemist**
Sample 1	12.7	13.5
2	13.0	13.1
3	12.0	13.1
4	12.9	13.2
5	12.6	13.4
6	13.3	13.1
7	13.2	13.2
8	11.5	13.7
9	15.0	13.2
10	12.5	13.2
	Trainee	**Forensic chemist**
% Error	–2.5	0.5
Mean	12.9	13.3
Standard (absolute) error	–0.3	0.1
Standard deviation (samples)	0.93	0.20
%RSD (CV) (sample)	7.2	1.5
Standard deviation (population)	0.88	0.19
%RSD (CV) (population)	6.8	1.4
Sample variance	0.86	0.04
Range	3.5	0.6
Confidence level (95.0%)	0.66	0.14
95% Cl range	12.2–13.6	13.2–13.4

FIGURE 5 Hypothetical data for two analysts analyzing the same sample 10 times each, working independently. The chemists tested a white powder to determine the percent cocaine it contained. The accepted true value was 13.2%. In a small data set ($n = 10$), the 95% CI would be a reasonable choice to estimate uncertainty. The absolute error for each analyst was the difference between the mean that analyst obtained and the true value. Note that here,"absolute" does not mean the absolute value of the error.

a 1g sample. A reasonable approach would be to homogenize the block and draw, for example, 5 1g samples. This is n; you took a subset sample of the parent population for the analysis. The average value for a subset (the sample mean, or $\bar{x}$) is an estimate of μ. In the cocaine purity example, your goal as an analyst is to obtain the best estimate of the true mean as possible based on your sample mean. As the number of measurements of the population increases, the average value approaches the true value. The goal of any sampling plan is twofold: first, to ensure that n is sufficiently large to appropriately represent characteristics of the parent population; and second, to assign quantitative, realistic, and reliable estimates of the uncertainty that is inevitable when only a portion of the parent population is studied.

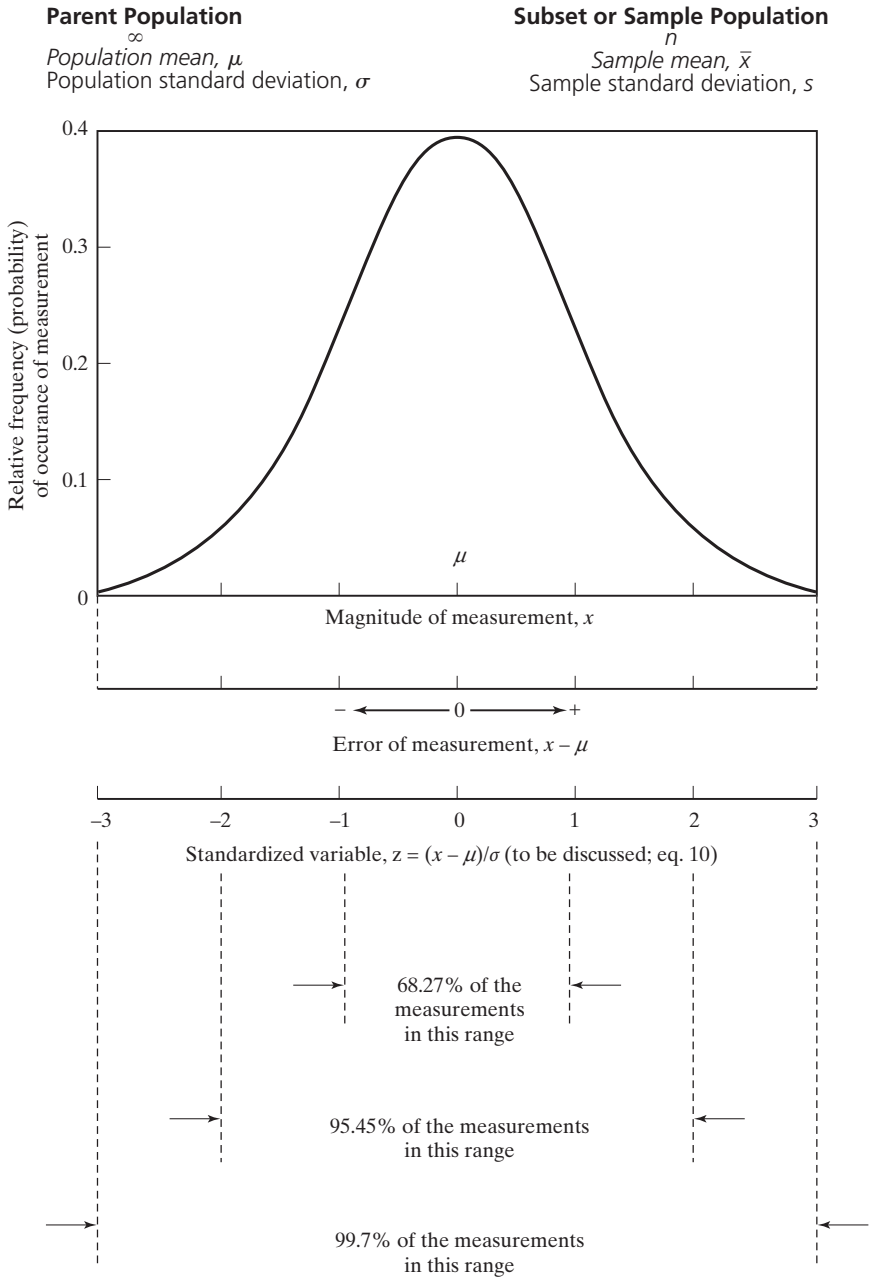
Consider the following example (see Figure 5), which will be revisited several times throughout the chapter. As part of an apprenticeship, a trainee in a forensic chemistry laboratory is tasked with determining the concentration of cocaine in a white powder. The powder was prepared by the QA section of the laboratory, but the concentration of cocaine is not known to the trainee (who has a blind sample). The trainee's supervisor is given the same sample with the same constraints. Figure 5 shows the result of 10 replicate analyses ($n = 10$) made by the two chemists. The supervisor has been performing such analyses for years, while this is the trainee's first attempt. This bit of information is important for interpreting the results, which will be based on the following quantities now formally defined:

The sample mean $\bar{x}$: The sample mean is the sum of the individual measurements, divided by n. Most often, the result is rounded to the same number of significant digits as in the replicate measurements.[3] However, occasionally an extra digit is kept, to avoid rounding errors. Consider two numbers: 10. and 11. What is the sample mean? 10.5, but rounding would give 10, not a terribly helpful calculation. In such cases, the mean can be expressed as $10._5$, with the subscript indicating that this digit is being kept to avoid or address rounding error. The 5 is not significant and does not count as a significant digit, but keeping it will

reduce rounding error.[3] Having said that, in many forensic analyses rounding to the same significance as the replicates is acceptable and would be reported as shown in Figure 5. **The context dictates the rounding procedures.** In this example, rounding was to three significant figures, given that the known has a true value with three significant figures. The rules pertaining to significant figures may have allowed for more digits to be kept, but there is no point to doing so on the basis of the known true value and how it is reported.

EXHIBIT B

Statistics "Cheat Sheet"



Absolute error: This quantity measures the difference between the true value and the experimentally obtained value with the sign retained to indicate how the results differ. Remember, error is not the same thing as uncertainty, as these applications of the term will clearly demonstrate. For the trainee, the absolute error is calculated as 12.9 − 13.2, or −0.3% cocaine. The negative sign indicates that the trainee's calculated mean was less than the true value, and this information is useful in diagnosis and troubleshooting. For the forensic chemist, the absolute error is 0.1 with the positive indicating that the experimentally determined value was greater than the true value.

% Error: While the absolute error is a useful quantity, it is difficult to compare across data sets. An error of −0.3% would be much less of a concern if the true value of the sample was 99.5% and much more of a concern if the accepted true value was 0.5%. If the true value of the sample was indeed 0.5%, an absolute error of 0.3% would translate to an error of 60%. To address this limitation of absolute error, the % error is employed. This quantity normalizes the absolute error to the true value:

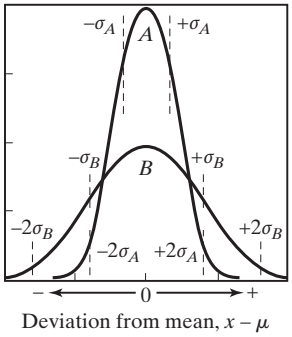


FIGURE 6 Two Gaussian distributions centered about the same mean, but with a different spread (standard deviation). This approximates the situation with the forensic chemist and the trainee.

$$\%\ \text{error} = [(\text{experimentally determined value} - \text{true value})/\text{true value}] \times 100 \qquad \textbf{(2)}$$

For the trainee, the % error is −2.5%, whereas for the forensic chemist, it is 0.5%. The percent error is commonly used to express the accuracy of an analysis when the true value is known. The technique of normalizing a value and presenting it as a percentage will be used again for expressing precision (reproducibility), to be described next. The limitation of % error is that this quantity does not take into account the spread or range of the data. A separate quantity is used to characterize the reproducibility (spread) and to incorporate it into the evaluation of experimental results.

Standard deviation: The standard deviation is the average deviation from the mean and measures the spread of the data. (See Figure 6.) The standard deviation is typically rounded to two significant figures.[3] A small standard deviation means that the replicate measurements are close to each other; a large standard deviation means that they are spread out over a larger range of values. In terms of the normal distribution, ±1 standard deviation from the mean includes approximately 68% of the observations, ±2 standard deviations includes about 95%, and ±3 standard deviations includes around 99% (see Exhibit B). A large value for the standard deviation means that the distribution is relatively wide; a small value for the standard deviation means that the distribution is relatively narrow. The smaller the standard deviation, the closer is the grouping and the smaller is the spread. In other words, the standard deviation expresses quantitatively the reproducibility of the replicate measurements. The experienced chemist produced data with more precision (less of a spread) than those produced by the trainee, as would be expected based on their skill levels.

In Figure 5, two values are reported for the standard deviation: that of the **population** (σ) and that of the sample (s). The population standard deviation is calculated as

$$\sigma = \sqrt{\frac{\sum_{i=1}^{N}(x_i - \mu)^2}{n}} \qquad \textbf{(3)}$$

where i is the number of replicates, 10 in this case.* As we will see, 10 samples in such a case is a very small subset of the parent population. The value σ is the standard

*Although σ is based on sampling the entire population, it is sometimes used in forensic and analytical chemistry. One rule of thumb is that if $n > 15$, population statistics may be used. Similarly, if all samples in a population are analyzed, population statistics are appropriate. For example, to determine the average value of coins in a jar full of change, every coin could be included in the sampling, and population statistics would be appropriate.

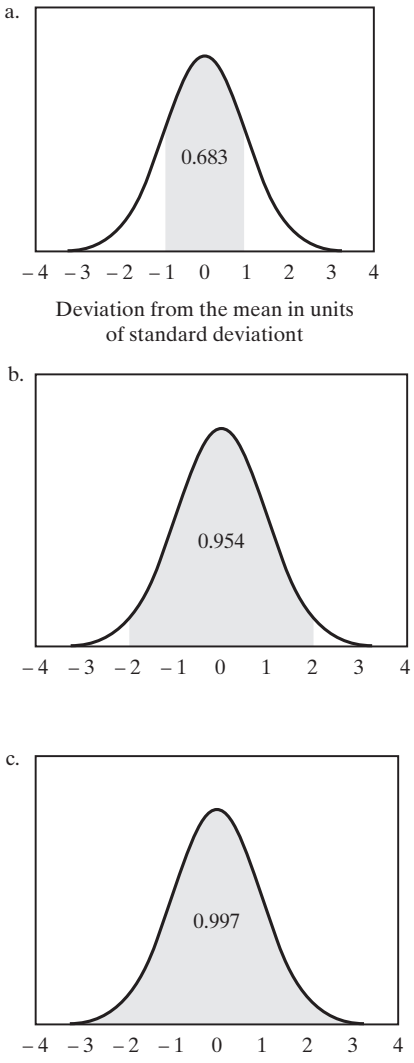


FIGURE 7 Area under the Gaussian curve as a function of standard deviations from the mean. Within one standard deviation (a), 68.3% of the measurements are found; (b) 95.4% are found within two standard deviations, and (c) 99.7% are found within three standard deviations of the mean. The *y*-axis is frequency of occurence.

deviation of the parent population. The use of σ with small sample sets underestimates the true standard deviation that is the squared value of sigma σ.[2] In such cases, a better estimate of σ is given by

$$s = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}{n-1}} \tag{4}$$

The value of s is the standard deviation of the selected subset of the parent population. Calculators and spreadsheet programs differentiate between s and σ, so it is important to make sure that the correct formula is applied.

The sample standard deviations (s) provides an empirical estimate of the expected spread and is frequently used for that purpose. If a distribution is normal, 68.3% of the values will fall between ± 1 standard deviation ($\pm 1s$), 95.4% within $2s$, and 99.7% within $\pm 3s$ from the mean. This concept is shown in Figure 7. This spread provides a range of measurements as well as a probability of occurrence. Frequently, the uncertainty is

TABLE 1 Comparison of Ranges for Determination of Percent Cocaine in QA Sample, True Value $\mu = 13.2\%$

Calculation Method	Trainee, $\bar{x} = 12.9$	Forensic Chemist, $\bar{x} = 13.3$
Min–Max (range)	11.5–15.0	13.1–13.7
$\pm 1s$	12.0–13.8	13.1–13.4
$\pm 2s$	11.0–14.8	12.9–13.7
$\pm 3s$	10.1–15.7	12.7–13.9
95% CI	12.2–13.6	13.2–13.4

cited as ±2 standard deviations, since approximately 95% of the area under the normal distribution curve is contained within these boundaries. Sometimes ±3 standard deviations are used, to account for more than 99% of the area under the curve. Thus, if the distribution of replicate measurements is normal and a representative sample of the larger population has been selected, the standard deviation can be used as part of a reliable estimate of the expected spread of the data.

As shown in Table 1, the supervisor and the trainee both obtained a mean value within ±0.3% of the true value. When uncertainties associated with the standard deviation and the analyses are considered, it becomes clear that both obtained an acceptable result. This is also seen graphically in Figure 8. However, the trainee would likely be asked to practice and try again, not because of the poor accuracy, but to improve reproducibility.

Variance (v): The sample variance (v) of a set of replicates is simply s^2, which, like the standard deviation, gauges the spread within the data set. Forensic chemists favor

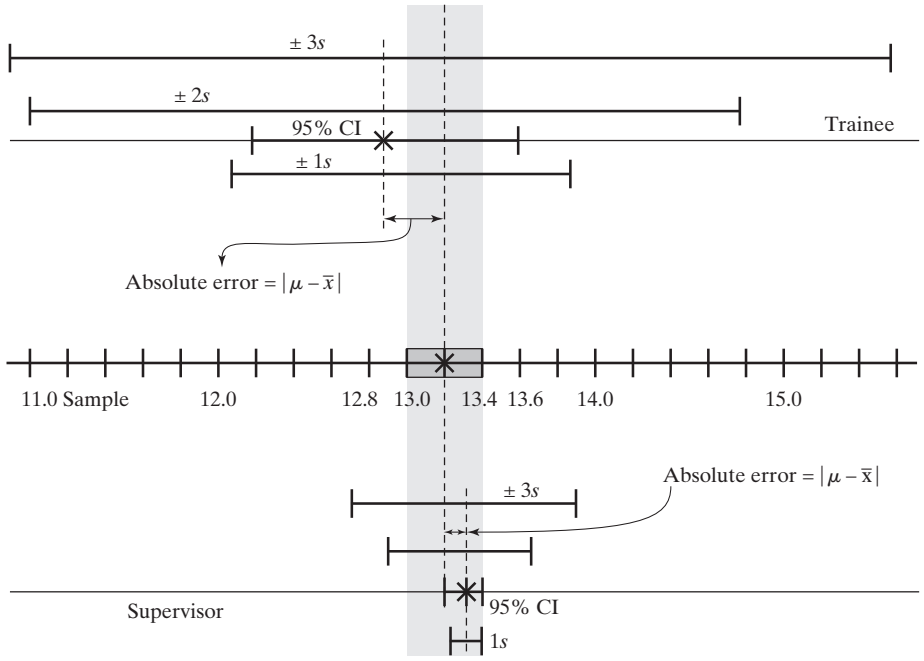


FIGURE 8 Results of the cocaine analysis presented graphically with spread reported several ways. The shaded region is the range around the true value of the sample.

standard deviation as their primary measure of reproducibility, but variance is used in analysis-of-variance (ANOVA) procedures as well as in multivariate statistics.

%RSD of coefficient of variation (CV or %CV): The standard deviation alone does not reflect the relative or comparative spread of the data. This situation is analogous to that seen with the quantity of absolute error. To compare the spread of one data set with another, the mean must be taken into account. If the mean of the data is 500 and the standard deviation is 100, that's a relatively large standard deviation. By contrast, if the mean of the data is 1,000,000, a standard deviation of 100 is relatively small. The significance of a standard deviation is expressed by the percent relative standard deviation (%RSD), also called the coefficient of variation (CV) or the percent CV:

$$\%\text{RSD} = (\text{standard deviation}/\text{mean}) \times 100 \quad \textbf{(5)}$$

In the first example, $\%\text{RSD} = (100/500) \times 100$, or 20%; in the second, $\%\text{RSD} = (100/1{,}000{,}000) \times 100$, or 0.01%. Thus, the spread of the data in the first example is much greater than that in the second, even though the values of the standard deviation are the same. The %RSD is usually reported to one or at most two decimal places, even though the rules of rounding may allow more to be kept. This is because %RSD is used comparatively and the value is not the basis of any further calculation. The amount of useful information provided by reporting a %RSD of 4.521% can usually be expressed just as well by 4.5%.

EXAMPLE PROBLEM 2

As part of a method-validation study, three forensic chemists made 10 replicate injections each in a GCMS experiment and obtained the following data for area counts of a reference peak:

Injection No.	A	B	C
1	9995	10640	9814
2	10035	10118	10958
3	10968	10267	10285
4	10035	10873	10915
5	10376	10204	10219
6	10845	10593	10442
7	10044	10019	10752
8	9914	10372	10211
9	9948	10035	10676
10	10316	10959	10057

Assuming that analyst technique is the only significant contributor to the spread of the data, which chemist had the most reproducible injection technique?

Answer:

This problem provides an opportunity to discuss the use of spreadsheets—specifically, Microsoft Excel. The calculation could be done by hand or on a calculator, but a spreadsheet method provides more flexibility and less tedium. The example shown in Figure 5 (trainee and experienced chemist) was created via a spreadsheet. Note that as a result, the significant figures are not necessarily rounded as they would be in a final calculation.

Reproducibility can be gauged by the %RSD for each data set. The data were entered into a spreadsheet, and built-in functions were used for the mean and standard deviation (sample). The formula for %RSD was created by dividing the quantity in the standard deviation cell by the quantity in the mean cell and multiplying by 100.

Injection #	A	B	C	
1	9995	10640	9814	
2	10035	10118	10958	
3	10968	10267	10285	
4	10035	10873	10915	
5	10376	10204	10219	
6	10845	10593	10442	
7	10044	10019	10752	
8	9914	10372	10211	
9	9948	10035	10676	
10	10316	10959	10057	
Mean	10247.6	10408.0	10432.9	**Function used: average()**
Standard deviation	379.1	340.8	381.6	**Function used: stdev()**
%RSD	3.7	3.3	3.7	**mean/stdev*100**

Analyst B produced data with the lowest %RSD and had the best reproducibility. Note that significant figure conventions must be addressed when a spreadsheet is used just as surely as they must be addressed with a calculator.

95% Confidence interval (95%CI): In most forensic analyses, there will be three or fewer replicates per sample, not enough for standard deviation to be a reliable expression of spread. Even the 10 samples used in the foregoing examples represent a tiny subset of the population of measurements that could have been taken. One way to account for a small number of samples is to apply a multiplier called the Student's *t*-value as follows:

$$\text{confidence interval(CI)} = \frac{s \times t}{\sqrt{n}} \quad \textbf{(6)}$$

where t is obtained from a table such as Table 2. Here, the quantity $\frac{s}{\sqrt{n}}$ is the measure of spread as an average over n measurements.The value for t is selected on the basis of the number of degrees of freedom and the level of confidence desired. In forensic and analytical applications, 95% is often chosen, and the result is reported as a range about the mean:

$$\bar{x} \pm \frac{s \times t}{\sqrt{n}} \quad \textbf{(7)}$$

For the trainee's data in the cocaine analysis example, results are best reported as 12.9 ± 0.7, or $12.2 - 13.6_{(95\%CI)}$. Rephrased, the results can be expressed as the statement

TABLE 2 Student's *t*-Values (Abbreviated)

$n - 1$	90% Confidence Level	95%	99%
1	6.314	12.706	63.657
2	2.920	4.303	9.925
3	2.353	3.182	5.841
4	2.132	2.776	4.604
5	2.015	2.571	4.032
10	1.812	2.228	3.169

that the trainee can be 95% confident that the true value (μ) lies within the reported range. Note that both the trainee and the supervisor obtained a range that includes the true value for the percent cocaine in the test sample. Higher confidence intervals can be selected, but not without due consideration. As certainty increases, so does the size of the range. Analytical and forensic chemists generally use 95% because it is a reasonable compromise between certainty and range size.[4] The percentage is *not* a measure of quality, only of certainty. Increasing the certainty actually *decreases* the utility of the data, a point that cannot be overemphasized.

EXHIBIT C

Is Bigger Better?

Suppose a forensic chemist is needed in court immediately and must be located. To be 50% confident, the "range" of locations could be stated as the forensic laboratory complex. To be more confident of finding the chemist, the range could be extended to include the laboratory, a courtroom, a crime scene, or anywhere between any of these points. To bump the probability to 95%, the chemist's home, commuting route, and favorite lunch spot could be added. To make the chemist's location even more likely, the chemist is in the state, perhaps with 98% confidence. Finally, there is a 99% chance that the chemist is in the United States and a 99.999999999% certainty that he or she is on planet Earth. Having a high degree of confidence doesn't make the data "better"; knowing that the chemist is on planet Earth makes such a large range useless. A confidence interval is not a grade or measure of goodness, it is just a range. Recall that our goal is to deliver data that is both useful and reliable. The data here may be reliable (because the chemist is on earth) but the range is so large that the data has lost its utility.

4 ACCURACY, PRECISION, AND ERROR

With a few basic statistical definitions in hand, we can introduce some important related terms that rely on the foundational statistics just discussed (Figure 9).

- ***Accuracy:*** The closeness of a test result or empirically derived value to an accepted reference value.[5] Note that this is not the traditional definition invoking the closeness to a *true* value; indeed, the true value is unknown, so the test result can be reported only as existing in a range with some degree of confidence, such as the 95%CI. Accuracy is often measured by the error (observed value minus accepted value) or by a percent error.
- ***Bias:*** The difference between the expected and experimental result; also called the total systematic error.[5] Biases should be corrected for, or minimized in, validated methods. An improperly calibrated balance that always reads 0.0010 g too high will impart bias to results.
- ***Precision:*** The reproducibility of a series of replicate measurements obtained under comparable analytical conditions. Precision is measured by %RSD.
- ***Random error:*** An inescapable error, small in magnitude and equally positive and negative, associated with any analytical result. Unlike systematic error, random error is unpredictable. Random error, which can be characterized by the %RSD (precision), arises in part from the uncertainties of instrumentation. Typical micropipets have uncertainties in the range of 1–2%, meaning that, no matter how much care is taken, each use will produce a slightly different volume. The variation may be too small to measure, but it will be present. When all such discrepancies involved in a procedure are combined, the relative variation increases, decreasing reproducibility in turn and adding to random error. True random

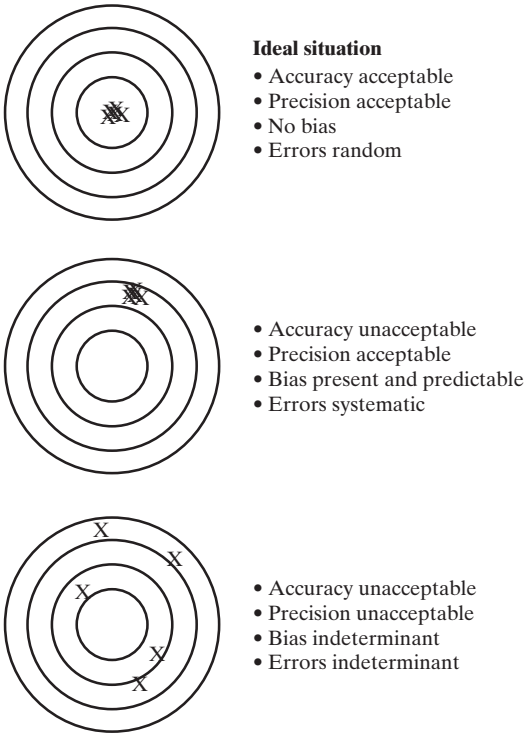


FIGURE 9 Relationships among accuracy and precision, on the one hand, and random and systematic error, on the other.

errors of replicate measurements adhere to the normal distribution, and analysts strive to obtain results affected only by such small random errors.

- ***Systematic error:*** Analytical errors that are the same every time (i.e., predictable) and that are not random. Some use this term interchangeably with "bias." In a validated method, systematic errors are minimized, but not necessarily zero, as illustrated in Figure 9.

4.1 Types of Analytical Errors

In any analytical or forensic measurement, two central goals of method development, validation, and implementation are (1) minimization of errors and (2) development of a defensible uncertainty. To fix errors, they must first be found and diagnosed. An overview of the different sources that contribute to analytical errors is shown in Figure 10. Errors associated with the matrix cannot be controlled, but they can be taken into account.

One way to divide errors is to separate them into two broad categories: those originating from the analyst and those originating with the method. The definitions are as the names imply: The former is an error that is due to poor execution, the latter an error due to an inherent problem with the method. Method validation is designed to minimize and characterize method error. Minimization of analyst error involves education and training, peer supervision and review, and honest self-evaluation. Within a forensic laboratory, new analysts undergo extensive training and work with seasoned analysts in an apprentice role for months before taking responsibility for casework. Beyond the laboratory, there are certification programs administered by professional organizations such as the American Board of Criminalistics (ABC) and the American Board of Forensic Toxicologists (ABFT). Requirements for analyst certification include education, training, professional experience, peer recommendations, and passing certain written and laboratory tests. Certification must be periodically renewed.

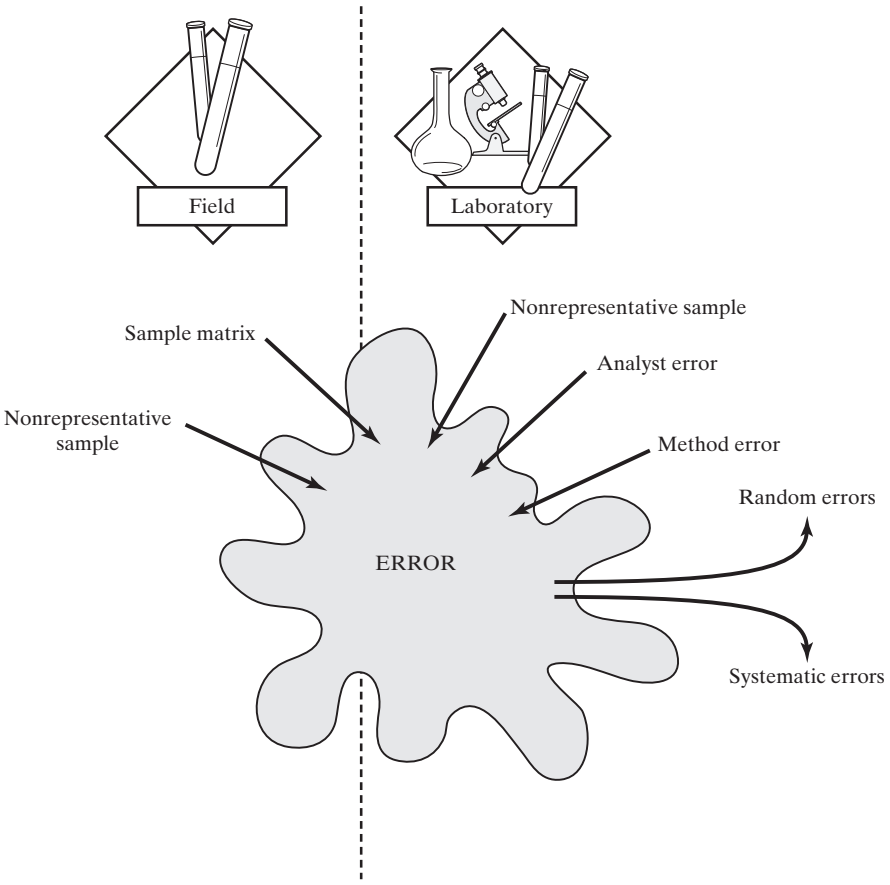


FIGURE 10 Contributions to errors that lead to random and systematic errors in the data produced.

A second way to categorize errors is by whether they are systematic. **Systematic errors** are predictable and impart a bias to the reported results. These errors are usually easy to detect using laboratory checks and quality control procedures. In a validated method, bias is minimal and well characterized. Random errors are equally positive and negative and are generally small. Large random errors are sometimes categorized as gross errors* and often are easy to identify, such as a missed injection by an autosampler. Small random errors cannot be completely eliminated and are due to inherent and inescapable variations due to uncertainties such as illustrated in Example Problem 1.

A whimsical example may help clarify how errors are categorized and why doing so can be useful. Suppose an analyst is tasked with determining the average height of all adults, not just in a town this time, but every human adult. For the sake of argument, assume that the true value is 5 feet, 7 inches. The hapless analyst, who of course does not know the true value, has selected a large subset of the population to measure, gathered data, and obtained a mean of 6 feet, 3 inches, plus or minus 1 inch. Clearly, there is bias (a notable difference between the true value

*Large random errors are also known as OMG errors, for "Oh, my God!" or similar expressions that accompany their discovery.

and the experimentally determined value), but what type of bias? The possibilities include the following:

- An improperly calibrated measuring tape that is not traceable to any unassailable standard. Perhaps the inch marks are actually less than an inch apart. This is a method error that is systematic and can be traced to the instrument being used. To detect it, an object of known height or length would be measured.
- The selected sample (n) included members of a professional basketball team. This is sample bias introduced by a poor sampling plan and is a case where n does not accurately represent the parent population.
- The tape was used inconsistently and with insufficient attention to detail. This is analyst error and would be classified as random. This is an example of a procedural, methodological, and/or analyst error. To detect it, the analyst would be tasked with measuring the height of the same person 10 times under the same conditions. A large variation (%RSD) would indicate poor reproducibility and would also suggest that the analyst needs to have extensive training in the use of a measuring tape and obtain a certification in height measurement.

5 HYPOTHESIS TESTING

5.1 Overview

One of the most useful forensic applications of statistics is **hypothesis testing**, also called **significance testing**. The goal of a significance test is to answer a specific question using calculations and statistical distributions. Through the selection of critical values (α or p value), levels of confidence can be assigned to the decision made. The steps involved in hypothesis testing are outlined in Figure 11. We will use several examples to illustrate the processes and concepts involved.

First, let's return to the data associated with the two forensic chemists, the experienced analyst and the trainee (Figure 5). Let's alter the scenario and say that these data, rather than coming from a proficiency test, originate from an actual case. The experienced analyst performed 10 analyses of white powder drawn from a homogenized exhibit, while the trainee analyzed 10 different samples drawn from the same parent exhibit. The true value is unknown; the goal of the analysis is to estimate it. Because all the samples originated from the same exhibit, all 20 should be representative of the same parent population. A reasonable question to ask would be: Is there any statistically significant difference between the value obtained by the trainee and the value obtained by the experienced analyst? Because we know that the spread of the trainee's data is much larger than that of the trained analyst, our hunch would be that these two means are probably representative of the same population. The way to convert a hunch to a defensible decision is through a hypothesis test.

As shown in Figure 11, the first step, definition of the question, is to state the question as a hypothesis that can be proven or disproven. Here, the *null hypothesis* (H_0) is that there is *no statistically significant difference* between the mean obtained by the trainee and the mean value obtained by the experienced chemist. In other words, $\bar{x}_{\text{trainee}} = \bar{x}_{\text{experienced chemist}}$. Any difference between them is due only to small random variations reflected in the normal distribution curve. The next step is to select the appropriate significance test. Several references can be used for this purpose, including most introductory statistics books. In this case, we have two data sets, both with $n = 10$ and known values of standard deviation and variance. Furthermore, the values of s and v are different. This information is important for selecting the best test. According to a typical reference,[6]

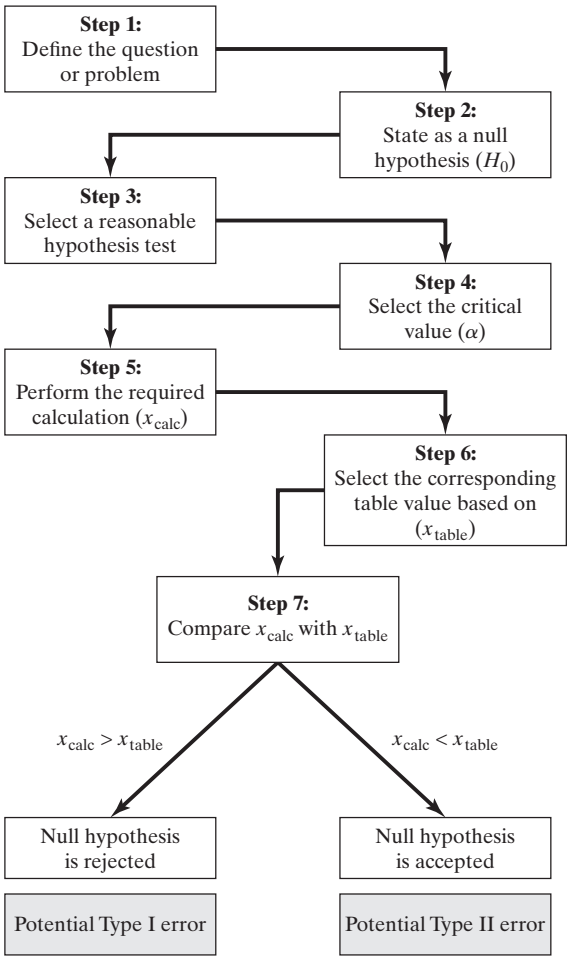


FIGURE 11 A flow chart for hypothesis testing.

several tests for comparing means are available. One that fits is the *t-test of means*. Step 4 (Figure 11) requires selection of a critical value, here 0.05 corresponding to 95% confidence or 95% of the area under the normal distribution curve. The test statistic is given as

$$z = \frac{(\bar{x}_1 - \bar{x}_2)}{\left(\frac{\sigma_{x_1}^2}{n_1} + \frac{\sigma_{x_2}^2}{n_2}\right)^{1/2}} \tag{8}$$

Step 5 involves doing the calculation:

$$z = \frac{(12.9 - 13.3)}{\left(\frac{0.86}{10} + \frac{0.04}{10}\right)^{1/2}} = \frac{0.4}{0.300} = -1.3 = x_{\text{calculated}}$$

The table for the normal distribution that is specified for the test gives a critical value of 1.96. Our calculated value is less than the table value ($x_{calc} < x_{table}$ in Figure 11), meaning that the null hypothesis (i.e., the two means are not different) is accepted. There is only a slight (5% chance) that our acceptance is mistaken. The important point is that there is now a quantifiable level of certainty and risk associated with the decision

reached. Our hunch that the two means are not statistically significantly different has become a defensible probabilistic statement.

5.2 Outliers and Statistical Significance Tests

The identification and removal of **outliers** is dangerous,[4] given that the only basis for rejecting one is often a hunch. A suspected outlier has a value that "looks wrong" or "seems wrong," to use the wording heard in laboratories. The outlier issue can be phrased as a question: Is the data point that "looks funny" a true outlier? The point is (is not) an outlier. The corresponding null hypothesis is that the point is not an outlier, it falls within a range that is expected based on what we have observed for all of the other points.

Once again, let's return to the trainee and experienced chemist data set (Figure 5) and return to the scenario where both are doing replicate analyses of a proficiency test sample. Suppose the supervisor and the trainee both ran one extra analysis independently under the same conditions and obtained a concentration of 11.0% cocaine. Is that datum suspect for either of them, neither of them, or both of them? Should they include it in a recalculation of their means and ranges? This question can be tested by assuming a normal distribution of the data. As shown in Figure 12, the trainee's data have a much larger spread than those of the supervising chemist, but is the spread wide enough to accommodate the value 11.0%? Or is this value too far out of the normally expected distribution? Recall that 5% of the data in any normally distributed population will be on the outer edges far removed from the mean—that is expected. Just because an occurrence is rare does not mean that it is unexpected. After all, people do win the lottery. These are the considerations the chemists must balance in deciding whether the 11.0% value is a true outlier or a rare, but not unexpected, result.

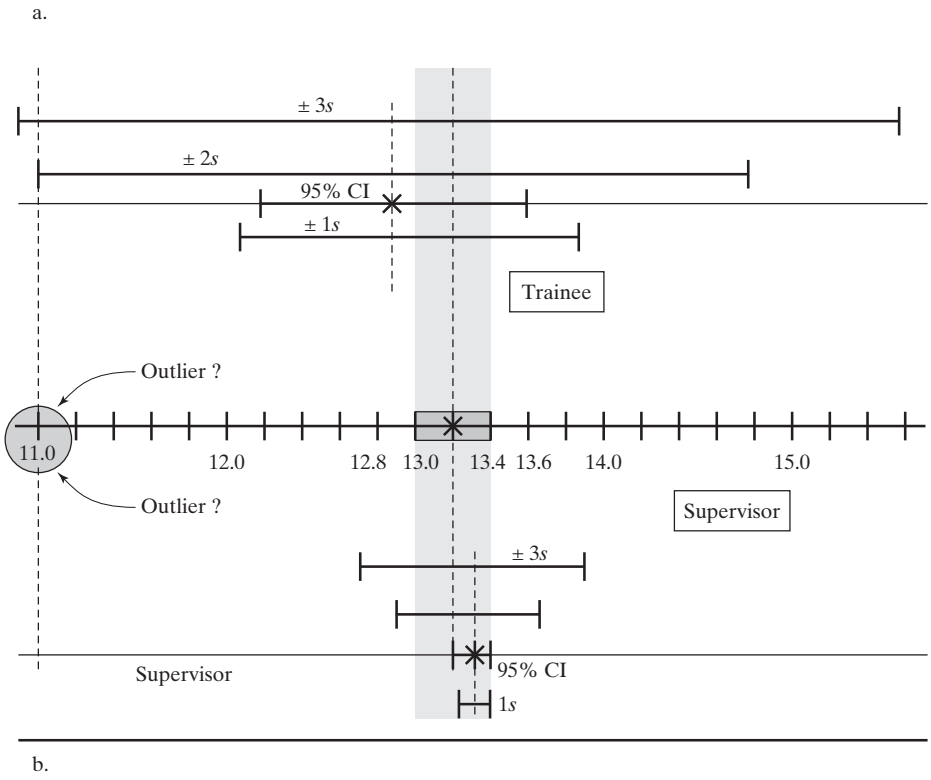
Regarding the outlier in question, the null hypothesis for the chemist and the trainee states that the 11.0% value is not an outlier and that any difference between the calculated and expected value can be attributed to normal random variation. Both want to be 95% certain that retention or rejection of the data is justifiable. Another way to state this is to say that the result is or is not significant at the 0.05 ($p = 0.05$ or $\alpha = 0.05$), or 5%, level. If the calculated value exceeds the value in the table, there is only a 1 in 20 chance that rejecting the point is incorrect and that it really was legitimate based on the spread of the data.

With the hypothesis and confidence level selected, the next step is to apply the chosen test (Step 5 in Figure 11). For outliers, one test used (perhaps even abused) in analytical chemistry is the Q or **Dixon test:**

$$Q_{calc} = |\text{gap}/\text{range}| \quad \textbf{(9)}$$

To apply the test, the analysts would organize their results in ascending order, including the point in question. The gap is the difference between that point and the next closest one, and the range is the spread from low to high, also including the data point in question. The table used is that for the Dixon's Q parameter, two tailed.[7-10]* If $Q_{calc} > Q_{table}$, the data point can be rejected with 95% confidence. The Q_{table} for this calculation with

*Many significance tests have two associated tables: one with one-tailed values, the other with two-tailed values. Two-tailed values are used unless there is reason to expect deviation in only one direction. For example, if a new method is developed to quantitate cocaine, and a significance test is used to evaluate that method, then two-tailed values are needed because the new test could produce higher or lower values. One-tailed values would be appropriate if the method were always going to produce, for example, higher concentrations.



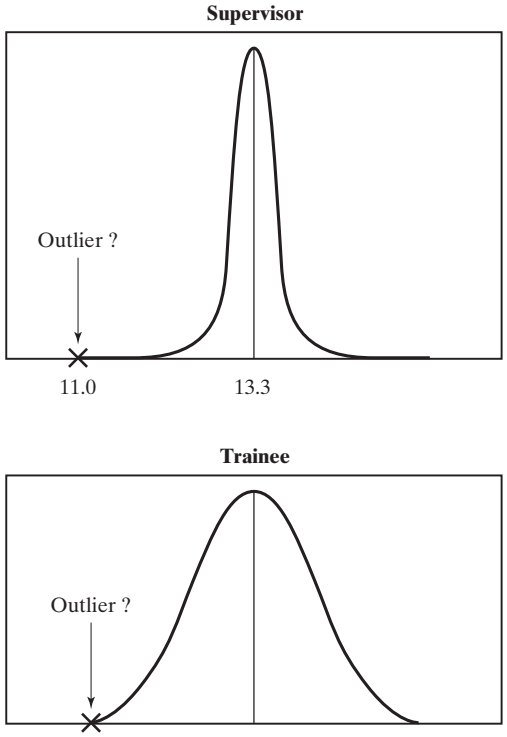


FIGURE 12 On the basis of the spread seen for each analyst, is 11.0% a reasonable value for the concentration of cocaine?

TABLE 3 Outlier Tests for 11.0% Analytical Results

Test	Trainee	Chemist
Q test $Q_{table} = 0.444$	$Q_{calc} = \frac{[11.5 - 11.0]}{[15.0 - 11.0]} = 0.125$	$Q_{calc} = \frac{[13.1 - 11.0]}{[13.7 - 11.0]} = 0.778$
Grubbs test	$Z = \frac{\lvert 12.9 - 11.0 \rvert}{0.93} = 2.04$	$Z = \frac{\lvert 13.3 - 11.0 \rvert}{0.20} = 11.5$
Critical $Z = 2.34$		

$n = 11$ is 0.444. The calculations for each test are shown in Table 3. The results are not surprising, given the spread of the experienced chemist's data relative to that of the trainee. The trainee would have to include the value 11.0 and recalculate the mean, standard deviation, and other quantities associated with the analysis.

In the realm of statistical significance testing, there are often several tests for each type of hypothesis. The **Grubbs test,** recommended by the International Organization for Standardization (ISO) and ASTM International, formerly known as the American Society for Testing and Materials (ASTM), is another approach to the identification of outliers:

$$G = |\text{questioned} - \bar{x}| / s \quad \textbf{(10)}$$

Analogously to obtaining Dixon's Q parameter, one defines H_0, calculates G, and compares it with an entry in a table. The quantity G is the ratio z that is used to normalize data sets in units of variation from the mean.

EXAMPLE PROBLEM 3

A forensic chemist analyzed a blind-test sample with high-performance liquid chromatography (HPLC) to determine the concentration of the explosive RDX in a performance test mix. Her results were as follows (all in units of parts-per-million or ppm):

Laboratory data	
56.8	57.2
57.0	57.2
57.0	57.8
57.1	58.4
57.2	59.6

Are there any outliers in these data at the 5% level (95% confidence)? Take any such outliers into account if necessary, and report the mean, %RSD, and 95% confidence interval for the results.

Answer:

For outlier testing, the data are sorted in order such that potential outliers are easily located. Here, the questionable value is the last one: 59.6 ppb. It seems far removed from the others, but can we justify removing it from the data set? The first step is to determine the mean and standard deviation and then to apply the two outlier tests mentioned thus far in the text: the Dixon and Grubbs approaches.

Mean = 57.5_3°°Standard deviation $(s) = 0.86$°°$n = 10$

Dixon Test (eq. 8)

$$Q = \frac{\text{map}}{\text{range}} = \frac{(59.6 - 58.4)}{(59.6 - 56.8)} = 0.429$$

Table value = 0.477

$Q_{calc} < Q_{table}$ = keep

Grubbs Test (eq. 9)

$$G = \frac{(59.6 - 57.5_3)}{0.8642} = 2.39$$

Table value (5%) = 2.176

$G_{calc} > G_{table}$ = reject

This is an example of contradictory results (compared to the Q test), and in such cases, ASTM recommends that the Grubbs test take precedence. Accordingly, the point is retained and the statistical quantities remain as is. The 95% confidence interval is then calculated:

95% confidence interval

$$\bar{x} \pm \frac{ts}{\sqrt{n}}$$

0.8642

10

from table of *t* values
2.26

$$\bar{x} \pm \frac{(2.26)(0.8642)}{\sqrt{10}} = 0.618$$

57.5 ± 0.6 or 56.9 – 58.1 ppm

For example, one of the data points obtained by the trainee for the percent cocaine was 15.0. To express this as the normalized *z* value, we have

$$z = (15.0 - 12.9)/0.93 = 2.26 \quad \textbf{(11)}$$

This value is 2.26*s*, or 2.26 standard deviations higher than the mean. A value less than the mean would have a negative *z*, or negative displacement. By comparison, the largest percentage obtained by the experienced forensic chemist, 13.7%, is 2.00*s* greater than the mean. The Grubbs test is based on the knowledge that in a normal distribution, only 5% of the values are found more than 1.96 standard deviations from the mean.* For the 11.0% value obtained by the trainee and the chemist, the results agree with the *Q* test; the trainee keeps that value and the forensic chemist discards it. However, different significance tests may produce different results, with one indicating that a certain value is an outlier and another indicating that it is not. When in doubt, the typical practice in a forensic context is to use the more conservative test. Absent other information, if one says to keep the value and one says to discard it, the value should be kept. Finally, note that these tests are designed for the evaluation of a single outlier. When more than one outlier is suspected, other tests are used, but this situation is not common in forensic chemistry.

Finally, there is a cliché that "statistics lie" or that they can be manipulated to support any position desired. Like any tool, statistics can be applied inappropriately, but that is not the fault of the tool. The previous example, in which both analysts obtained the same value on independent replicates, was carefully stated. However, having both

*The value ±2 standard deviations used previously is a common approximation of 1.96*s*.

obtain the exact same concentration should at least raise a question concerning the coincidence. Perhaps the calibration curve has deteriorated or the sample has degraded. Perhaps there an error has occurred in the generation of the report in which one result was reported twice. The point is that the use of a statistical test to eliminate data does not, and should not, take the place of laboratory common sense and analyst judgment. A data point that "looks funny" warrants investigation and evaluation before anything else. In summary, chemistry before statistics. Statistical testing and analysis is used to identify problems, but chemistry and lab work should be used to address them if at all possible.

5.3 Hypothesis Tests for Comparison of Data Sets

Another hypothesis test used in forensic chemistry is one that compares the means of two data sets. In the supervisor–trainee example, the two chemists are analyzing the same unknown, but obtain different means. The *t*-test of means can be used to determine whether the difference of the means is significant. The *t*-value is the same as that used in equation 6 for determining confidence intervals. This makes sense; the goal of the *t*-test of means is to determine whether the spread of two sets of data overlap sufficiently for one to conclude that they are or are not representative of the same population.

In the supervisor–trainee example, the null hypothesis could be stated as "H_0: The mean obtained by the trainee is not significantly different than the mean obtained by the supervisor at the 95% confidence level $(p = 0.05)$." Stated another way, the means are the same and any difference between them is due to small random errors.

EXAMPLE PROBLEM 4

A toxicologist is tasked with testing two blood samples in a case of possible chronic arsenic poisoning. The first sample was taken a week before the second. The toxicologist analyzed each sample five times and obtained the data shown in the following table. Is there a statistically significant increase in the blood arsenic concentration? Use a 95% confidence level.

	Week 1	**Week 2**	
Possible arsenic poisoning	16.9	17.4	
Q: Has there been a statistically significant	17.1	17.3	**[As] ppb**
increase in the arsenic concentration?	16.8	17.3	**in blood**
	17.2	17.5	
	17.1	17.4	
Excel → **Use Tools → Data analysis → t test unequal variance**			
p = 0.05, hypothesized mean = 0			t_{table}:
Output:			**2.365** ←

	Week 1	***Week 2***	
Mean	17.02	17.38	
Variance	0.027	0.007	
Observations	5	5	
Hypothesized mean difference	0		
df	6		
t Stat	*–4.37*	← $t_{cal} = 4.37$	
P(T<=t) one-tail	0.00		
t Critical one-tail	1.94		
P(T<=t) two-tail	0.00		
t Critical two-tail	2.45		

$t_{cal} >> t_{table}$:

Null hypothesis that means are the same is rejected.

Answer:

Manual calculations for the *t*-test are laborious and prone to error. The best way to work such problems is with Excel, as shown in the accompanying figure. The feature used is under "Data Analysis," found in the "Tools" menu. This analysis pack is provided with Excel, although it has to be installed as an add-in. (See Excel help for more information on installing it.)

Once the data are entered, the analysis is simple. Notice that it was assumed that the variances were different; if they had been closer to each other in value, an alternative function, the *t*-test of means with equal variances, could have been used. Also, the *t*-statistic is an absolute value; the negative value appears when the larger mean is subtracted from the smaller. For this example, $t_{calc} = 4.37$, which is greater than $t_{table} = 2.365$. This means that the null hypothesis must be rejected and that the concentration of arsenic has increased from the first week to the second.

The equation used to calculate the test statistic is

$$t_{calc} = \frac{|\text{mean}_1 - \text{mean}_2|}{s_{pooled}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \tag{12}$$

where s_{pooled}, the pooled standard deviation from the two data sets, is calculated as

$$s_{pooled} = \sqrt{\frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}} \tag{13}$$

This calculation can be done manually or preferably with a spreadsheet, as shown in Example Problem 4. The result for the supervisor/trainee is a t_{calc} of 1.36, which is less than the t_{table} of 2.262 for 10 degrees of freedom. Therefore, the null hypothesis is accepted and the means are the same at the 95% confidence level, or $p = 0.05$. This is a good outcome, since both chemists were testing the same sample. Note that the *t*-test of means as shown is a quick test when two data sets are compared. However, when more data sets are involved, different approaches are called for. It is important to reiterate that the selected test must fit the situation and that before use, all caveats and limitations of the test be considered. If not, the test is no better (and sometimes even worse) than a hunch.

5.4 Types of Error

Whenever a significance test is applied such that a null hypothesis is proposed and tested, the results are always tied to a level of certainty, 95% in the example in the previous section. With the forensic chemist's data, the 11.0% data point was identified as an outlier with 95% certainty, but that still leaves a 1-in-20 chance that this judgment is in error. This risk or possibility of error can be expressed in terms of types. A **Type I** error is an error in which the null hypothesis is incorrectly rejected, whereas a **Type II** error is an error in which the null hypothesis is incorrectly accepted. Put in terms of the experienced chemist and the 11.0% value, H_0 states that that value is not an outlier, but the null hypothesis was rejected on the basis of the calculations for the *Q* test and the Grubbs test. If that were in fact a mistake, and the 1-in-20 long shot came true, throwing out the 11.07% value would be a Type I error. In effect, the chemist makes a defensible judgment in rejecting that value, deciding that the harm done by leaving it in would be greater than the risk of throwing it out. This point is illustrated in Figure 11.

Summary

The NUSAP framework combined with basic statistics forms a fundamental and quantitative component of quality assurance. Statistics is also a powerful tool for the forensic chemist, in that it can describe the accuracy and precision of data, assist in answering hypothesis-based questions, and identify trends and patterns in data. With this foundational information in hand, we are ready to explore quality assurance in detail, including topics such as method validation, figures of merit, and uncertainty. Together, these constitute the pedigree (P) of forensic data that are relevant and reliable.

Key Terms and Concepts

Absolute error
Absolute uncertainty
Accuracy
Bias
95% Confidence interval (95%CI)
Dixon's test
Error
Grubbs test
Hypothesis testing
Measurand
Metrology
NUSAP
Outlier
Population
Precision
Propagation of uncertainty
Random error
Reliability
Relative uncertainty
Significance test
Significant figures
Standard deviation
Systematic error
Type I error
Type II error
Uncertainty
Utility
Variance

Problems

FROM THE CHAPTER

1. A standard of Pb^{2+} for a gunshot residue analysis using atomic absorption is prepared by first dissolving 1.0390 g dried $Pb(NO_3)_2$ in distilled water containing 1% nitric acid. The solution is brought to volume in a class A 500-mL volumetric flask with an uncertainty of ± 0.20 mL. The solution is then diluted 1:10 by taking 10 mL (via an Eppendorf pipet, tolerance ± 1.3 μL) and diluting this in 1% nitric acid to a final volume of 100 mL in a volumetric flask with a tolerance of ± 0.08 mL. The balance has an uncertainty of ± 0.0002 g.
   a. Using conventional rounding rules, calculate the concentration of the final solution of Pb^{2+}, in ppm.
   b. Determine the absolute and relative uncertainties of each value obtained in part a. Select the largest and report the results as a concentration range.
   c. Report the results as a range by the propagation-of-error method.
   d. Comment on your findings and why this case is unique.
2. If an outlier is on the low side of the mean, as in the example in the chapter, could a one-tailed table be used?
3. Find the following reference for *Q*-value tables: Rorabacher, D. B. "Statistical Treatment for Rejection of Deviant Values: Critical Values of Dixon's 'Q' Parameter and Related Subrange Ratios at the 95% Confidence Level." *Analytical Chemistry*, 63 1991, 139–148. For the trainee/forensic chemist example used throughout this chapter, determine what the percent cocaine would have to be in the 11th sample for it to be eliminated at the following significance levels: 0.20, 0.10, 0.05, 0.04, 0.02, and 0.01. Present your findings graphically and comment on them.
4. Differentiate clearly between statistical and analytical errors.
5. Justify or explain the use of the factor 2 in equation 13.
6. In the quotation from the NAS report, what aspects of NUSAP are specifically addressed?
7. A forensic chemist prepares a standard of caffeine in chloroform for use in a quantitative assay. The caffeine is purchased from a reputable supply house and arrives with a certificate stating that it is 99.5% pure or better. The analyst needs to make a stock solution at a concentration near 1 mg/mL. To do so, he obtains a class A 50.00-mL volumetric flask (± 0.05 mL) and uses a microbalance to accurately weigh out 49.6 mg. The balance uncertainty is listed as ± 0.0003 g near the 50-mg range. The analyst quantitatively transfers the powder to the flask and carefully dilutes the solution to volume. Report the concentration of the solution in mg/mL and the propagated uncertainty associated with the value. Be sure to use the proper number of significant figures.
8. An analyst proposes a new method for analyzing blood alcohol. As part of a method validation study,

she analyzes a blind sample five times and obtains the following results: 0.055%, 0.054%, 0.055%, 0.052%, and 0.056%.
   a. Are there outliers in the data?
   b. On the basis of the results of the outlier analysis and subsequent actions, calculate the mean and the %RSD of the analyst's results.
   c. If the true value of the blind sample is 0.053% ±0.002% (based on a range of ±2*s*), is the mean value obtained by the analyst the same as the true value at $p = 0.05$?

9. In the scenario presented in Figure 5 and discussed in Section 5, would it matter if the data were normally distributed for application of a hypothesis test?

10. Using the Excel functions skewness and kurtosis, calculate these values for each data set and comment on their meaning. Is either or neither a hypothesis test? Explain.

INTEGRATIVE

1. Locate one or more hypothesis tests that can be used to determine whether a distribution is normal. Describe and apply the test(s) to the two distributions presented in Figure 5. Discuss the results. *Hint:* There are many resources available for this, including the *R* statistical package, available online for free at The *R* Project for Statistical Computing, http://www.r-project.org/, last accessed February 2011.

2. What is the difference between a confidence level and a probability?

3. A university instructs its professors to assign grades in the range from 0.0 for an F to 4.0 for an A. The letter grade is not recorded; only the number grade is. When students' GPAs are calculated, they are reported to three decimal places. Can this practice be justified by the rules of significant figures?

4. A toxicology laboratory associated with a state medical examiner's office purchases a new type of extraction system with the hope of improving on its current method of screening for fentanyl in postmortem blood samples. To validate the change, lab analysts run 20 samples using the older extraction method and the same 20 samples using the new method (results given in µg/L). The laboratory director asks, "Is this new method more reproducible than the old method?" You elect to use a hypothesis test to answer the question, specifically the *F*-test. Use online or other resources to learn about the *F*-test and apply it to the question at hand. Explain why the *F*-test works, what your null hypothesis is, and whether the data support rejecting or accepting the null hypothesis at the 95% level ($p = 0.05$). Is the new method "better" in terms of reproducibility?

Old Method	New Method	Old Method	New Method
3.77	3.49	3.76	3.78
3.81	3.85	3.90	3.86
3.77	3.54	3.74	3.81
3.85	3.63	3.86	3.53
3.77	3.60	3.79	3.87
3.80	3.65	3.82	3.67
3.71	3.70	3.88	3.71
3.82	3.52	3.82	3.95
3.76	3.91	3.87	3.69
3.76	3.58	3.74	3.50

5. Why can there never be a true value?

6. A government agency, the National Institute of Standards and Technology (NIST), provides certified reference materials to forensic laboratories. Research how NIST certifies a reference material, and comment on how this approach facilitates reliability.

7. What is the difference between a hypothetical question and a hypothesis-based question?

8. What court cases apply to the concepts of utility and reliability? How?

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Quality Assurance and Quality Control

OVERVIEW AND ORIENTATION

In this chapter, we will expand on the concept of NUSAP as a model for supporting the reliability of data, integrating concepts of statistics as we go. As illustrated in Figure 1, when a police officer, agent, or other client submits evidence to a laboratory, what happens next is all but invisible to them, a "black box" process in which evidence goes in and a report comes out. **Quality assurance (QA)** is an all-encompassing, "cradle-to-grave" system that controls data generation. **Quality control (QC)** usually has to do with the procedures, policies, and practices designed to assure data quality. As shown in Figure 1, QA defines the triangle, and QC populates it, and taken as a whole, these elements define the pedigree (P) that we discussed as part of NUSAP. Validated analytical methods, laboratory accreditation, blanks, **replicates**, and legally defensible documentation are all part of quality assurance. The system incorporates multiple reviews and can be viewed as an integrated, layered structure of redundant checks and protocols that relate directly or indirectly to data generated at the bench level. Quality assurance is dynamic, is evolving, and requires daily care and maintenance to remain viable.

1 QUALITY ASSURANCE AND QUALITY CONTROL

1.1 Total Quality Management

As a systematic procedural approach, quality assurance and quality control (broadly defined) can be traced to the post–World War II years and to applications in the military and engineering. Most quality management systems have a business orientation, but the philosophy is applicable to laboratory work.

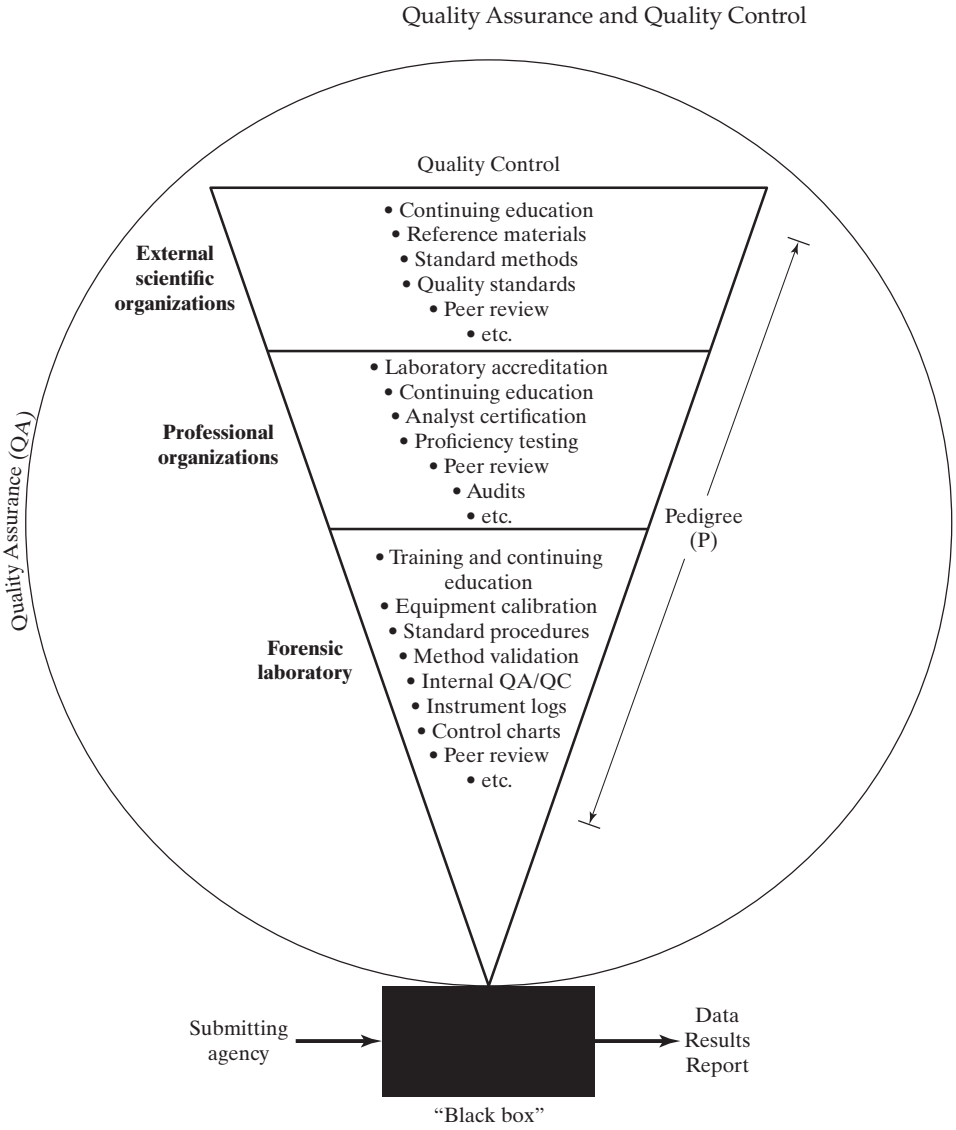

FIGURE 1 The layered nature of quality assurance. This chapter will discuss the specific elements shown in the layers. Taken collectively, these layers help define the pedigree of data emerging from a forensic laboratory.

The practices and procedures used within an organization (the lower level of the triangle shown in Figure 1) such as a forensic laboratory are often referred to as **total quality management**, or TQM. TQM, when combined with guidelines referred to as "good laboratory practice" (GLP), encompasses the entire process of generation of data and ensures both the utility and reliability of that data.[1] TQM consists of the protocols used by the laboratory and involves everyone from the analyst working at the laboratory bench through management. The structures and protocols within a TQM system are often adopted or adapted from external standardization and quality assurance entities. The International Organization for Standardization (ISO) was formed in 1947 as a voluntary organization consisting of member groups. The American National Standards Institute (ANSI) is the U.S. representative in ISO. Also within ANSI, the American Society of Quality (ASQ) oversees issues of quality management.[1] These organizations are not regulatory bodies, nor are they industry or product specific. As illustrated in Figure 1, these organizations have a hierarchy from global and general (such as ISO) to

profession-specific. Taken together, these organizations help develop the pedigree of data emerging from the forensic laboratory.

1.2 Who Makes the Rules? International Organizations, Accreditation, and Certification

As you have undoubtedly noted, definitions are central to quality and quality management. For a truly international system of quality to exist, all parties, from bench chemists to professional societies, need to be working from the same or similar definitions. A reasonable question is, Who makes the rules? A number of organizations are involved in setting these definitions and updating them as necessary. A few of these (ANSI, ISO, etc.) have already been mentioned. Other organizations and references include the following:

- The International Vocabulary of Basic and General Terms in Metrology (often abbreviated as the VIM)
- International Union of Pure and Applied Chemistry (IUPAC)
- International Organization of Legal Metrology (OIML)
- The Association of Analytical Communities (AOAC)
- EURACHEM
- Cooperation on International Traceability in Analytical Chemistry
- National Institute of Standards and Technology (NIST)

A journal published by Springer-Verlag also provides a forum for peer-reviewed publications relevant to this aspect of metrology: *Accreditation and Quality Assurance: Journal for Quality, Comparability and Reliability in Chemical Measurement*, ISSN 0949–1775 (print), 1432–0517 (online). Taken collectively, these organizations and references provide definitions, standards, and a foundation upon which individual laboratories build their TQM programs. Unless otherwise noted, we will use definitions derived from the VIM.

Within the forensic community, a number of professional organizations utilize these organizations and definitions to craft methods and procedures that are discipline specific. The following are some examples;

- Society of Forensic Toxicologists (SOFT)/American Academy of Forensic Sciences (AAFS): Forensic Toxicology Laboratory Guidelines
- Scientific Working Group for Seized Drug Analysis (SWGDRUG): SWGDRUG Recommendations
- Scientific Working Group for Materials Analysis (SWGMAT)
- American Society for Testing and Materials International (ASTM International); numerous standard procedures for forensic analysis
- American Society of Crime Laboratory Directors, Laboratory Accreditation Board (ASCLD/LAB)
- Forensic Quality Service International

At the laboratory level, information from the international, national, and professional organizations is integrated as needed to craft the overall quality management program. For example, a laboratory wishing to launch a toxicology section would likely first turn to the SOFT/AAFS guidelines to begin planning and then move outward and upward from there as it worked toward starting, operating, and eventually accrediting the toxicology section.

Two important aspects of QA/QC are **accreditation** and **certification**. As part of TQM, there has to be some way to ensure that the person on the bench and the organization he or she works for can perform the required analyses. Any mature profession, such as law or medicine, has professional organizations, and one of the roles of these organizations is to guarantee the ethics, responsibility, and trustworthiness

of their members. This process is generically called certification. In the forensic context, accreditation refers to laboratories, but both functions are often overseen by the same entity. This is an important distinction: analysts are certified; laboratories are accredited.

The different areas of forensic science have different certification bodies, depending on the specialty, but most are coordinated by or affiliated with the AAFS. For forensic chemists working in drug analysis, arson, and trace evidence, accreditation is administered by the American Board of Criminalistics (ABC), which was founded in 1989. Forensic toxicologists seek certification through the American Board of Forensic Toxicology (ABFT). Forensic laboratories are accredited by the American Association of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD/LAB). Accreditation is an arduous multistep process that examines everything from the physical plant of a laboratory through its analysts and management structure. As of June 2010, 375 crime labs had earned this accreditation, which, like analyst certification, must be renewed periodically.

Finally, on the subject of accreditation and certification, the National Academy Report of 2008 made an unambiguous and strong recommendation in regard to these two aspects of forensic practice:

> *Laboratory accreditation and individual certification of forensic science professionals should be mandatory, and all forensic science professionals should have access to a certification process.*[3]

These recommendations will undoubtedly affect the practice of forensic chemistry in the coming years.

1.3 Inside the Black Box of TQM

QA/QC envelops all aspects of forensic chemistry. As shown in Figure 2, much of this is invisible to the outside observer or laboratory client. Once the sample enters the laboratory, a number of interlocking and overlapping factors play a role in generating the report the client receives. In this chapter, we will focus on elements specific to the analysis (middle block of Figure 2) before addressing sampling plans and uncertainty determinations in detail in the next chapter. However, we will briefly mention them here as background for this chapter. Different laboratories and laboratory systems practice and implement QA/QC in various ways.

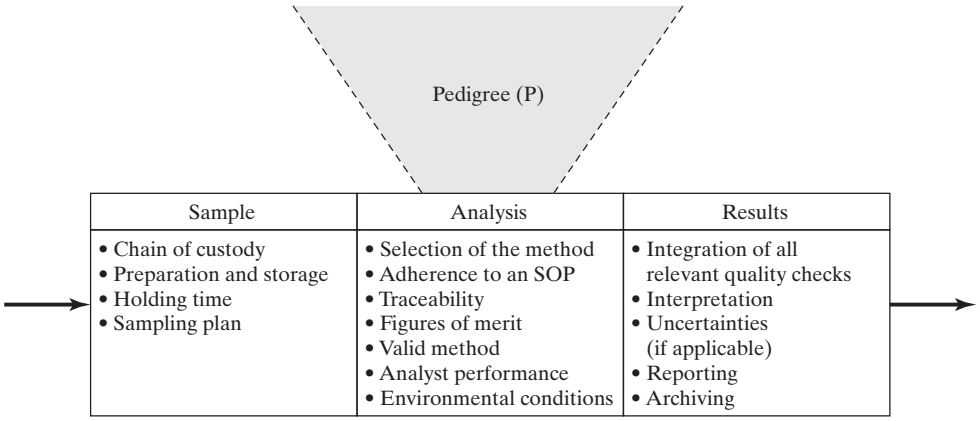

FIGURE 2 Details of the "black box."

When a sample is accepted at the laboratory, the cradle-to-grave monitoring of quality begins, building and supporting the pedigree of the data that emerge. The **chain of custody** (see section 1.3.1) of each exhibit of evidence must be preserved and verified, and the sample must be properly handled and stored. As an example, blood-stained clothing must be allowed to dry before long-term storage, and this drying must take place in a controlled environment that preserves the chain. Blood or tissue samples have to be refrigerated and, in some cases, frozen. Until the evidence is transferred to the analyst, it must be stored properly and securely. Some sample types must be analyzed within a certain time frame to avoid degradation, and these holding times must be monitored and met. Large seizures of evidence with multiple individual exhibits must be subsampled in a reasonable and defensible way. If any of these criteria are not met, then the reliability of the analysis, regardless of how well it is designed and executed, is already in question.

QA/QC also plays a critical role after the analysis is complete. If the data are quantitative, the uncertainty of the results should be estimated. Checks must be in place to insure that the link between data and sample is unquestioned and that no transcription error has occurred. Bar coding is frequently used for this purpose. The report generated must be concise, complete, accurate, and peer-reviewed. All the information that constitutes the pedigree of the reported results (Figures 1 and 2) must be collated and made available to the client or other parties as the case moves forward. For example, a defense attorney in a heroin case may request details about the method used, standards employed, and information regarding the raw data. This should be easily accessible and deliverable upon request. The laboratory must accurately and safely archive whatever information is necessary to do so, be it needed in a month from the date of completion or 5 years later. Thus, the actual analysis of an exhibit of evidence represents only one part of a complex process designed to yield useful and reliable analytical data.

As for the analysis itself, the first question to be addressed is: Which method is to be used? The answer to that question is dictated by the forensic question. In other words, the method selected depends on its **fitness for purpose** (or **fitness of purpose**). If the forensic question is, Could the questioned fiber (Q) have come from the blanket collected from the crime scene? Then the methods selected will involve microscopy and instrumental analysis. If the forensic question is: Are there drugs and metabolites present in this blood sample that could help determine the cause of death? Then a different set of methods will be chosen. Fitness for purpose is part of insuring the utility of the data produced. In the case of the blood sample, characteristics of the blood sample such as settling rate or white cell count are interesting but typically do not provide data that are relevant to answering the forensic question and would not be determined. If these properties were measured, then the method that was used would not meet the fitness for purpose criteria.

Once the method is selected, it must be executed properly. As illustrated in Figure 2, there are many factors that contribute to this process.

1.3.1 LABORATORY DOCUMENTATION Documentation within a laboratory and how it is managed is often an unappreciated and poorly understood aspect of forensic analysis. The best known example of laboratory documentation, the chain of custody, is one part of a larger collection of written materials that describe, prescribe, and document the day-to-day functioning of the laboratory. The purpose of the chain of custody documentation (often abbreviated as "the chain") is to provide an unassailable record of the history of an exhibit of evidence from its creation or collection through its final deposition, be it destruction or storage. Although the format varies among agencies and laboratories, the chain-of-custody document includes a description and number for an exhibit along with who collected it, when, where, and possibly how. These

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FIGURE 3 Examples of evidence labeling as part of laboratory documentation.

facts are attested to via a legal signature, time, and date. The exhibit is packaged with seals—usually evidence tape—that are marked in such a way to insure that any tampering with the package will be obvious. As seen in Figure 3, the plastic bag is sealed with tamper-resistant evidence tape and initialed such that breaking of the seal will be evident because the initials will be disturbed. Securely packaged, the exhibit is the responsibility of the signatory until delivered to the laboratory, where the transfer will be noted on the chain-of-custody document. The person surrendering custody signs, as does the person accepting custody, and writes the date and time of transfer. The same procedure is used at every transfer, and the last person signing is responsible for the safety and security of the exhibit.

When the analyst has custody and begins work, he or she must open the exhibit in such as way as to preserve the integrity of earlier seals. In the example shown in Figure 3, the analyst could cut the baggie along the bottom edge, noting this operation in the case file. This procedure affords access to the contents while preserving the earlier seal. After completing the analysis, the analyst returns whatever remains of the exhibit to the bag and seals the cut he or she made with evidence tape, marks it with dates and initials, and also records it in the case file. Should another analyst need access to the sample at a later date, he/she would have to make a third cut, protecting the other two seals in place. This process insures that anyone who handled the exhibit can look at it while testifying in court and immediately note whether his/her seal remains intact. If there is any question as to the integrity of any seal, the integrity of the exhibit itself comes into question.

How analysts record their working notes varies among laboratories. One common method is to use note sheets imprinted with a laboratory identifier. The analyst records notes on sheets and authenticates them using a legal signature, initials, and dates. The number of pages associated with a given case is noted; for example, the bottom of each sheet would include a notation such as "Page 6 of 8." These sheets are then placed in the case file folder along with any other pertinent information such as instrument printouts or calibration data. Forensic laboratories generally do not employ the classic laboratory notebook for documenting casework. Given that case notes must be made available

to parties outside the laboratory, such a notebook might have to be surrendered at a moment's notice. If more than one case was recorded in the notebook, all that information would be unavailable for the time that the notebook was surrendered. Therefore, a notebook as the primary record of laboratory work is impractical in most forensic laboratory settings.

One of the most important pieces of laboratory documentation is a **standard operating procedure (SOP)**. As the name implies, an SOP is a detailed description of how a procedure is to be executed. An SOP may describe something as simple as how a balance is to be used or as complicated as instrument maintenance or sample analysis. The key descriptor is "standard," which enforces uniformity of the procedure regardless of who performs it. SOPs should be meticulously designed and reviewed and updated frequently or as needs dictate. Older versions of the SOPs are archived so that it can always be known what SOP was used during what time periods. If a deviation occurs from an SOP, this should be documented in the appropriate place. As an example, suppose a drug analyst receives an exhibit that consists of a sticky tarlike material, and the SOP for weighing exhibits states that all visible traces must be transferred to a weighing dish for determination of weight. The consistency of the sample makes this impossible; the analyst would record in the case notes what was done instead and why.

How SOPs are structured and what they include varies widely across laboratories, agencies, and industries. As an example, the U.S. Environmental Protection Agency (EPA) suggests a format that includes the following sections:[4]

- Title page
- Table of Contents
- Procedures
- Quality Assurance and Quality Control
- References

Within the "Procedures" section, some or all of the following topics could be addressed, depending on the process being described:

- Scope and applicability
- Summary of the method
- Definitions and abbreviations
- Health and safety warnings
- Cautions
- Interferences
- Personnel qualifications/responsibilities
- Equipment and supplies
- Procedure (to include as appropriate):
  - Instrument or method **calibration** and standardization
  - Sample collection
  - Sample handling and preservation
  - Sample preparation and analysis
  - Troubleshooting
  - Data acquisition, calculations, and data reduction requirements
- Computer hardware and software
- Data and records management

Each laboratory or laboratory system will have its own guidelines for an SOP design and content, but the type of information included will be the same. An example SOP (minus title page and table of contents) is shown in Figure 4. Examples of more complex SOPs can be found online at http://www.epa.gov/quality/qs-docs/g6-final.pdf (last accessed October 2011).

Standard Operating Procedure:
Weekly Balance Check
Revision 2011.A.3

A. Purpose and Applicability

The purpose of the SOP is to describe the procedure to be used in weekly balance checks to insure that the balances are performing to specifications. Any deviation from this procedure must be duly noted in the balance log and reported to the laboratory's QAO.

This SOP applies to all analytical balances in the laboratory within an operational range of 0.01 g – 100 g and readability of 0.001g.

B. Summary of Method Page

The recorded weights of several certified weights are to be obtained weekly for each balance in the laboratory. Weights are removed from dry storage and the reference weight for each obtained on the balance being evaluated. This information is recorded in a logbook for incorporation into control charts and performance checks of each balance.

C. Definitions

QAO: Laboratory quality assurance officer

CWS: Certified weight set and forceps

PPE: Personal protective equipment

D. Health and Safety Warnings

None. Normal laboratory PPE is to be used.

E. Cautions

None.

F. Interferences

Moisture and dirt are the two primary concerns. The balance and balance pan must be clean and dry. The standard weights must be clean, dry, and handled with clean, dry forceps.

G. Personnel Qualifications

The QAO will assign primary and secondary personnel responsible for each balance. Only personnel who have completed the QA/QC training module and section-specific method training may execute this procedure.

H. Equipment and Supplies

- Analytical balance (linear range within 0.01 g and 100 g)
- Precision certified weight set (CWS) (100 g, 50 g, 20 g, 10 g, 5 g, 2 g, 1 g, 500 mg, 200 mg, 100 mg, 50 mg, 20 mg, 10 mg, 5 mg, 2 mg, and 1 mg).
- Forceps (supplied with weight set)

I. Procedural Steps

1. The weight sets are stored in a large dessicator in Evidence Storage Locker C3. Sign the weight set out immediately before the weighing operation. These weights should not be exposed to normal atmosphere and humidity for more than 10 minutes.
2. Visually inspect the weight set to insure cleanliness and dryness. Also inspect each weight as it is removed from the storage box and placed on the balance pan. Any issues with cleanliness are to be immediately reported to the QAO. Do not use any questionable weights for this weekly check.

FIGURE 4 Example SOP.

3. Turn on and zero the balance as per that balance's specific SOP for simple weight determinations. It is important to check the weights using the same procedure as is used for any other weighing operation.
4. Working from smallest weight to the largest, place the weight on the center of balance pan and allow the reading to settle. Record the value in the logbook along with the certified weight.
5. Immediately return the weight set to the controlled environment storage and sign it in. Note any issues or concerns with the condition of the weights and report any problems or concerns immediately to the QAO.

J. Data and Records Management

The analyst shall record his or her name, date, and time that the measurements were taken in the balance logbook, along with the readings obtained for each weight recorded to the 0.001 g place. If any weight reading exceeds the tolerance specified in the balance specifications sheet, the weighing operation is to be repeated and this information recorded. A second value outside specifications must be reported immediately to the QAO and the balance removed from service until corrective actions are taken.

K. Quality Assurance and Quality Control

The QAO will review all balance check data weekly and incorporate into the appropriate laboratory control chart data.

L. References

All these references are available in the laboratory library.

Gonzalez, A. G., and M. A. Herrador. 2007. The assessment of electronic balances for accuracy of mass measurements in the analytical laboratory. *Accredit. Qual. Assur.* 12 (1):21–9.

Jones, F. E., and R. M. Schoonover. 2002. *Handbook of Mass Measurement*. Boca Raton, Fl: CRC Press 0-8493-2531-5.

Lawn, R., and E. Pritchard. 2003. *Measurement of Mass Practical Laboratory Skills Training Guides Series*: Royal Society of Chemistry 0-8540-4463-9.

Salahinejad, M., and F. Aflaki. 2007. Uncertainty Measurement of Weighing Results from an Electronic Analytical Balance. *Meas. Sci. Rev.* (6), http://www.measurement.sk/2007/S3/Salahinejad.pdf.

Wunderli, S., G. Fortunato, A. Reichmuth, and P. Richard. 2003. Uncertainty evaluation of mass values determined by electronic balances in analytical chemistry: A new method to correct for air buoyancy. *Anal. Bioanal. Chem.* 376 (3):384–91.

1.3.2 TRACEABILITY AND STANDARDS Whenever a laboratory has to develop a procedure or an SOP, rarely is it done from scratch. Within the analytical and forensic communities, specific guidance for methods and procedures are provided by groups such as ASTM International and professional organizations, including the AAFS, SOFT, and ASCLD. ASTM develops procedures called standards that are applicable to specific industries and processes.* The organization was formed by engineers and chemists in 1898 and has grown to cover everything from the manufacture of amusement rides to that of wood products. Standards are developed by committees of professionals in their field. Committee E30, currently with about 300 members, drafts and approves forensic science standards. Selected examples of relevant ASTM standards relating to forensic science are given in Table 1.

Standardization and the adoption of standard methods and practices assure the comparability of analytical measurements. If one blood sample is submitted to 10 different laboratories for a determination of blood alcohol concentration, all of them should obtain the same result, within the estimated uncertainties. The odds that this will happen increase dramatically if procedures are standardized. The concept of standardization is

*Analytical chemists usually associate the term *standard* or standards with a solution or some other mixture, but in ASTM parlance, a standard is a practice, method, or procedure, not a chemical standard.

TABLE 1 Partial List of ASTM Standards for Forensic Science

Standard (year)	Title
E620-97	Standard Practice for Reporting Opinions of Technical Experts
E1388-00	Standard Practice for Sampling of Headspace Vapors from Fire Debris Samples
E1413-00	Standard Practice for Separation and Concentration of Ignitable Liquid Residues from Fire Debris Samples by Dynamic Headspace Concentration
E1422-01	Standard Guide for Test Methods for Forensic Writing Ink Comparison
E1588-5(2001)	Standard Guide for Gunshot Residue Analysis by Scanning Electron Microscopy/Energy-Dispersive Spectroscopy
E1610-02	Standard Guide for Forensic Paint Analysis and Comparison
E1968-98	Standard Guide for Microcrystal Testing in the Forensic Analysis of Cocaine
E2123-01	Standard Practice for the Transmittal of Evidence in Sexual Assault Investigation
E2154-01	Standard Practice for Separation and Concentration of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Solid Phase Microextraction (SPME)
E2224-02	Standard Guide for Forensic Analysis of Fibers by Infrared Spectroscopy
E2227-02	Standard Guide for Forensic Examination of Nonreactive Dyes in Textile Fibers by Thin-Layer Chromatography
E2228-02	Standard Guide for Microscopic Examination of Textile Fibers

vital to ensure what informally is called "data comparability." For example, instruments such as Fourier transform infrared (FTIR) spectrophotometers and gas chromatography/mass spectrometry (GC/MS) systems are supplied with spectral libraries. Usually, the mass spectral library is provided by NIST, an organization will we discuss in more detail shortly. This library would be useless but for standardized instrument conditions. In mass spectrometry, instruments are typically adjusted (or "tuned") with the use of a standard compound such as perfluorotributylamine (PFTBA). This compound is introduced into the mass spectrometer, and instrument settings are adjusted until the mass spectrum is comparable to one produced by standard specifications within specified tolerances. Analogous methods are used for other instruments.

A second advantage of standard methods is that they are optimized and validated. As the name implies, a validated method has been thoroughly tested and characterized. The performance limitations of the method are understood, and quantifiable criteria for its satisfactory performance exist. As with mass spectrometers, tests can determine whether the method is working to specifications. If it is, then the data are comparable and reliable. Finally, because data generated by standard methods are comparable, it is possible to measure performance at the analyst and laboratory levels. This standardization feeds back into the processes related to certification and accreditation, as previously discussed. Fundamental to both of these are reliable standard reference materials recognized as such by the forensic community. One important source of these standards is NIST.

Unlike ANSI and ISO, NIST is an agency of the U.S. government. Founded in 1901, it is part of the Department of Commerce, but it does not have any regulatory functions. Rather, the role of NIST is to promote the standardization of weights and measures to serve the needs of industry, commerce, science, and technology. Forensically, one of the most important functions of NIST is the creation, maintenance, and supply of standard reference materials (SRMs). There are several types of SRMs, including certified SRMs (CRMs). According to NIST,[5] a certified reference value for an analytical parameter meets the following criteria:

NIST Certified Value—Value and its uncertainty assigned by NIST in conformance with the NIST uncertainty policy. A NIST certified value is obtained by one or more of the following measurement modes:

1. A definitive (or primary) method using specialized instrumentation capable of high accuracy and precision and whose errors have been thoroughly investigated and corrected; or,
2. Two or more independent methods at NIST using commercial instrumentation that is calibration based and with differing sources of systematic errors; or,
3. Interlaboratory data from selected laboratories using multiple methods and NIST SRMs as controls.

A few NIST CRMs are used directly as QA samples in forensic science. For example, SRM 1511 (Figure 5) is freeze-dried urine containing drugs and metabolites. Although this particular SRM is no longer available, the method of preparation and documenta-

FIGURE 5 Data sheet for traceable NIST standard reference material for forensic toxicology.

Source: US Department of Commerce, National Institute of Standards and Technology (NIST). This and other data sheets are available from the NIST website,www.nist.gov.

National Institute of Standards and Technology
Certificate of Analysis

Standard Reference Material 1511
Multi-Drugs of Abuse in Freeze Dried Urine

The Standard Reference Material (SRM) is intended primarily for verifying the accuracy used for the determination of morphine, codeine, cocaine metabolite (benzoylecgonine), marijuana metabolite (THC-9-COOH), and phencyclidine in human urine. SRM 1511 consists of three (3) bottles of freeze-dried urine with all of the analytes in each bottle. (See reconstitution procedure for reconstitution to 25 mL). There is no blank urine with this SRM.

Certified concentration: The certified values and uncertainties for the analytes, as free bases, are calculated and given in the table below. For benzoylecgonine, morphine, codeine, and phencyclidine, GC/MS and LC/MS data were used and the uncertainty is a 95% confidence interval for the mean. For the THC-9-COOH, the mean concentration was computed from GC/MS and GC/MS/MS measurements taken at NIST and the uncertainty is also a 95% confidence interval for the mean. However, this confidence interval also includes variability observed between NIST and five military labs which had been used to demonstrate the suitability of the material. It is assumed that systematic errors are very small compared to random errors.

Analyte	Concentration mmol/L	ng/mL
Morphine	$1.08 \times 10^{-3} \pm 0.07 \times 10^{-3}$	309 ± 20
Codeine	$9.62 \times 10^{-4} \pm 0.37 \times 10^{-4}$	288 ± 11
Benzoylecgonine	$5.60 \times 10^{-4} \pm 0.28 \times 10^{-4}$	162 ± 8
THC-9-COOH	$4.09 \times 10^{-5} \pm 0.23 \times 10^{-5}$	14.1 ± 0.8
Phencyclidine	$8.51 \times 10^{-5} \pm 0.82 \times 10^{-5}$	20.7 ± 2.0

The certified concentrations apply *only* to urine reconstituted as specified under "Reconstruction Procedure" and are based upon the concordant results from two different analytical methods for each analyte. Brief descriptions of the methods are given under "Analytical Methods." *Note:* This material also contains amphetamine and methamphetamine, but these analytes were not certified as analytical results that indicated probable degradation of these constituents with time.

The overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by M.J. Welch of the Organic Analytical Research Division.

Analytical measurements were performed by R.G. Christensen, P. Elerbe, C.S. Phinney, L.C. Sander, and S.S.-C. Tai of the Organic Analytical Research Division.

Statistical analysis was provided by K.J. Coakley of the Statistical Engineering Division.

tion is representative of the forensic reference materials supplied by NIST. Other NIST materials are used for instrument verification and validation.

Consider infrared spectroscopy, in which absorbance is plotted as a function of a wavenumber. Most users take for granted that if a peak appears at 1700 cm^{-1} on a screen or a printout, an actual absorbance of electromagnetic radiation with a frequency of 1700 cm^{-1} occurred. That assumption, however, rests on another: that the instrument has a reliable wavelength and frequency calibration. What if there were problems with the instrument, and a peak at 1700 cm^{-1} really represented the sample absorbing light at 1600 cm^{-1}? The only way to detect such a problem would be to obtain a spectrum with known and reliable absorbances of a material in which the frequencies of absorbance are certified. NIST provides such CRMs. Laboratories that use those CRMs (or other accepted calibration procedures) and obtain results comparable to NIST values can be confident that their spectrophotometers are calibrated properly and will produce reliable and acceptable spectra. Spectrophotometric data obtained on such instruments are comparable to each other and can be linked back to NIST. This linkage, called **traceability**, is a central premise of quality assurance.

In a nutshell, "traceability" means that analytical results, such as the concentration of a drug metabolite in urine, can be linked, related, or traced to an unassailable original source, such as an NIST SRM. Traceability is also interpreted more broadly to include the calibration of instrumentation and equipment. For example, analytical balances can be calibrated with certified weights that are traceable to NIST standard weights. According to the VIM, traceability in this context (also called metrological traceability) is "a property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations each contributing to the measurement uncertainty." In other words, traceability can be thought of as a chain of custody for quality: data that are traceable can be linked to reliable sources through what may be a long, but unbroken, documented chain of relationships and dependencies. Ideally, the calibration and performance of every piece of laboratory equipment and every instrument should be traceable to an unassailable standard, be it defined by a professional accreditation body or by NIST. The example given in the previous section referring to the wavenumber calibration of an IR spectrometer is typical of a traceability operation. According to laboratory policy, an IR spectrometer used in routine drug analysis would be periodically evaluated using an NIST SRM with certified wavenumbers and absorbances. If the calibration fell within the uncertainty specified on the *Certificate of Analysis*, that fact would be documented and the instrument kept in service. Failure of the check would result in recalibration or repair, followed by another validation analysis, all documented. If the instrument passes a validation test, does it mean that every spectrum obtained on such an instrument is fully trustworthy? Not at all, because validation of the instrument is just one step in a long and complex analytical process. Traceability and quality management of the entire analysis insures the ultimate reliability and utility of the spectrum and the data derived from it.

Traceability is also a foundation of method validation. When a method is validated, it means that, properly applied, the method will produce reliable and trustworthy data with a known performance and known limitations. This does not mean that each sample will be the same, but the analytical *protocols* will be, thereby insuring optimal performance and comparability of data. Novel samples may require novel approaches, but a standardized and validated method will, at the very least, provide a starting point. Method validation is one of the key components of total quality management in a laboratory.

1.3.3 METHOD VALIDATION AND FIGURES OF MERIT A validated method is intended to fit for purpose and produce the best possible data for a given analyte or, more likely, group of analytes such that the data are acceptably accurate, precise, quantifiable, timely, and reliable.[6]

Specifically, method validation is a process in which its fitness for purpose is verified, and the operational parameters and limitations are developed, defined, and documented. The operational parameters of a method, sometimes referred to as ***figures of merit***, vary based on the goals of the method; the figures of merit for a presumptive color change test would differ from the figures of merit associated with a method designed to quantitate the concentration of ethanol in blood. Table 2 provides definitions for several figures of merit frequently employed in forensic chemistry.

To validate a method, the first questions are directed toward fitness for purpose. To determine blood alcohol content, a heated headspace method is ideal. The method will produce the data needed to answer the forensic question and can do so in an efficient and affordable manner. That many laboratories already use these procedures

TABLE 2 Figures of Merit

Term	Definition
Accuracy	Closeness of agreement between a measured quantity and a true quantity of a measurand. Accuracy is defined by a combination of trueness and precision, meaning that accuracy has systematic and random aspects.
Bias	The difference between an experimentally determined value and an accepted reference value; the expression of trueness. This is the systematic component of accuracy.
Limit of detection (LOD)	The lowest concentration of an analyte that can be unambiguously detected, but not necessarily quantitated.
Limit of quantitation (LOQ)	The lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy for a given method.
Precision	Closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. This quantity is usually expressed quantitatively as the standard deviation or %RSD. Precision is the random component of accuracy.
Repeatability	"Closeness of the agreement between the results of successive measurements of the same measurand carried out subject to all of the following conditions: • The same measurement procedure • The same observer • The same measuring instrument used under the same conditions • The same location • Repetition over a short period of time" (quoted from SWGDRUG)
Reproducibility	"Closeness of the agreement between the results of measurements of the same measurand, where the measurements are carried out under changed conditions such as: • Principle or method of measurement • Observer • Measuring instrument • Location • Conditions of use • Time" (quoted from SWGDRUG)
Robustness	A measure of a method's capacity to remain unaffected by small but deliberate variations in method parameters; provides an indication of its reliability during normal use. Variations might include changes in the source for purchasing solvents, changes in environmental conditions such as temperature, or new pieces of equipment such as syringes.

TABLE 2 Figures of Merit (*Continued*)

Term	Definition
Ruggedness	The degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, analysts, instruments, lots of reagents, elapsed assay times, assay temperatures, or days. Ruggedness is normally expressed as the lack of influence on test results of operational and environmental variables of the analytical method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and analyst to analyst, in contrast with robustness, which is typically inside a single laboratory.
Sensitivity	Quotient of the change in an indication of a measuring system and the corresponding change in a value of a quantity being measured. The term is often incorrectly used to express detection or quantitation limits. In quantitative analytical chemistry, a change in the slope of a given calibration curve is associated with a change in sensitivity.
Trueness	Closeness of agreement between the expectation of a test result or a measurement result and a true value. It is usually expressed in terms of a bias. Trueness is the systematic component of accuracy.
Uncertainty	Parameter associated with a measurement result that characterizes the dispersion (spread) of the values that could reasonably be attributed to the particular quantity being measured. Note that uncertainty is not an expression of error, only of a range.

Sources: ISO. 2006. ISO 3534-1 Statistics—Vocabulary and Symbols. Part 1: General Statistical Terms and Terms Used in Probability. In *Statistics—Vocabulary and Symbols.* Switzerland: ISO, 2006.

ISO. ISO 3534-1 Statistics—Vocabulary and Symbols. Part 2: Applied Statistics. In *Statistics—Vocabulary and Symbols.* Switzerland: ISO, 2006.

ISO. ISO Guide 99:2007, The International Vocabulary of Basic and General Terms in Metrology. Switzerland: ISO, 2007.

SWGDRUG. *Recommendations*, 5th ed. U.S. Department of Justice, Drug Enforcement Administration, 2010. Available online at www.swgdrug.org.

adds to the fitness-for-purpose considerations. Once the general methodology is identified, the next step is to develop a method validation plan.[7–10] The goal of the plan is to characterize the method in terms of the appropriate figures of merit. Once the plan is reviewed and approved, laboratory work begins. Method validation may sound simple, but a complete method validation from inception to completion may take months of work by several individuals, depending on the goals of the method and its complexity. Examples of method validation plans specific to forensic chemistry can be found online.[11] Note that in some of these documents the wording of definitions of figures of merit varies, but the core concepts are similar.

We will now expand and improve on relevant definitions in the context of forensic chemical methods. Most should make intuitive sense, but there are some points worth noting. One of the interesting definitions is the first one—**accuracy**. Analytical chemistry books often define this as the difference between a measured value and a true value, which is correct but incomplete for our purposes. Suppose a laboratory has a supply of the NIST SRM 1511 and uses this SRM as an accepted true value. An experienced analyst using a validated laboratory method obtains a value of $9.13 \times 10^{-4} \pm 0.10 \times 10^{-4}$ mmol/L

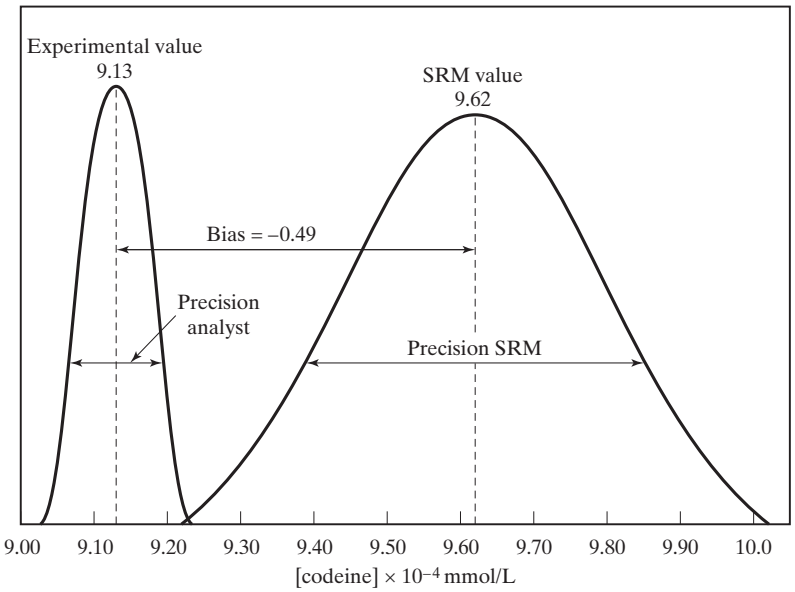


FIGURE 6 Comparing an experimental value with an accepted true value.

for codeine. We can calculate the difference between the experimental value and the true value as -0.49×10^{-4} mmol/L, but is this the accuracy? No, it is the trueness (bias) of the measurement and only one part contributing to accuracy. Accuracy must take into account not only this bias but also the spread around each mean value. This is what is meant by the systematic and random components of accuracy; the bias (-0.49×10^{-4} mmol/L here) is loosely analogous to a systematic error, and the spread around each data point is analogous to the random error component. This concept is illustrated in Figure 6. Here, the spread of each measurement is such that there is a zone of overlap, albeit a very small one, which suggests that there is a definable probability that the means are the same.

The definition of **precision** fits within our earlier discussion, but we have added two new terms to the concept: **repeatability** and **reproducibility**. Assume that the analyst in the previous example performed not 3 but 10 replicate analyses during a single afternoon of work. The standard deviation (or %RSD) of the mean of the 10 concentration values determined would constitute the precision and also would be representative of *repeatability*. Now, suppose that during that same afternoon a different analyst performed 10 independent replicates using the same instrument and equipment. The spread of the combined data could now be used to represent *reproducibility*, since the observer (i.e., the analyst) changed. These differences may seem to be splitting hairs, but when considered across a laboratory with many analysts, or a laboratory system with many analysts and many laboratories, these differences are important and describe different aspects of method performance.

2 QUALITY CONTROL PROCEDURES

By now it should be clear that no sample stands alone and that every analysis is accompanied, directly and indirectly, by a multitude of quality assurance steps. These steps are themselves tested by means of quality control samples, procedures, and checks. Analytical quality control samples almost always accompany a sample run, and the

collection (sample to be tested and quality control samples) is usually referred to as a **batch**. A batch cannot be broken apart without losing relevance; it presents a snapshot of method, analysts, and sample, but only at a given moment in time. A picture taken a few hours or a few days later will never be the same. Similarly, analytical conditions vary with time. A batch run on Monday will not be run under the same conditions as a batch run on Thursday. When validated methods and adequate quality assurance and quality control procedures are used, differences should be minimal, but not necessarily negligible.

What constitutes a batch depends on the type of samples being analyzed and whether quantitative, qualitative, or both types of data are required. At the very least, a batch should contain positive and negative controls. Even simple color tests should be performed alongside a sample that produces a positive result **(positive control)** and one that produces no result **(negative control** or **blank)**. In a more complex example, suppose a stain suspected of being blood is found on a dark-blue bedsheet. Then another essential control is a background or matrix control taken from an unstained portion of the sheet. Recall the overriding goals of quality assurance: reliability *and* utility. If something in the bedsheet itself produces a positive result, that presumptive test is useless, indicative of absolutely nothing.

The number of control samples required per batch increases proportionately if quantitative information is sought. Consider a GC/MS analysis of a drug sample extract that, based on initial presumptive tests and screening, is thought to contain heroin. Such a batch would likely include the following types of quality assurance samples:

EXHIBIT A

Trade-offs

Forensic chemists always balance the need for extensive quality assurance and quality control with more mundane limitations of time and money. For example, many students wonder why replicates are not done on known samples. After all, that would generate a measure of reproducibility that could be compared with that obtained from the unknown. Similarly, why are no replicates done on blanks? While such steps would provide useful information, they would require more analysis time and reagents and would generate more waste. Thus, a tradeoff is made: The quality assurance and quality control are acceptable and provide the necessary information without being excessive. This approach is further justified by performing a thorough method validation. If you have a properly validated method and known figures of merit, then it should be relatively easy to determine whether the method and analyst are meeting these criteria.

- *Blank samples:* These are used to make sure that glassware, equipment, and instrumentation are free of the analyte of interest and potential interferences. It does no good to analyze drinking water for trace amounts of acetone when laboratory glassware and procedures contribute 10 ppb per sample. Blanks provide information about the accuracy of the technique used and can help detect systematic errors such as a contaminated reagent or contaminated glassware.
- *Open controls or knowns:* These can be viewed as practice samples for the analyst that have known reliable values. In the drug screen example we are considering here, an open control could be a heroin standard prepared by the analyst from a reliable, but independent, source of heroin. The known provides a measure of expected bias and is usually gauged for accuracy by the percent error. Knowns are valuable for detecting and diagnosing systematic errors.
- ***Blinds or blind controls:*** These are samples in which the true value may be known to someone within the laboratory, such as the QA/QC officer, or to someone outside or above the laboratory hierarchy, but not to the analyst. If no one in the laboratory knows the true value, the sample may be referred to as a "double blind."

When forensic chemists go through proficiency testing, blind samples are used. In some cases, the blind sample may be disguised to be a case sample. This is done so that no extraordinary care is taken with the sample, since handling the sample is, in a sense, a test. Double blinds such as this are often the only way to accurately gauge how routine samples are treated.

- ***Calibration checks:*** Using **calibration curves**, analyses are performed on calibrated instruments. Any calibration has a limited lifetime and thus should be checked regularly, either as part of each batch or on a time schedule, such as daily. A calibration check solution should be prepared from material that is completely independent of that used to prepare the calibration standards. We will examine the calibration process in detail in the next section.
- *Replicates:* A replicate is a subsample, also occasionally referred to as an *aliquot*. Performing at least three replicates allows for simple statistics to be calculated (mean, *s*, %RSD). Often, time constraints within the laboratory dictate which samples are done in replicate. Replicates are frequently, but not always, analyzed at different times.[12]
- ***Duplicates:*** Duplicates differ from replicates in that a duplicate is a separate sample from a separate source. Replicates are aliquots taken from the same initial homogeneous sample. Duplicates are often taken in the field. For example, when urine samples are taken at sporting events, two separate containers are often filled and sealed for delivery to the laboratory. Since the containers arrive as two samples, they would be labeled as duplicates. A replicate would be a subsample created in the laboratory from one homogeneous sample. In practice, the three terms "duplicate," "replicate," and "aliquot" are sometimes used interchangeably, but technically there is a difference, as shown in Figure 7.
- *Spikes:* **Spikes** are compounds purposely added at known levels and used to gauge the recovery of an analyte from a specific matrix and sample. As a matrix, blood and urine are similar from person to person, so potential matrix effects,

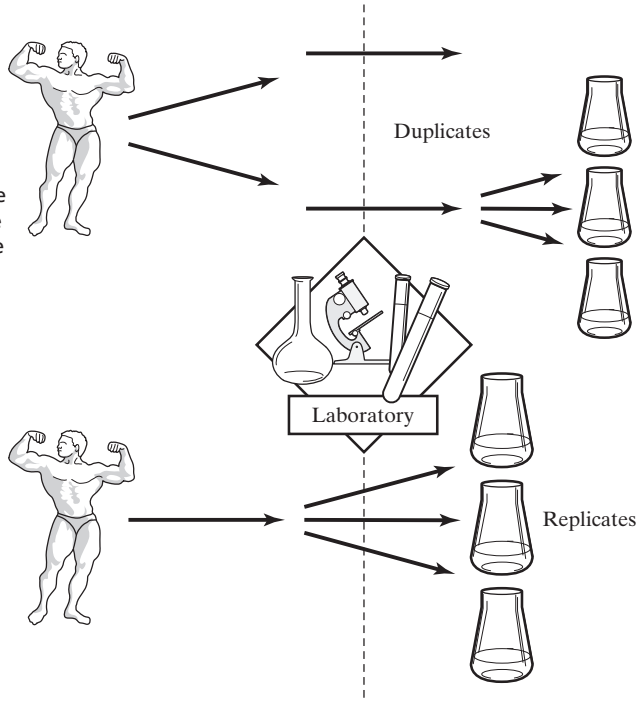


FIGURE 7 "Duplicate" is the term used to describe samples collected outside of the laboratory but from the same source or population. Suppose an athlete is required to take urine tests to check for the presence of banned substances. Replicates are taken either inside or outside the laboratory and are subsets of one sample, whereas duplicates are two separate samples. A duplicate can also be further divided into replicates in the lab.

although significant, are nearly constant when the same matrix is used. However, when matrices vary, there is often a need for a quantitative assessment of their effect. Spikes are employed in this role.

As an example, suppose a can containing fire debris is collected. Because the material is burned, the sample will contain significant levels of charcoal. Charcoal adsorbs most organics, including accelerants such as gasoline, a complex mixture of hydrocarbons. Furthermore, each fire and each location within a fire will have different sample characteristics, depending on what was burned, how long it burned, how much accelerant was present, and more. Unlike the situation with blood and urine, the matrix effect for fire debris is neither consistent nor predictable. One way to measure how the matrix will influence the recovery of accelerant residues is to spike every sample with a known amount of a compound that is chemically similar to the components of gasoline. If the recovery of the spike compound is 90%, it implies that recovery of the accelerants will be high as well. A challenge with spikes is finding compounds chemically similar to the target analytes yet that are unlikely to be found in the sample. When mass spectrometry is used for the analysis, deuterated compounds can be used. Deuterated benzene (benzene-D6) is chemically identical to benzene but can be distinguished from it on the basis of mass. Consequently, deuterated compounds are frequently used as matrix spikes.

3 QUALITY CONTROL AND CALIBRATION

The process of calibration consists of linking an instrumental response or readout to performance. To calibrate instruments and devices, a trustworthy, traceable standard, procedure, or comparison is mandatory. Forensic laboratories calibrate equipment such as balances and pipets, as well as instruments, via calibration curves. All calibrations are transitory and must be repeated at intervals defined by a laboratory SOP or known performance limitations. For example, the performance of an analytical balance might be checked weekly via NIST-traceable weights (as in Figure 4), while a calibration curve from a gas chromatograph might require recalibration or a calibration check every 12 hours. Calibration and calibration checks ensure that the device's output is reliable. Typically, the more complex the device, the more often and more detailed the calibration checking must be.

3.1 Calibration of Equipment and Use of Control Charts

The calibration of analytical balances is one of many calibrations that must be done routinely in a forensic laboratory. Some laboratories contract out calibration services, but all are required to keep such records as part of total quality management. The accuracy and precision of such devices must be known and monitored. Devices requiring calibration include refrigerators, balances, pipets, syringes, pH meters, microscopes, and so on. In short, if the equipment provides a measurement that is related to generating data, it must be calibrated. In the case of a balance, the process is as simple as obtaining traceable weights, recording the displayed weight and certified weight, and seeing if the difference falls within the expected range, based on uncertainty and significant figures. With new equipment, manufacturers provide specifications that document the accuracy and precision of that equipment if it is used properly. If a balance fails the check, it must be taken out of service until it is repaired, which may be simple (cleaning and leveling) or may require a return to the factory.

Other devices, such as pipets or refrigerators, may be monitored with control charts. **Control charts** identify failures and also predict and, ideally, diagnose such failures. There are a number of ways charts are implemented. The first step is

Initial performance	
Stable water temperature	26.0°C
Date	2/2/2011
Analyst	S.H.
Pipette serial number	1098CX-3
Received	2/1/2011
Density H_2O	
CRC 2004	0.9967867

Trial	Weight (g)	Volume (calc, μL)
1	0.2003	200.9
2	0.1995	200.1
3	0.2004	201.1
4	0.1993	199.9
5	0.2004	201.0
6	0.2007	201.3
7	0.1998	200.4
8	0.2003	200.9
9	0.2010	201.6
10	0.1999	200.5

Volume (calc, μL)	
Mean	200.8
Standard error	0.168
Standard deviation	0.531
Sample variance	0.282
%RSD	0.265
Range	1.7
Minimum	199.9
Maximum	201.6
Count	10
Confidence level (95%)	0.38

UWL: 201.1
LWL: 200.5
UAL: 201.3
LAL: 200.3

FIGURE 8 Data used to determine the performance limits of the example pipet and to establish the warning and action limits of the control chart.

the establishment or adoption of an expected range of variation. Once these limits are established, the chart is used to monitor the device's performance over time. Suppose an autopipet arrives at the lab certified to deliver 200 μL $\pm$ 2.0 μL ($\pm$ 1%). The analyst immediately validates this performance by repeatedly pipetting what the device records as 200 μL aliquots into dried, tared containers on a calibrated analytical balance. By recording the water temperature and using a chart that relates density to temperature, the analyst converts the weight of water, in milligrams, to a volume delivered by the pipet. This example is illustrated in Figures 8 and 9.

A control chart usually has two sets of lines: warning limits and action limits. The term control limit is also seen for the $\pm$ 3s limit. The ranges are typically calculated as ± 2 or 3σ (if known) or 95% and 99.7% confidence intervals:[11]

$$\bar{x} \pm \frac{2\sigma}{\sqrt{n}}(95\%) \tag{1}$$

$$\bar{x} \pm \frac{3\sigma}{\sqrt{n}}(99.7\%) \tag{2}$$

The pipet is tested initially with 10 replicates and is found to meet the manufacturer's specifications and to have a %RSD of -0.3% On the basis of the variation observed, warning and action (control) limits are established and performance is monitored. Whenever a warning line is crossed, a second test is made. If performance falls below the action limit, the pipet is removed from service. A close look at the control chart (Figure 9) presaged the failure; if a similar pattern is seen with other pipets, preventive action would be undertaken. Possible outcomes are summarized in Figure 10.

3.2 Calibration of Instruments: Concentration and Response

A good **regression line** is required for a valid and trustworthy calibration but still is only one of many requirements. A calibration curve has a lifetime that is linked to the stability of the instrument and the calibration standards. A curve run on Monday

Date Checked	Volume (μL)	UWL	LWL	UAL	LAL	Nominal
2/9/2011	200.7	201.1	200.5	201.3	200.3	200.8
2/17/2011	200.8	201.1	200.5	201.3	200.3	200.8
2/25/2011	200.9	201.1	200.5	201.3	200.3	200.8
3/5/2011	201.1	201.1	200.5	201.3	200.3	200.8
3/13/2011	200.7	201.1	200.5	201.3	200.3	200.8
3/21/2011	201.0	201.1	200.5	201.3	200.3	200.8
3/29/2011	200.8	201.1	200.5	201.3	200.3	200.8
4/6/2011	200.8	201.1	200.5	201.3	200.3	200.8
4/14/2011	200.8	201.1	200.5	201.3	200.3	200.8
4/22/2011	200.9	201.1	200.5	201.3	200.3	200.8
4/30/2011	200.5	201.1	200.5	201.3	200.3	200.8
Dup 4/30/2011	200.6	201.1	200.5	201.3	200.3	200.8
5/16/2011	200.6	201.1	200.5	201.3	200.3	200.8
5/24/2011	200.4	201.1	200.5	201.3	200.3	200.8
Dup 5/24/2011	200.6	201.1	200.5	201.3	200.3	200.8
6/9/2011	200.4	201.1	200.5	201.3	200.3	200.8
Dup 6/9/2011	200.2	201.1	200.5	201.3	200.3	200.8
Dup 6/9/2011	200.1	201.1	200.5	201.3	200.3	200.8

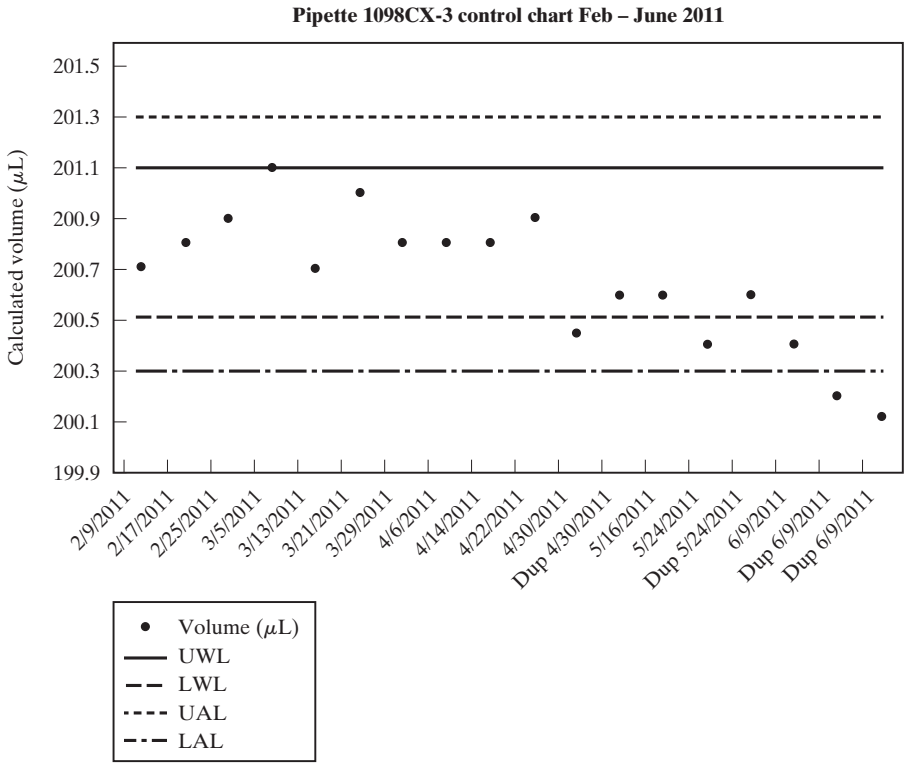


FIGURE 9 Weekly control chart for the pipet. The data from June 9 were at the warning limit, and two duplicates fell below the action limit. The action (retesting) indicated that the pipet was no longer "in control" and would have to be taken out of service for repair or replacement.

morning on an ultraviolet/visible-range (UV/VIS) spectrophotometer probably will not be reliable Tuesday. Therefore, procedures must account for the passage of time in considering the validity of a calibration curve. Modern instruments such as gas chromatographs and mass spectrometers can produce calibration curves that are stable over many days, but they must be tested periodically. Many instruments allow for

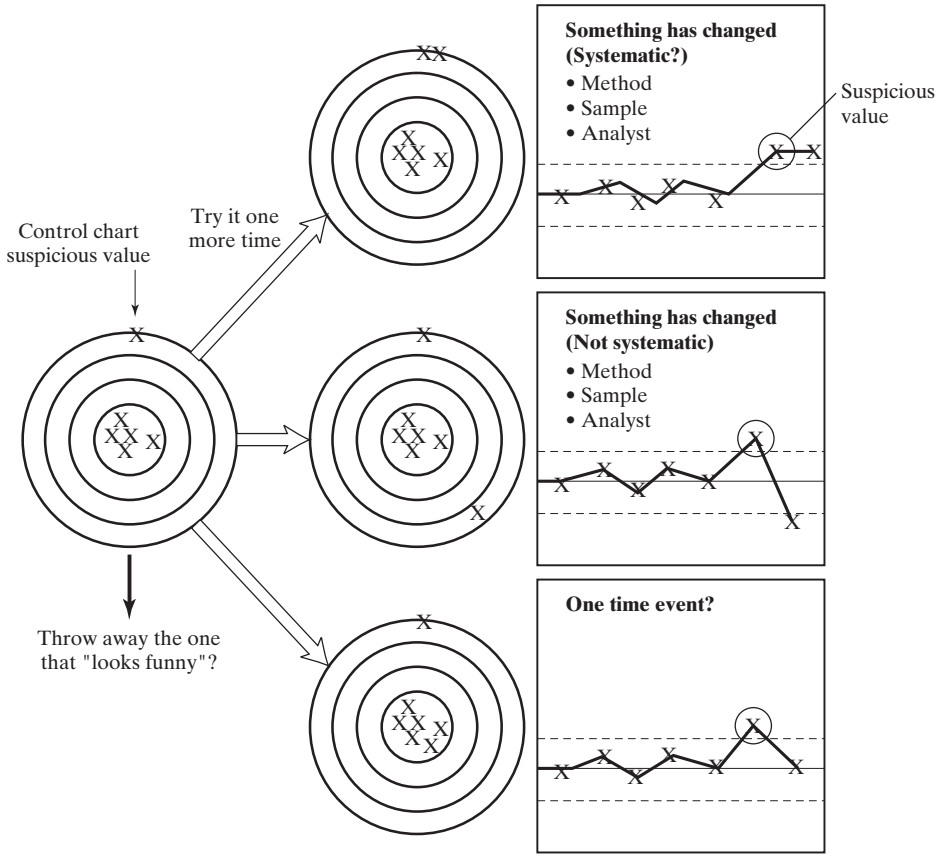


FIGURE 10 Relationship of a control chart to the target analogy. If a sample exceeds warning or control limits, it should not be discarded arbitrarily but rather should be used to diagnose and correct problems. Once again, chemistry before statistics.

automatic curve checks and updates, such as replacing the middle point of the curve with a fresh run every 12 hours.

Regression lines are used to calibrate instruments and take the familiar form $y = mx + b$, where m is the slope and b is the y-intercept, or simply intercept. The variable y is called the **dependent variable**, since its value is dictated by x, the **independent variable**. All linear calibration curves share certain generic features, as shown in Figure 11. The range in which the relationship between concentration and response is linear is called the *linear range* and is typically described by "orders of magnitude." A calibration curve that is linear from 1 ppb to 1 ppm, a factor of 1000, has a **linear dynamic range (LDR**, also a figure of merit**)** of three orders of magnitude. At higher concentrations, most detectors become saturated and the response flattens out; the calibration curve is not valid in this range of higher concentrations, and samples with concentrations above the last linear point on the curve must be diluted before quantitation. The concentration corresponding to the lowest concentration in the linear range is the **limit of quantitation (LOQ)**. Instruments may detect a response below this concentration, but it is not predictable and the line cannot be extrapolated to concentrations smaller than the LOQ. The concentration at which no significant response can be detected is the **limit of detection (LOD)**.

The line generated by a **least-squares regression** results from fitting empirical data to a line that has the minimum total squared deviation from all the points. The method is called *least squares* because distances from the line are squared to prevent points that

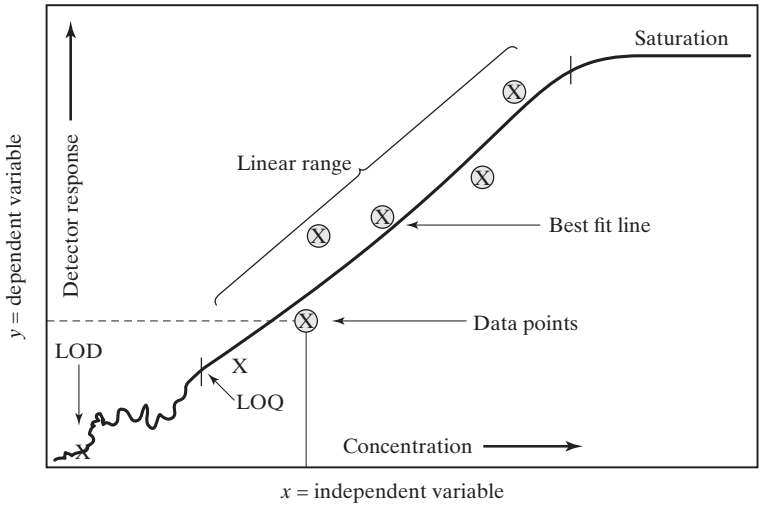


FIGURE 11 General features of linear calibration curves. Note that below the LOQ, the behavior of concentration vs. response is no longer linear and rarely predictable.

are displaced above the line (signified by a plus sign, +) from canceling those displaced below the line (signified by a minus sign, −) and to increase the weight of the outliers. Most **linear regression** implementations have an option to "force the line through the origin," which means forcing the intercept of the line through the point (0,0). This might seem reasonable, since a sample with no detectable cocaine should produce no response in a detector. However, forcing the curve through the origin is not always recommended, since most curves are run well above the instrumental limit of detection (LOD). Arbitrarily adding a point (0,0) can skew the curve because the instrument's response near the LOD is not predictable and is rarely linear, as shown in Figure 11. As illustrated in Figure 12, forcing a curve through the origin can, under some circumstances, bias results.

The goodness of fit of the line is measured by the **correlation coefficient** or more frequently as its squared value (R^2):

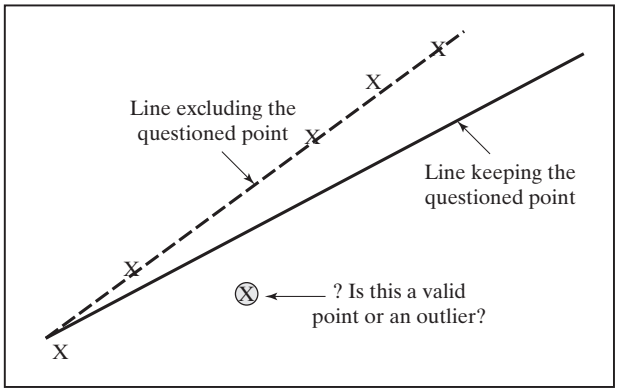
$$R^2 = \frac{\left[\sum(x_i - \bar{x})(y_i - \bar{y})\right]^2}{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2} \quad \textbf{(3)}$$

The value of R^2 will range between −1 and +1 and is a measure of linearity of the points. If $R^2 = 1.0$, the line is perfectly correlated and has a positive slope, whereas $R^2 = -1$ describes a perfectly correlated line with a negative slope (Figure 13). If there is no correlation, $R^2 = 0$. It is important to remember that R^2 is but one measure of the goodness of a calibration curve, and all curves should be inspected visually as a second level of control.

EXHIBIT B

Just Do It (Again)

There are a number of statistical tests and analyses that can be used to evaluate a calibration curve and to weigh the "goodness" of each calibration data point. As shown in the accompanying figure, one of the calibration points appears to be an outlier and including it would skew the calibration curve significantly. What is the best course of action? Clearly the point "looks funny," but, what should the next action be? Just because it looks funny, the point should not be discarded without further scrutiny.

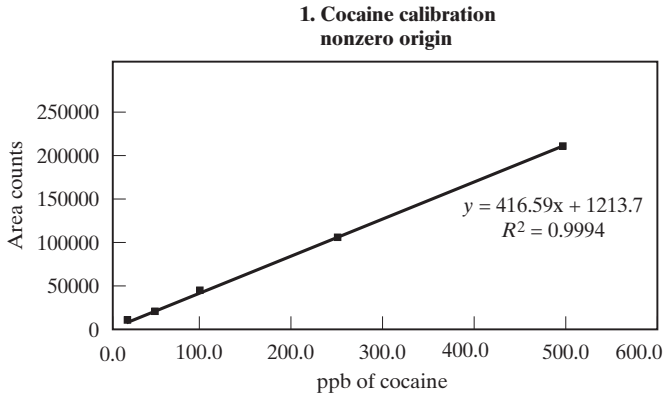


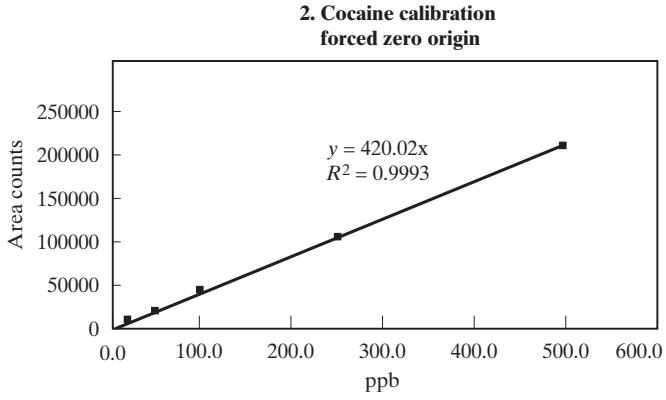
There is no justification for throwing the point away on a hunch. Of course it looks suspicious, but the question is, Why? Is it a symptom of an underlying problem, or is it just a fluke? There is only one way to resolve the issue, and it does not involve a calculation or a hypothesis test. The next step involves laboratory work. The suspicious solution would be rerun to see if the results were replicated. If so, then a fresh solution should be prepared and retested. Depending on the results, the entire calibration series may have to be prepared fresh and reanalyzed. Even then, though, the answer to this question has little to do with calculations, identifying outliers, or weighting the questionable point less than that of the other calibration points. Once again, it's a matter of *chemistry before statistics.*

FIGURE 12 Problems associated with forcing a calibration curve through the origin. The bottom line includes the point (0,0) while the top line uses only the empirical data. In general, calibration lines should not be arbitrarily forced through the origin, as shown by the two different values calculated for the sample.

ppb cocaine	Peak area
20.0	9599
50.0	21456
100.0	45326
250.0	102391
500.0	210561

Sample data:
Peak area = 72003
Concentration (1): 171.4
Concentration (2): 169.9





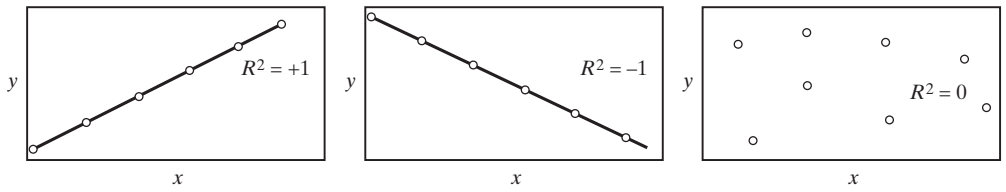


FIGURE 13 Relationship of correlation coefficient (R^2) to linear fit. Typical calibration curves are at least "two nines," or 0.99. The value of (R^2) is an important criterion, but not the only one, for describing the goodness of the calibration curve.

Another aspect of curve validation is the use of calibration checks. The ideal calibration check (CC) is obtained from an accepted reference standard that is independent of the solutions used to prepare the calibration standards, as shown in Figure 14. This is the only method that facilitates the detection of a problem in the stock solution. Finally, blanks must be analyzed regularly to ensure that equipment and instrumentation have not been contaminated. Thus, four factors contribute to the validation of a calibration curve: correlation coefficient (R^2), the absence of a response to a blank, the time elapsed since the initial calibration or update, and performance on an independent **calibration check sample**. These criteria should be met regardless of the type of curve being used, the most common of which are as follows:

- ***External standard:*** This type of curve is familiar to students as a simple concentration-versus-response plot fit to a linear equation. Standards are prepared in a generic solvent, such as methanol for organics or 1% acid for elemental analyses.

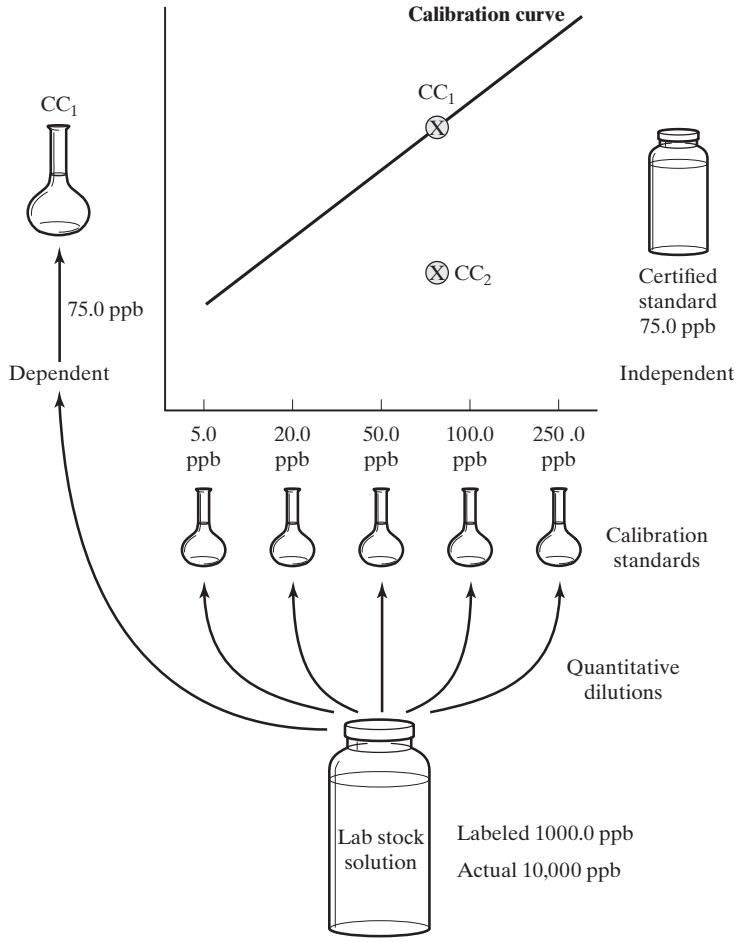


FIGURE 14 The value of an independent calibration check (CC) solution. Any dilutions, be they calibration standards or test solutions taken from tainted stock, will be compromised. Only an independently prepared solution will identify the problem.

ppb cocaine	Peak area
20.0	9599
50.0	21456
100.0	45326
250.0	102391
500.0	210561

Calibration check:	
True ppb =	125
Area counts:	65.8
Calculated:	28632
% error:	–47.3

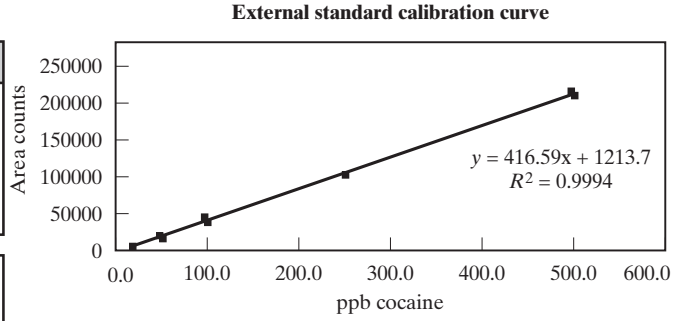


FIGURE 15 Demonstration of a matrix effect. The matrix reduces the response of the analyte cocaine by half, and as a result, the percent error is nearly 50%.

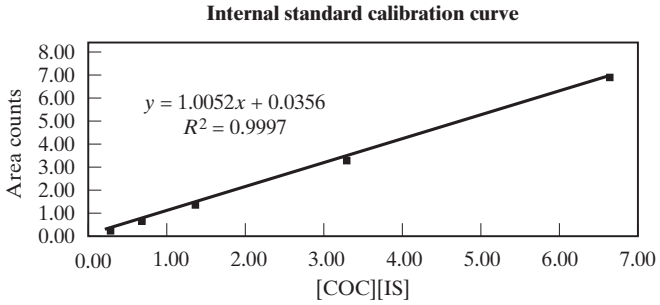
Such curves are easy to generate, use, and maintain. They are also amenable to automation. External standard curves work well when matrix effects are minimal. For example, if an analysis is to be performed on cocaine, some sample preparation and cleanup is done and the matrix removed or diluted away. In such cases, most interference from the sample matrix is inconsequential, and an external standard is appropriate. External standard curves are also used when internal standard calibration is not feasible, as in the case of atomic absorption spectrophotometry.

- ***Internal standard:*** External standard calibrations can be compromised by complex or variable matrices. In toxicology, blood is one of the more difficult matrices to work with, because it is a thick, viscous liquid containing large and small molecular components, proteins, fats, and many materials subject to degradation. A calibration curve generated in an organic solvent is dissimilar from that generated in the blood matrix, a phenomenon called **matrix mismatch**. Internal standards provide a reference to which concentrations and responses can be ratioed. The use of an internal standard requires that the instrument system respond to more than one analyte at a time. Furthermore, the internal standard must be carefully selected to mimic the chemical behavior of the analytes. This property is important, since internal standard calibration rests on the assumption that whatever happens to the analyte in a matrix also happens to the internal standard. However, as with spikes, the internal standard cannot be a compound that might occur in a sample. An example (Figure 16) illustrates how an internal standard protocol can correct for matrix effects or other problems.

Suppose an external standard curve is generated for an analysis of cocaine. The calibration standards are prepared from a certified stock solution of cocaine in methanol, which is diluted to make five calibration standards. The laboratory prepares a calibration check in a diluted blood solution using certified standards, and the concentration of the resulting mixture is 125.0 $\pm$ 1.2 ppb cocaine. When this solution is analyzed with the external standard curve, the calculated concentration is found to be about half of the known true value. The reason for the discrepancy is related to the matrix, in which unknown interactions mask nearly half of the cocaine present. The mechanism could be protein binding, degradation, or a myriad of other possibilities, but the end result is that half of the cocaine in the sample goes undetected. With external standard methodology, there is no mechanism of identifying the loss or correcting for it, since the matrix is not accounted for in calibration.

Now consider an internal standard approach (Figure 16). An internal standard (IS) of xylocaine is chosen because it is chemically similar to cocaine but unlikely to be found in typical samples. To prepare the calibration curve, cocaine standards are

ppb cocaine	Peak area	ppb xylocaine	Peak area	Conc. ratio	Area ratio
20.0	9599	75.0	31063	0.27	0.31
50.0	21456	75.0	30099	0.67	0.71
100.0	45326	75.0	32051	1.33	1.41
250.0	102391	75.0	31004	3.33	3.30
500.0	210561	75.0	31100	6.67	6.77



True =	125
Area counts cocaine =	28832
Area counts IS =	16723
Area ratio (x)	1.712
Calculated [] ratio	1.668
Calculated cocaine:	125.1
% error:	0.1

FIGURE 16 An internal standard corrects for the matrix effect as long as the internal standard is affected similarly to the way the analyte is affected.

prepared as before, but 75.0 ppb of xylocaine is added to each sample, including blanks, calibration solutions, and other quality assurance/quality control samples. Since the added standard is always at the same level, the response should be the same, within expected uncertainties. Xylocaine is chemically similar to cocaine, so if half of the cocaine is lost to the matrix, half of the xylocaine will be lost as well. Area counts still decrease as with the external standard curve, but here the *ratio* of the two will be unchanged, since both are reduced proportionally. Put another way, 8/4 is the same as 4/2 and 2/1—the individual numbers differ, but all three ratios equal 2. This is the principle of internal standard calibration: *Ratios* of concentrations and responses are used, rather than uncorrected concentrations and responses. The improvement in performance, versatility, and ruggedness can be significant. As with external standards, **internal standard curves** are easily automated and can be adapted to multi-analyte testing.

- *Standard addition:* Although not widely used in forensic chemistry, the method of **standard addition** provides the perfect matrix match. To execute a standard addition, the sample is typically divided into four or five portions. Nothing is added to the first sample. Minute volumes of concentrated analyte are added in increasing increments to the remaining samples and the results plotted as shown in Figure 17. The added standard volume must be small such that the dilution effect is neglible. The *x*-axis intercept corresponds to the negative equivalent of the concentration.

ppb added	Response
20.0	30101
50.0	42000
100.0	53998
200.0	78013

True:	125
Calculated:	125.4
% error:	0.3

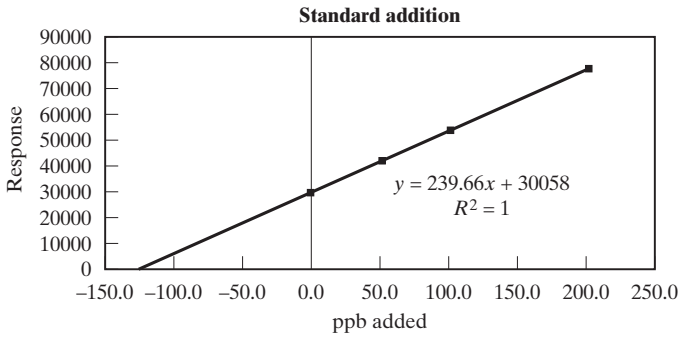
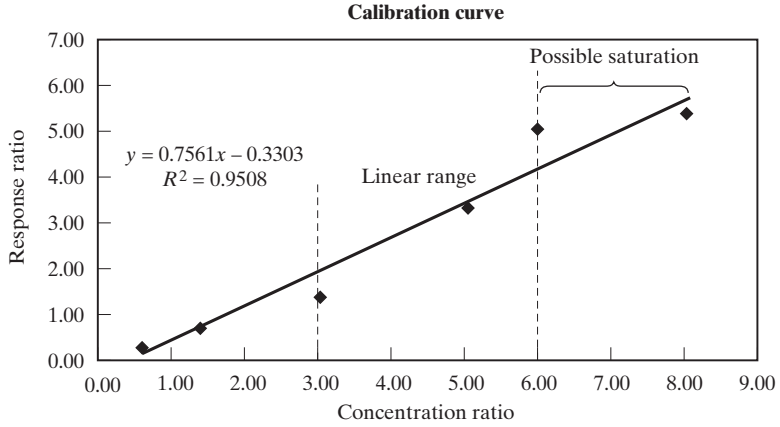


FIGURE 17 Standard addition calibration. The displacement along the *x*-axis to the left corresponds to the rise at $x = 0.0$; the equation is used to determine the associated concentration.

EXAMPLE PROBLEM 1

Given the following data, construct an internal standard calibration curve and evaluate the results:

ppb Codeine	Peak Area	ppb Internal Standard	Peak Area	Conc. Ratio	Area Ratio
15.0	9599	25.0	29,933	0.60	0.32
35.0	21,456	25.0	30,099	1.40	0.71
75.0	45,326	25.0	32,051	3.00	1.41
125.0	102,391	25.0	31,004	5.00	3.30
150.0	157,342	25.0	31,100	6.00	5.06
200.0	162,309	25.0	30,303	8.00	5.36



Answer:

This calibration has problems that become clear when the curve is plotted. The response at the upper concentrations is flattening out, indicating possible detector saturation. At lower concentrations, there is what appears to be a linear relationship, but it differs from that in the middle of the curve. The LDR is approximately 35–150 ppb codeine; the calibration should be redone in this range.

Recall that the slope of any line, including a calibration curve, is defined as "rise over run." If the curve fit is good ($R^2 = 0.99$ or better), the rise over run is consistent, and the rise (the offset from the origin) corresponds to the run along the *x*-axis. The rise along the *y*-axis in the first sample ("0 added") correlates with the run on the *x*-axis, or the concentration of the original sample. Two disadvantages of standard addition are the large amount of sample consumed and the difficulty in adapting the approach to routine analysis. It is also not amenable to typical quality control samples, such as blanks and calibration checks. However, for unusual cases with difficult matrices, standard addition is a ready tool.

Summary

QA/QC is one of the most important and least discussed aspects of modern forensic chemistry. The goal of QA/QC is simple: to insure the goodness of data— that is, their utility and reliability. Utility is defined by fitness for purpose, while reliability is controlled by many factors such as method validation, analyst certification, calibration, and laboratory accreditation.

All the discussion in this chapter has focused on the analysis, but what about the sample? Inevitably, it will play a role in determining the goodness of data. Therefore, the sample has to be collected in a reasonable and defensible manner. Also, when quantitative data are produced by a validated method, we still need to know how to estimate the uncertainty of that result.

Key Terms and Concepts

Accreditation
Accuracy
Batch
Bias
Blank
Blind samples
Calibration
Calibration check sample
Calibration curve
Certification
Chain of custody
Control chart
Correlation coefficient
Dependent variable
Duplicates
External standard curve
Figures of merit
Fitness for purpose
Independent variable
Internal standard curve
Knowns
Least-squares regression
Limit of detection (LOD)
Limit of quantitation (LOQ)
Linear dynamic range (LDR)
Linear regression
Matrix mismatch
Negative control
Positive control
Precision
Quality assurance (QA)
Quality control (QC)
Regression line
Repeatability
Replicates
Reproducibility
Spike
Standard addition
Total quality management
Traceability

Problems

FROM THE CHAPTER

1. Provide definitions of the reliability and utility of chemical data that could be presented on the witness stand. Why are both considered part of quality assurance?
2. A micropipet is certified by the manufacturer at 50.0 μL TD, $\pm$1.5% (95% confidence). An analyst performs a routine calibration check by pipetting a series of 5 aliquots of deionized water into a tared vial. The water is at a temperature of 25.0°C. The following data are obtained:

n	Weight (cumulative), g
1	0.0494
2	0.0997
3	0.1484
4	0.1985
5	0.2477

   Is the micropipet performing according to specifications? Cite sources.
3. In complex environmental samples such as soils, spikes are used to measure the matrix effect. The range of allowable recoveries for 1,2-dichloroethane-*d*4, a spike compound, is 80–120%. How is it possible to obtain a recovery greater than 100%? (*Hint*: This happens frequently, and it does *not* imply that the compound in question is in the sample.)
4. Using the example in the text as a starting point, create an SOP for weighing plastic bags that contain green plantlike material suspected to be marijuana. The SOP should describe how to obtain the net weight of the plant matter only, not including the packaging. Write the SOP assuming only a single bag is to be weighed.
5. Differentiate between *accuracy* and *trueness.*
6. Differentiate among *precision*, *repeatability*, and *reproducibility*.
7. Differentiate between *robustness* and *ruggedness*.
8. A forensic laboratory operates in an older building in which the environmental systems are poorly controlled. In winter, the lab tends to overheat into the 90s (°F), while in summer, the air conditioning can drive temperatures into the low 60s (°F). Which figure(s) of merit will be affected?
9. If you had to work in the laboratory described in the previous question and had to develop a method validation plan for a new method, what procedures and steps would you employ to characterize this/these figures of merit?
10. A laboratory analyzes the CRM 1511 (Figure 5) and generates data for all components. Complete the table:

Can you calculate the accuracy in this case? Why or why not?

Analyte	Certified Value (mmol/L $\times 10^{-4}$)	Laboratory Value (mmol/L $\times 10^{-3}$)	Bias	%error
Morphine	1.08	0.99		
Codeine	9.62	9.50		
Benzoylecgonine	3.60	3.72		
THC-9-COOH	4.09	4.25		
Phencyclidine	9.74	9.74		

11. Suppose the analyst who did the analysis described in the previous question performed 10 independent replicate analyses each day for 5 days. Which figure of merit would the standard deviation of all these values represent?

12. A laboratory generates the following data for a morphine assay. Construct an external standard curve and complete the table that follows the curve data.

[Morphine] (ppb)	Area Counts (extracted ion)
20.0	1452
80.0	6108
150.0	11309
250.0	17009
400.0	30072
600.0	44567
800.0	61234

Data table to complete:

	Counts	ppb Morphine
Unknown 1:	6070	
Unknown 2:	8878	
Unknown 3:	19185	

13. The following data were generated as part of the calibration of a mass spectrometer to be used for a morphine assay:

[Morphine] (ppb)	Area Counts (extracted ion)
20.0	1400
80.0	6108
150.0	12419
250.0	17009
400.0	30072
600.0	42393
800.0	64098

The analyst runs an independent calibration check sample with an accepted true value of 100.0 ppb of morphine and obtains 7084 counts for the area of the morphine peak. The SOP stipulates that the calculated concentration of the calibration check solution must be within $\pm 2.5\%$ of the accepted true value. The analyst makes a mistake and, contrary to the SOP, forces the external standard calibration line through the origin (0,0). Calculate the concentration of the calibration check solution for this case as well as the case in which the line is not forced through the origin. What is the impact of this mistake?

14. Why are deuterated compounds frequently used as internal standards?

15. If an analyst inadvertently generated a least-squares-fit calibration curve that was forced through the origin, what type of error would be introduced? Would accuracy or precision be affected? What types of quality assurance or quality control samples and procedures could identify this error?

16. In Exhibit B the point was made that a calibration result from the middle of a curve should never be arbitrarily thrown away. The same is not necessarily true of calibration points that define the upper and lower extremes of the curve. Why?

17. A toxicologist receives a blood sample thought to contain a low level of a new poison just identified. The quantitation is of paramount importance because the treatment can cause severe side effects if it is given to a healthy person. What would be the best calibration method for determining whether the sample contains the new poison?

INTEGRATIVE

1. What levels of quality assurance are there in typical freshman chemistry laboratories? In organic laboratories?

2. Draw a diagram that employs two Gaussian distribution curves to illustrate the concept of bias. What does the diagram have in common with the *t*-test of means? In what ways does it differ?

3. A forensic toxicologist receives a postmortem blood sample and performs a routine screening analysis followed by GC/MS to confirm the presence of cocaine and its metabolites. The toxicologist also performs a quantitative analysis for these analytes. He writes his report and sends it through the laboratory system. The report arrives on the desk of the medical examiner. Identify at least three levels of peer review that would have occurred in this example. (*Hint*: Much of the applicable peer review would have occurred offstage and in the past.)

4. Research how NIST is able to apply the label "certified" and "standard" to CRMs and SRMs. What particular step is crucial and why does it lend such authority? Discuss this step in terms of error types as well as in terms of accuracy and precision.

5. Deuterated compounds are useful as internal standards, while isotopes such as ^{14}C and tritium are not. Why?

6. The following data are produced as part of a laboratory accreditation-check sample:

	ppb Codeine	Peak Area	ppb Internal Standard	Peak Area	Conc. Ratio	Area Ratio
	15.0	9599	50.0	29,933	0.30	0.32
	35.0	21,456	50.0	30,099	0.70	0.71
	75.0	45,326	50.0	32,051	1.50	1.41
	125.0	82,100	50.0	32,912	2.50	2.49
	150.0	95,003	50.0	31,100	3.00	3.05
	200.0	122,409	50.0	30,303	4.00	4.04
Blank:		3100	50.0	31,954		
Known (100.0 ± 1.0 ppb)		51,208	50.0	33,000		
Cal check (Independent, 125.0 ± 0.1 ppb)		74,912	50.0	32,844		

Critique these results. "[ ]" is the concentration.

7. A laboratory creates a control chart for an analytical balance by weighing a certified, traceable weight of 20.00 mg each week for a year. Enter the following data into an Excel spreadsheet (two-column format) and answer the following questions:

a. Calculate the mean, population, sample standard deviation, and range of values using the 95% confidence interval.
b. Which standard deviation is best suited to this data set and why? Calculate the %RSD based on this value.
c. Calculate the skew and kurtosis and comment.
d. If the warning limits are set at $\pm 2\sigma$ and the control limits at $\pm 3\sigma$, at what weights will the control limits be exceeded? Report to two decimals to match the significant figures of the weights.
e. Create the control chart.

First Quarter (Q1)		Q2		Q3		Q4	
Week #	Weight	Week #	Weight	Week #	Weight	Week #	Weight
1	19.91	14	20.04	27	20.03	40	20.02
2	20.10	15	20.00	28	20.14	41	20.19
3	20.13	16	20.14	29	20.04	42	20.10
4	19.99	17	20.19	30	20.13	43	20.07
5	20.01	18	20.11	31	19.93	44	20.13
6	20.16	19	20.08	32	19.94	45	19.97
7	19.95	20	20.19	33	20.09	46	19.96
8	19.97	21	20.21	34	20.11	47	19.96
9	19.92	22	19.98	35	20.05	48	20.16
10	19.96	23	20.02	36	20.00	49	19.94
11	20.08	24	20.00	37	20.15	50	20.04
12	19.88	25	20.11	38	20.13	51	19.91
13	19.96	26	20.10	39	19.99	52	20.15

8. A forensic toxicology laboratory associated with a medical examiner's office routinely screens postmortem blood samples for morphine, which is a metabolite of heroin. To address the difficult matrix, the laboratory uses an internal standard method. The following curve was generated for a typical assay:

The SOP requires the analysis of an independent calibration check solution. In this case, the accepted true value of the check solution is 200.0 ppb. The calculated value of the calibration check solution must be within ±5% of the accepted true value for the assay to proceed. There is also a requirement that the %RSD of the internal standard

[Morphine] (ppb)	Area Counts	[IS] (ppb)	Area Counts
20.0	1452	250	15023
80.0	6108	250	15865
150.0	13309	250	14998
250.0	17009	250	15000
400.0	30072	250	15299
600.0	42234	250	15308
800.0	61234	250	15333

areas in the curve be less than 5%. An analyst performs three replicate analyses on a single blood sample in the batch and obtains the following data (at right):

a. What are the mean and %RSD of the internal standard areas? Should the assay have been stopped based on this finding?
b. Why is this consistency a reasonable expectation?
c. What was the bias found between the observed concentration of the calibration check solution and the accepted true value? Should the assay have been stopped based on this finding?
d. Report the concentration of morphine calculated as a range based on the 95% confidence interval.

	Counts (Morphine)	Counts (IS)
Cal check 200 ppb	14503	15203
Unknown replicate 1	37249	15235
Unknown replicate 2	37326	15172
Unknown replicate 3	36636	15299

FOOD FOR THOUGHT

1. Is there such a thing as a "true" value for the concentration of a prepared blind quality assurance sample?
2. Are standard desktop calculators traceable? Can a user be absolutely sure that every operation is correct, assuming that the key entry is?
3. It is often said that the R^2 of a calibration curve is a measure of how well you do dilutions. Why?
4. A forensic toxicology laboratory hires a new chemist who starts work in the sample preparation section. The laboratory is accredited and has validated methods (as SOPs) that are used for each type of sample preparation. Do these have to be revalidated now that a new person is to be using them? Explain and justify your answer.

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Reporting Defensible Uncertainty and Obtaining Representative Samples

OVERVIEW AND ORIENTATION

This chapter addresses two topics that at first might seem unrelated; however, in forensic chemistry, and particularly in seized drug analysis, they most definitely are related. Generically, we can define the uncertainty associated with a quantitative analysis as

$$U_{\text{total}} = U_{\text{sample \& sampling}} + U_{\text{analysis}} \quad \textbf{(1)}$$

The latter is easier to define and control, whereas the former is a challenge in any analysis, not just forensic. Not surprisingly, given the complexity of the challenges, the estimation of uncertainty and sampling practices are rapidly evolving topics in forensic chemistry, with several national and international bodies working toward the development of recommended best practices. As we will see, there are many viable and defensible approaches to sampling and uncertainty estimates. In all cases, the goal should be to develop reasonable, rational, and defensible methodologies that are fit for purpose, meet jurisdictional requirements, and adequately and honestly answer the forensic question at hand.

1 UNCERTAINTY

Whenever quantitative data are reported, there is an associated uncertainty that should be estimated. Uncertainties are always estimated because, like a true value, they are inherently unknowable. Recall the NUSAP framework for reporting data, where uncertainty is the S or spread that creates a range in which we expect to find the true value.

Along with S comes A, or the assessment of that range, such as at the 95% confidence level. In this chapter, we will learn how to generate a defensible spread (S) along with a quantitative assessment (A).

The approach introduced here to estimation of uncertainty is relatively new. In the 1970s, uncertainty, if estimated at all, was often calculated using the propagation-of-uncertainty (also called propagation of error) method. The gradual transition from the propagation of error philosophy to the estimation of uncertainty approach began in the mid-1990s. The international metrology and analytical communities recognized and began to address the problem, and in 1997, the **Joint Committee for Guides in Metrology (JCGM)** was formed representing several international metrology organizations, IUPAC and ISO. A guide to uncertainty was published, the latest version of which is the *Evaluation of Measurement Data—Guide to the Expression of Uncertainty in Measurement*,[1] which is available online at no charge. This document is frequently referred to as "the **GUM**." The principles outlined in this document have become central to estimation of uncertainty in analytical procedures.

Within forensic chemistry, there are two general categories of data that require frequent or routine quantitative analysis and uncertainty estimates. In seized drug analysis, the weight of a seizure is often critical in that the seriousness of the crime and severity of the penalties may be tied to amount. When this is the case, the weight in question is called a **threshold weight**. Estimating the uncertainty of weights can be more complicated than you might expect, because obtaining a weight is more complicated than you might expect. We will use this as an example to illustrate the key points of uncertainty estimation. The second category of quantitative data is that related to concentrations in toxicological samples and drug purities in seized drug analyses. An obvious example is blood alcohol determinations; with a typical legal threshold of 0.08%, the estimated uncertainty of a result such as 0.081% is critical.

1.1 Uncertainty: What It Is and What It Is Not

Uncertainty exists whenever a quantitative measurement is made. It is inevitable and inescapable but not inherently bad or undesirable. Uncertainty is a consequence of using any measurement tool, procedure, or protocol. It arises from many sources including procedures, analyst, environment, sample, and instrumentation, and any honest reporting of data requires an equally honest estimation of uncertainty. Uncertainty is *not* error, even though those terms are related and often used interchangeably. *Error* is defined as the difference between a measured value (the measurand) and an accepted true value, while *uncertainty* is a spread or a range in which we expect the true value to lie. We can never know the true value, but we report a defensible range in which we expect it to be found and apply an associated probability or confidence. Just as we cannot report *the* true value, we cannot ever provide *the* uncertainty associated with a measurement. All we can do is estimate it and defend our methods for doing so.

Uncertainty arises from many sources, both inside and outside the laboratory. We will focus on sources that arise from the analysis itself, reserving a discussion of sampling and uncertainty for later in the chapter. These sources of uncertainty can be categorized in several ways. For example, there is uncertainty associated with instrumentation and equipment, and uncertainty arising from the method or procedure and how it is performed. One of the most important goals of method validation and QA/QC is to reduce uncertainty and error to the minimal achievable value and to provide the information needed to reliably estimate what remains. But even if a validated method is executed perfectly on equipment and instrumentation performing perfectly and operated by an analyst on his or her best day, uncertainty remains. That remaining uncertainty has both random and systematic error components. This may at first seem

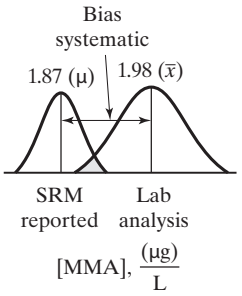

FIGURE 1 Comparison of an NIST SRM and laboratory analysis.

troubling because it was stated that error and uncertainty are not the same. Indeed, they are not, but it is reasonable to think of uncertainty as arising from a collection of many different types of small errors, some random and some systematic. Sometimes it is difficult to tease apart random and systematic contributions to uncertainty, but it is important to realize that both exist.

Consider the example shown in Figure 1. NIST has a standard reference material for arsenic species in frozen urine, SRM 2669. One of the components in this SRM is monomethylarsonic acid (MMA) with a certified concentration of 1.87 μg/L (ppb) ±0.39 ppb.[†] Suppose a toxicology laboratory uses this material as part of its arsenic poisoning detection method and performs analyses on 10 replicate samples under the same conditions, obtaining the results shown in the Figure. At first, it would seem that the systematic error (bias) is easily calculated as 0.11 μg/L. This is acceptable and consistent with all earlier statements. But have we, by this one subtraction, really identified and quantified *all* of the systematic error? No, because we are making the assumption that the reported value of the SRM is the true value. In actuality, the value we are calling true (μ) is the estimate of μ that came out of the NIST laboratory. We are comfortable accepting this as a true value because of the meticulous care and multiple analytical techniques used by NIST. But it is still not *the* true value, which is reflected in the uncertainty that is reported along with the mean. Because the true value (μ) is by definition unknowable, so is the systematic error ($\bar{x}$-μ). We strive to minimize it, but we can never remove it all, nor can we pin it down to an exact number. Figure 1 also illustrates the random components of uncertainty. We can estimate the uncertainty associated with the laboratory analysis based on the standard deviation of the replicates or the 95% confidence interval. Using a validated method and proper controls, the spread of the data is minimized but will never be zero. This is not a problem as long as we can reliably estimate that spread. Therein lies the challenge.

Broadly speaking, there are two approaches to estimating the uncertainty of an analytical process. The first is the **bottom-up model**, in which each factor that can contribute to the uncertainty is identified, described, assigned a quantitative value, and added to the whole. In contrast, the **top-down model** starts with some general measure of repeatability or reproducibility that captures the uncertainty of the process as a whole. We will discuss each, as well as combinations of the two, which are becoming more common in forensic laboratories. Keep in mind that there is no one right method to estimate an uncertainty; but whatever method is used must be reasonable, defensible, and fit-for-purpose.

1.2 Uncertainty Budgets and the Bottom-Up Model

The first step in estimating the uncertainty of a measurement process is to understand that process completely. This requires breaking down the process and identifying individual contributions to the total uncertainty and deciding whether they are significant. In the bottom-up approach, we first specify every factor that may contribute to the overall uncertainty. Next, we decide how to characterize this portion of the contribution, and then we judge its relative contribution to the overall uncertainty. Once we identify the significant contributors, we add them up and express the estimate based on the desired confidence level. In the top-down method, the goal is to capture and aggregate as many uncertainty contributors as possible using tools such as control charts and reference materials. We will illustrate the process with several examples.

The classic GUM bottom-up approach can be broken into thel steps illustrated in Figure 2:

1. Specify what is being measured (the measurand) and how.
2. Study the method and identify individual contributors (u_i) to the total uncertainty.

[†]Level 1 as per data sheet

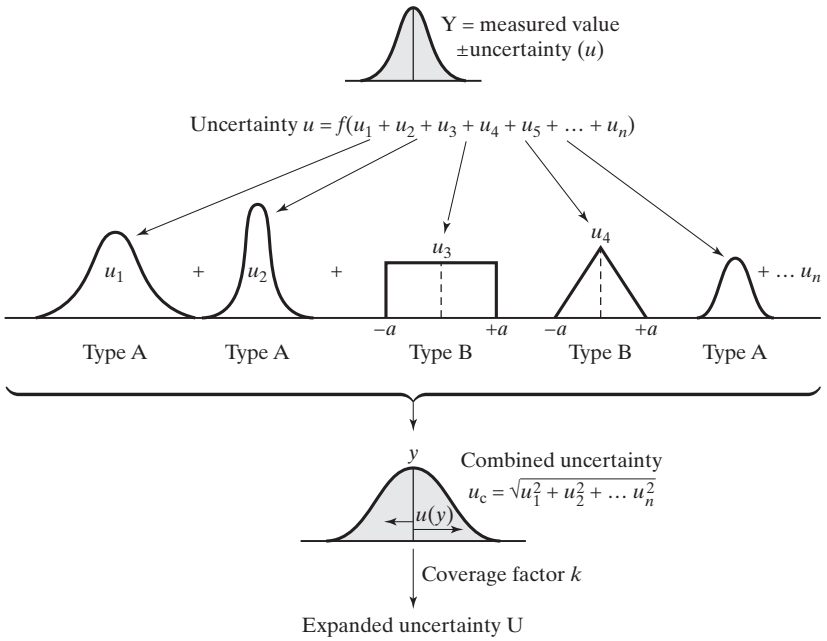

FIGURE 2 Individual contributions to uncertainty are summed to generate an estimate of the overall uncertainty in the GUM approach.

3. Classify each contributing factor as Type A or Type B.
4. Express each significant contributor as a standard deviation or equivalent.
5. Adjust to relative contributions (standard uncertainties).
6. Assess contributions and retain the most significant.
7. Sum the contributions to obtain the combined standard uncertainty (u_c)
8. Apply a **coverage factor (*k*)** to obtain **expanded uncertainty (*U*)**.

Step 1, identifying the mechanism of the measurement, is essential. This is one of the strengths of this model—it forces the analyst to understand the process literally from the bottom up (and everywhere between). Contributing factors may not be immediately apparent and may require thought and study. For example, we cannot estimate the uncertainty associated with an electronic analytical balance unless we *really* understand how it works, including limiting factors and operational constraints. With this knowledge, most of the individual contributors to the overall uncertainty can be identified.

A **cause-and-effect diagram** (also called a *fishbone diagram* or an *Ishikawa diagram*) is a useful tool for visualizing the measurement process and the uncertainty contributions. A simple example is presented in Figures 3 and 4. Here the process is the preparation of a standard solution of cocaine in methanol that is to be used in a subsequent quantitative analysis. The quantity to be reported, along with the uncertainty, is the concentration of cocaine. The calculation of the concentration is a straightforward $C_1V_1 = C_2V_2$ type, and it might be tempting to use a propagation-of-uncertainty calculation. However, there is a better way to estimate the uncertainty associated with the concentration of the diluted solution (C_2). The cause-and-effect diagram for this process is shown in Figure 4.

The central line represents the measured value (C_2), and the lines coming in at an angle represent general contributions to the uncertainty of that measurement. Factors coming in from the left or right of these slanted arrows are contributing factors that branch as required. Here, the top portion of the figure shows the skeleton and major factors,

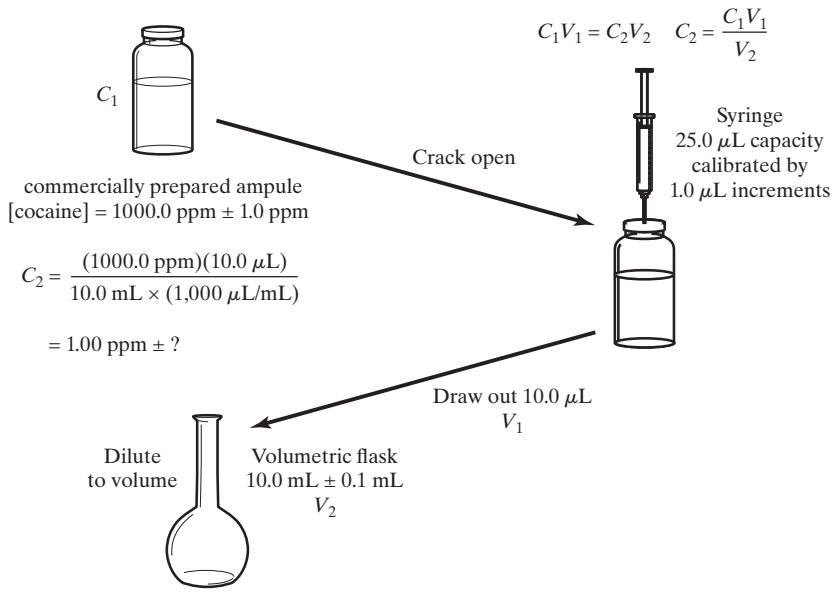

FIGURE 3 A dilution showing each step. This level of detail is needed to identify potential contributing factors to uncertainty.

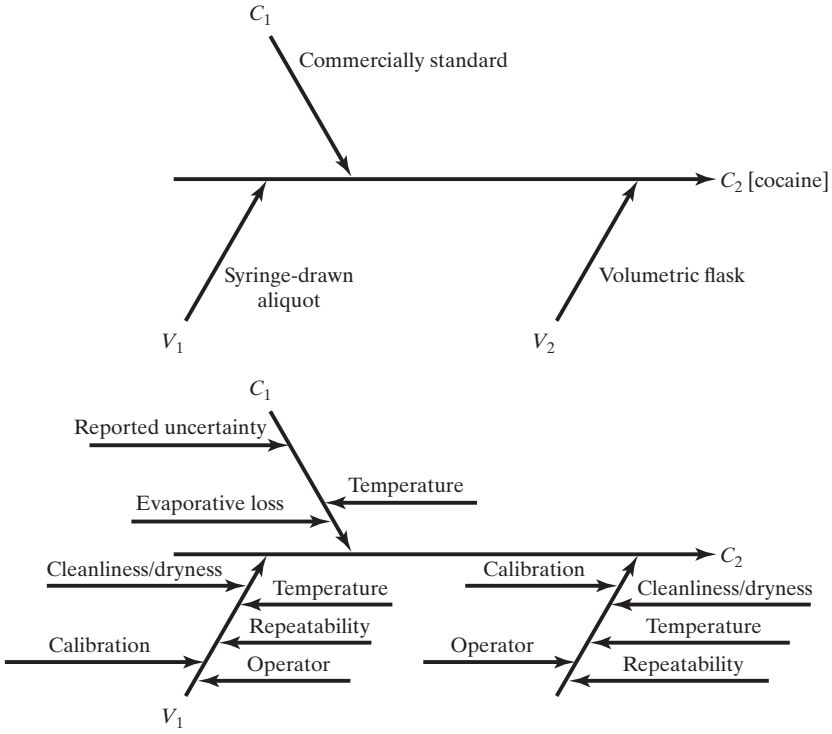

FIGURE 4 A cause-and-effect diagram for the dilution shown in Figure 3.

while the bottom shows a more complete (but not exhaustive) rendition. For example, temperature is a factor because it influences density and thus volume. Temperature could thus influence C_1 as well as the volume of solution drawn up in the syringe and the volume placed in the volumetric flasks. Both of these devices are calibrated at a set temperature (typically 20.0°C). If the lab is not at this temperature, then uncertainty is contributed. Is this a significant factor? It depends on the situation and on the laboratory operating conditions. Undoubtedly, temperature effects contribute to uncertainty; *how much* is the key question. A propagation-of-uncertainty calculation would not capture this uncertainty, and even using brute-force cause and effect, it would be difficult to assess the temperature contribution directly. We will see later in the chapter how to address this type of factor using a control chart. The advantage of creating a cause-and-effect diagram is that it forces you to think about every aspect of an analytical procedure and to develop ways to reduce error and uncertainty through a systematic attack on each contributor. Once this is accomplished, a quantitative estimate of the remaining uncertainty can be undertaken.

With reference to the GUM procedure, each contributing factor has an associated distribution, as shown in Figure 2. Not all are normal distributions. If the uncertainty of the factor can be captured and described by a standard deviation, then it is a reasonable estimate of the spread associated with that contribution. This is called a **Type A contribution**, but there are cases in which a standard deviation does not apply. As an example, consider an electronic balance, where a rounding operation occurs electronically to arrive at the displayed last digit. The rounding typically determined by standard rounding rules, not by the mean of a normal distribution. **Readability of a balance** is an example of a **rectangular distribution**, in which there is an equal chance that any digit will appear in the last display position. In other words, if rounding were dictated by a normal distribution, 5 would most often appear in the last place because that is near the center of a 0–9 range. This is not the case here—the rounding is not based on a normal distribution. Such nonstatistical responses are categorized as Type B contributors, and we cannot use the standard deviation as a measure of the spread.

The most common **Type B distributions** are the *rectangular* and the *triangular*. A **triangular distribution** implies that within a range, we expect the value to be near the center most of the time, but at the same time we cannot describe the spread as a normal distribution. Consider the volumetric flask shown in Figure 3. Assuming this is Type A glassware,[†] the true volume will be the calibrated volume ± 0.1 mL, assuming the temperature of use is the same as the temperature of calibration. However, with careful filling and meticulous attention to bringing the meniscus exactly to the line, we would expect the true value to be near the center of the range of ± 0.10 mL. This contribution to uncertainty could reasonably be considered to be a Type B triangular distribution. We do not have sufficient data or information to assume that the distribution is normal, but we can make a reasonable and defensible assumption that values near the center of the range are more likely than those at the extremes. As a general rule, if there is no prior knowledge of the distribution, a rectangular shape is assumed.[2]

The contributions to the total uncertainty can be listed in a table or spreadsheet called an **uncertainty budget**. This representation allows for calculations needed to obtain the relative contributions of each factor to the total uncertainty. Recall that to do the propagation-of-uncertainty calculation, we had to put each factor into a relative value to insure that the units matched; we cannot add an uncertainty expressed in milliliters to one expressed in grams. The same is true with an uncertainty budget: contributions

[†]This is in reference to the ASTM designation of glassware tolerance. It is just an unfortunate coincidence that it has the same designation as a Type A contribution to uncentainty.

have to be expressed in terms of contribution to the whole as a unitless value. Once these relative contributions are defined, those that are deemed insignificant can be discarded and the rest summed to give the combined uncertainty.

The combined uncertainty is not the final reported value; one step remains—the application of the coverage factor *(k)*. This concept is analogous to the use of the Student's *t*-value. Recall in that case we used the Student's *t*-distribution because we recognized that we had a small sample compared with the population size. Using the *t*-value corrected for the underestimation of the standard deviation that results with small sample sizes. In the uncertainty calculation, we are expanding the coverage (size of the range) to provide a desired confidence. For example, a *k*-value of 2 correlates to approximately the 95% confidence interval and a *k*-value of 3 to approximately a 99% confidence -interval. Recall that within a normal distribution, about 68% of all values are included within $\pm 1s$, about 95% within $\pm 2s$, and about 99% within $\pm 3s$. Conceptually, the *k*-factor accomplishes the same thing and provides a confidence level to the interval selected—the A (assessment) in NUSAP.

1.3 Uncertainty Budgets and the Top-Down Model

Recall the simple dilution example illustrated in Figures 3 and 4. We discussed the potential contribution of temperature to uncertainty and noted that it would be difficult to quantify this in a normal working environment. Even in the most modern laboratories, temperature fluctuation is inevitable. In an honest estimate of uncertainty, how do we account for it? The top-down model offers an alternative to account for this and other contributions that are challenging to tease out individually. It also allows for the determination of several uncertainty contributors using one tool. The tool is some type of repeatability or reproducibility measurement, often a control chart. We cannot isolate these individual contributions from one another, but in this model this is not necessary. We will see why shortly.

An examination of the lower frame of Figure 4 shows that there are several contributors we could capture using a control chart. A control chart focuses on replicate measurements taken over time and as a result, can capture time-related uncertainty as well as factors that are difficult to determine any other way. Recall we discussed how changes in laboratory temperature would effect our calculation of a final concentration illustrated in Figure 3. If we utilize a control chart that spans several weeks, we can "capture" the effect of temperature fluctuations in the laboratory over time. For this example, the laboratory could begin by establishing a control chart that determines volumes based on mass determinations. Each working day for 2 weeks, three different analysts would be assigned to measure volume delivered by the syringes used for standard preparations and the volumetric flasks used for the final dilution. The true volume of water in each case would be determined using the weight and density of water at the laboratory temperature. The volume would fluctuate as a function of temperature, analyst, and device used—this is normal expected uncertainty. This variation can be captured by the control chart, and the standard deviation can be used to represent the spread associated with these factors. We cannot isolate the contribution of temperature to this variation, but we can be confident that it is included in the overall uncertainty expressed as the standard deviation. Establishing control charts can be labor intensive, but once operating, they are invaluable. The key to using control charts in this way is to understand which factors a control chart captures and which factors it does not.

A variation on the top-down model being adapted by some forensic laboratories is the use of reference materials as a means of estimating uncertainties.[3–5] The idea is that because a SRM has a value that is accepted as the true value, deviations from that value obtained experimentally can be used as an indirect measure of uncertainty. In these

applications, the reference material is used as the basis of a control chart that establishes reproducibility/repeatability of the method over time. This concept is similar to that illustrated in Figure 1, with many variations in selection of reference materials and methods. As of this writing, these models are still being developed and bear watching in the future as another promising approach to estimating uncertainty.

1.4 Example Uncertainty Calculations: Obtaining a Weight

Weights of seized drugs are among the most important and consequential types of quantitative data to come out of a forensic laboratory. Nowhere is the need for reasonable uncertainty estimates more pressing. Prior to tackling this question, it is essential to understand how an electronic laboratory balance works, something that even seasoned analytical chemists may take for granted. Books have been written on obtaining reliable weights and uncertainties.[6,7] Accurate and comparable weights are critical for commerce and trade, as well as for science and technology. A kilogram in the United States has to be the same as a kilogram in Europe or Canada or the Middle East. Ultimately, the kilogram is traced back to a reference kept in France,[‡] and this establishes traceability. For this discussion, the term *weight* will be used synonymously with *mass*.

Older analytical balances consisted of two pans, one containing a known reference weight and one in which the sample was placed. The results were reported relative to the reference weights needed to balance the weight of the sample. Modern electronic balances measure the downward force exerted on a plate by a weight that is placed on it. The measurement of force is related to weight through a calibrated value obtained from a standard weight. This measurement is analogous to the way a pH meter or any other calibrated device works. A pH meter is manually calibrated with two or three solutions with accepted true values of pH of (typically) 4.0, 7.0, and 10.0. This operation associates a voltage on the electrode with a pH value, and if we assume a linear relationship, any voltage in the calibrated range can be associated with a pH value. Similarly, a balance must be properly calibrated to yield reliable weights, ideally using a traceable weight. Not surprisingly, this calibration process has an associated uncertainty that contributes to the overall uncertainty of any mass measurement. Several other factors can contribute to the uncertainty of a weight determination, including:

- *Temperature and the environment such as air currents:* Most balances have shields to limit air movement, but temperature is harder to control. Some balances are supplied with a value for a temperature sensitivity coefficient that can be used in estimating uncertainty.
- *Off-center loading:* Balances are designed with the assumption that the load is on the center of the pan.
- *Repeatability:* This is measured by replicate measurements of a standard weight.
- *Level:* Balances are designed to operate when level; any tilting can cause a distortion in the force on the pan.
- *Static electricity:* The presence of static can lead to erratic and unstable readings and contribute to uncertainty.
- *Linearity:* Balances may have different accuracies at low mass compared with those at high mass within their operating range. A plot of accepted true weight (from a traceable mass standard) is plotted against the measured value to obtain a linearity plot. An example is shown in Figure 5

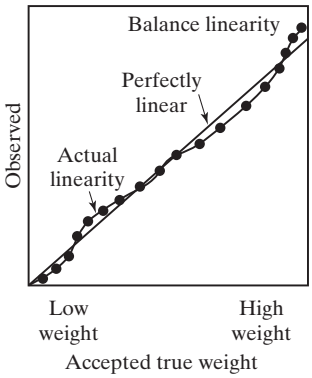

FIGURE 5 Linearity of a hypothetical analytical balance.

[‡]You can see "the" kilogram at the BIPM website (http://www.bipm.org/en/scientific/mass/prototype.html) Last accessed October 2011.

- *Buoyancy:* Air offers some support to any object weighed. The greater the density of the object, the less it is supported by air. Envision air as if it were like water. Once the density of water is exceeded, objects sink in it; anything that is less dense than water floats. In air, the downward force exerted on a balance pan is affected by air buoyancy. Less dense material is buoyed upward, resulting in less force on the pan and a lower mass reading than the true value. Consider an empty balloon. We can place one on the pan and obtain a weight. Now, we fill that same balloon with helium and try again. Even though mass has been added, the buoyancy makes obtaining an accurate weight problematic. The opposite is true for very dense material. Steel is used as the reference point[6,7]. Thus, for most forensic applications, where objects being weighed are less dense than steel, the observed weight is less than the true weight, and uncertainty is generated.
- *Readability*: How the balance rounds the last digit contributes to the uncertainty. With such analog devices, the user (you in this case) estimated the first uncertain digit. Electronic devices also estimate the first uncertain digit. In the case of the analytical balance, the first uncertain digit is the readability, and it is obtained by some type of electronic operation. This electronic rounding should be considered to be a source of uncertainty just as surely as how we read a bathroom scale would have to be considered.

These factors are summarized in the cause-and-effect diagram shown in Figure 6. Once the factors are identified, the relative impact on uncertainty should be estimated and procedures optimized to minimize contributions. Here the laboratory would establish an SOP for weighing and would train all analysts in the proper use of the balance. This would minimize uncertainty contributions from improper leveling, off-center loading, and using the balance without the draft shields closed. An anti-static chip would be purchased and used before each weighing as part of the SOP. This would minimize the uncertainty contribution from static. The laboratory would establish a routine calibration procedure to be conducted by outside contractors to insure the balances are working properly and reliably.

To address buoyancy, the laboratory could decide that given that there is no way to account for buoyancy effects across all possible sample types, this factor will not be considered, as long as the items being weighed are less dense than steel (as nearly everything weighed in a forensic laboratory is). This decision is further justified by recognizing that for anything less dense than steel, the recorded weight is less than the true weight.[7] This is a reasonable and a defensible conservative approach that fits the reality of work in a forensic laboratory. With this assumption, what factors remain? Four are meaurable contributing factors: the temperature sensitivity, linearity, repeatability, and readability. We are now ready to construct a budget and assess these contributions individually.

For sake of example, assume an exhibit is weighed using the SOP at 50.000g, and the analyst will use an uncertainty budget to report the estimated uncertainty of this result. The temperature sensitivity in this example could be supplied by the manufacturer of the balance and would be found in the specification sheet. One way this is reported is in ppm/°C. In this example, the uncertainty contributed by temperature is 1 ppm (10^{-6}g per gram) per °C fluctuation in temperature from 20°C, or 1 µg per gram per °C. Thus, if an exhibit weighs 50.000g at a laboratory temperature of 26°C, the uncertainty contribution would be estimated as

$$\frac{1\mu g}{g^{o}C} \times 50.000g \times 6^{o}C = 300\mu g = 0.3mg = 0.003g \quad \textbf{(2)}$$

This value is still not amenable to use in the budget, since the temperature in the lab is not measured as part of the weighing SOP. Instead, the SOP could state that the balances can be used only when room temperature is between 15°C and 25°C, which means in the worst case, the deviation would be ±5°C:

$$\frac{1\,\mu g}{g^{o}C} \times \text{balance reading g} \times 5^{o}C = \frac{50\,\mu g}{g} \quad \textbf{(3)}$$

This formula would be applied to any weight within the working range of the balance, so for this example

$$\frac{50\,\mu g}{g} \times 50.000g = 2500\mu g = 2.50mg = 0.0025g \quad \textbf{(4)}$$

The last step in constructing the uncertainty budget is to determine the **standard uncertainty (*u*)** for the contribution of temperature variations to the total uncertainty. Recall that, as shown in Figure 2, this is a Type B contribution, and the value we have arrived at (equation 4) must be expressed as a spread. For a rectangular distribution, the value *a* (Figure 2) is divided by the square root of 3 to obtain a standard uncertainty (*u*) of 1.44×10^{-3} grams or 1.44 mg.

For linearity and readability, the laboratory could turn to the calibration report obtained each time the balance is checked, using the most recent and current value. Finally, for the repeatability, there are several options. It could be obtained from the calibration check report, or it could be obtained from a control chart.

Continuing with this example, the SOP for weighing could require that once a week every analyst using the balance weigh a series of traceable SRM weights and record these values in a logbook. The analysts could do this anytime on any day as long as they did it once every week. The values would be incorporated into control charts across the mass range. The standard deviation associated with the weight closest to the exhibit weight could then be used to express the uncertainty associated with the repeatability. As a bonus, this approach would also capture uncertainty associated with different environmental conditions and different operators. Because this value is statistically based, we can use *s* as the standard uncertainty of repeatability.

We can now build an uncertainty budget, as illustrated in Table 1. Two important notes about the budget: 1) units are all in or converted to grams; and 2) this budget was constructed using a spreadsheet and so some extra digits are included in some of the intermediate calculations for illustrative purposes. The final rounding will be done at the end of the process and will be dictated by the precision of the balance.

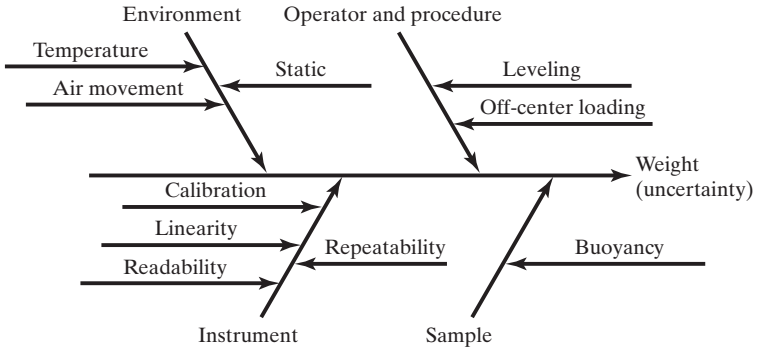

FIGURE 6 Cause-and-effect diagram for a hypothetical analytical balance.

TABLE 1 Uncertainty Budget for Weighing

Contributing Factor	Value (units)	Type of Distribution	Standard Uncertainty (u)	u^2	Relative Contribution (%)
1. Temperature sensitivity	1 ppm/°C deviation from 20°C (convert to grams as in eq. 2)	Rectangular	1.443×10^{-4}	2.083×10^{-8}	0.1
2. Linearity	0.002 g	Rectangular	1.115×10^{-3}	1.333×10^{-6}	6.9
3. Repeatability	For a 50.000-g standard weight, control chart $s = 0.0042$ g	Normal	0.0042	1.76×10^{-5}	91.3
4. Readability	0.001 g	Rectangular	5.774×10^{-4}	3.333×10^{-7}	1.7
		Sum of standard uncertainties: $\sum u_{1-4}$	6.076×10^{-3}	1.933×10^{-5}	100%

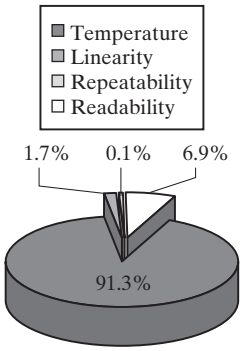

FIGURE 7 Relative contributions to uncertainty for the analytical balance.

The relative contribution of each factor to the total is determined by comparing the variances (recall that the variance is the square of the standard deviation). Thus, for temperature, the relative contribution to the total uncertainty (100%) would be

$$\frac{2.083 \times 10^{-8}}{1.933 \times 10^{-5}} \times 100 = 0.1\% \quad \textbf{(5)}$$

In this example, it would be reasonable to ignore the contribution from the temperature fluctuation, since its contribution to the overall uncertainty is less than 1% (Figure 7). This decision is based on the data and could be made only *after* the uncertainty budget was complete and the relative contributions known.

With these two contributors removed, the uncertainty is dominated by linearity and repeatability and can be expressed mathematically as the **combined standard uncertainty (u_c)**:

$$u_c = \sqrt{\mathrm{u(linearity)}^2 + \mathrm{u(repeatability)}^2 + \mathrm{u(readability)}^2} \quad \textbf{(6)}$$

or 0.00439. The final step is to calculate the expanded uncertainty U as a function of the coverage factor k. We select $k = 2$ to correspond to 95% confidence:

$$U_c = 2.0 \times 0.00439 = 0.0088 \quad \textbf{(7)}$$

We can now report the weight of the exhibit as 50.000g ±0.009 at the 95% confidence level and rounded to the precision of the balance. If we elected to use a coverage factor of 3 (~ 99 confidence), the weight would be reported as 50.000g +/− 0.013g. Has this rigorous approach captured every conceivable contribution to uncertainty? No, but it has captured what would reasonably be expected to be the major contributions. This is an example of a rational and defensible estimate of uncertainty that is fit for purpose. It is by no means the only way that could work. The process of developing the budget allowed the laboratory to look at each step of the process, train analysts, develop an

SOP, and implement a thoughtfully planned control chart to capture elements of uncertainty that would be missed in a simple propagation-of-uncertainty approach. It also assisted the laboratory in designing the best possible process to obtain measurements and report results.

1.5 Increasing Complexity

As involved as this weighing example may seem, it barely scratches the surface of weighing as an analytical operation. Consider one of the most common exhibits in seized drug analysis—a plastic bag containing green plant material that is shown through analysis to be marijuana. Because penalties are usually tied to amount, a reliable weight and uncertainty estimate are vital. How is the weight obtained? Typically, a plastic weighing dish is placed on the balance pan and tared (display is zeroed). Does this constitute a weighing event? If so, how is the uncertainty associated with the taring taken into account? Next, the contents of the bag are transferred as completely as possible to the dish and the weight recorded. This is a sampling event. Inevitably, some of the material is lost in the transfer, but in practical terms this quantity is difficult to estimate and impossible to generalize except to say that some mass will be lost. Suppose the case consists of not one plastic bag but a hundred? How are the individual weights and uncertainties taken into account to report a total net weight? You will be happy to learn that these topics exceed the scope of this text, but they are the subject of debate and discussion in the forensic community. Anyone working in the field should monitor developments and recommendations as they emerge.

If we extrapolate from the weighing example, it is not surprising that uncertainty estimations derived exclusively from the bottom-up model turn ugly in a hurry. A recent paper described an uncertainty budget created for determination of pK_a values using pH titrations and identified nearly 90 contributing factors.[8] For quantitative operations such as determination of blood alcohol or percent purity of a drug seizure, the bottom-up model becomes problematic. In such situations, the top-down model combined with a simplified cause-and-effect diagram is a good alternative; Figure 8 shows an example derived from the process of measuring breath alcohol concentrations (BrAC) using a calibrated instrument.[9] Three major factors contribute to the uncertainty—the sampling event, the instrument and its use, and the calibration. Calibration is accomplished using traceable NIST reference standards consisting of ethanol in water and a device that simulates an exhalation into the device. This information provides the starting place for optimization of the method and construction of the uncertainty budget. In this example, the laboratory utilized the traceable standards, duplicate samples taken from individuals, and a coverage factor (k) of 2.58. The latter was derived from historical data from more than 90,000 sample collection events. The relationship revealed between uncertainty and concentration is shown in Figure 9.

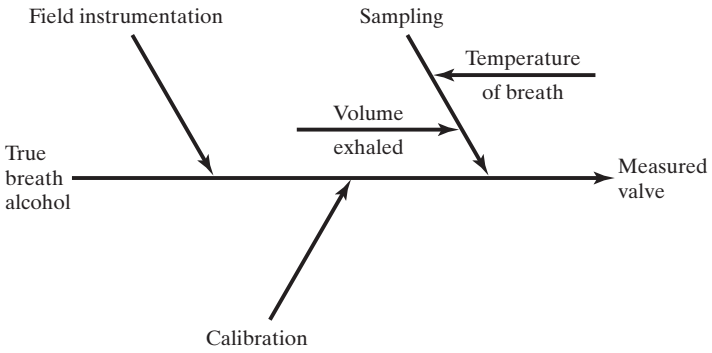

FIGURE 8 A cause-and-effect diagram for breath alcohol measurements. Reproduced with permission from Gullberg, R. G. 2006. "Estimating the measurement uncertainty in forensic breath-alcohol analysis." *Accreditation and Quality Assurance* no. 11 (11):562–568. doi: 10.1007/s00769-006-0176-y. Copyright 2006 Springer Publications.

APPLYING THE SCIENCE 1

Sampling and Method Uncertainty in Urine

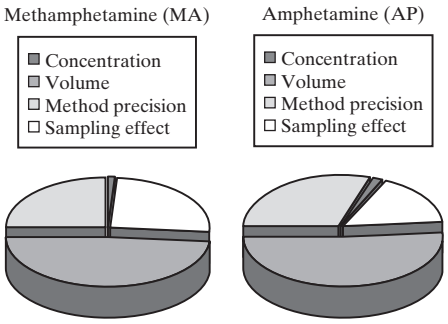

FIGURE A. Cause-and-effect diagram for the quantitation of methamphetamine and amphetamine in urine. Reproduced with permission from Lee, S., H. Choi, E. Kim, H. Chung, and K. H. Chung. 2010. "Estimation of the Measurement Uncertainty by the Bottom-Up Approach for the Determination of Methamphetamine and Amphetamine in Urine." *Journal of Analytical Toxicology* no. 34 (4): 222–228. Copyright Preston Publications, 2010.

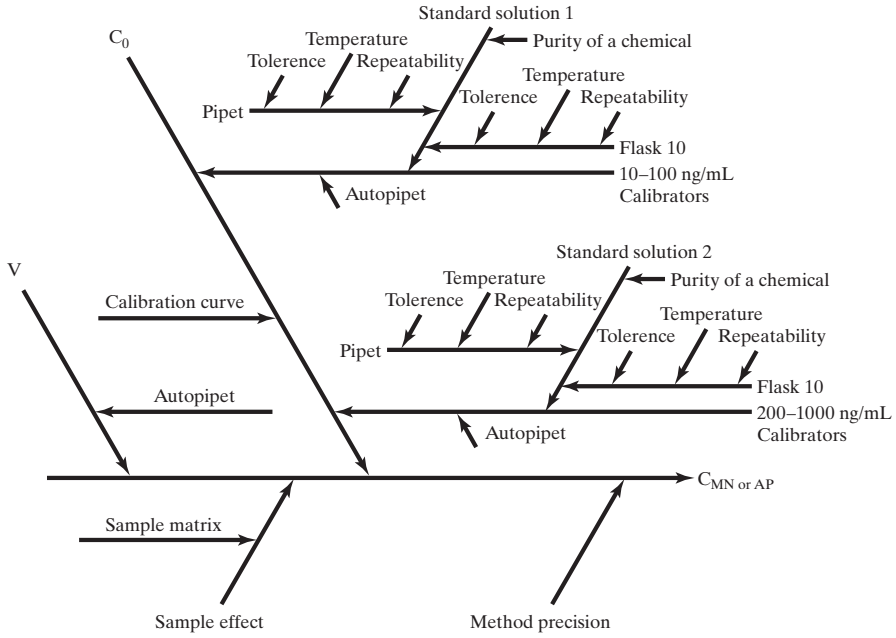

FIGURE B. Contributions to overall uncertainty in this assay. Reproduced with permission from Lee, S., H. Choi, E. Kim, H. Chung, and K. H. Chung. 2010. "Estimation of the Measurement Uncertainty by the Bottom-Up Approach for the Determination of Methamphetamine and Amphetamine in Urine." *Journal of Analytical Toxicology* no. 34 (4):222–228. Copyright Preston Publications, 2010.

Urine is a common sample matrix in forensic toxicology. Although not as complex as blood, urine is still a challenging material, and inevitably, this sample matrix contributes to the overall uncertainty of the measurement according to the equation that opened this chapter:

$$U_{total} = U_{sample\ \&\ sampling} + U_{analysis}$$

A recent study examined these uncertainties in assays designed to detect methamphetamine (MA) and amphetamine (AP). The analysis involved a solid phase extraction followed by derivatization and GC/MS analysis using an internal standard method for quantitation. The authors started with a GUM-type bottom-up model to estimate method uncertainty and used a repeatability measurement to capture and combine several individual contributors. The results are shown in the bar chart. Notice the significant contribution to uncertainty that arises from the sample matrix.

Source: Lee, S., H. Choi, E. Kim, H. Chung, and K. H. Chung. "Estimation of the Measurement Uncertainty by the Bottom-Up Approach for the Determination of Methamphetamine and Amphetamine in Urine." *Journal of Analytical Toxicology* 34, no. 4 (2010): 222–28.

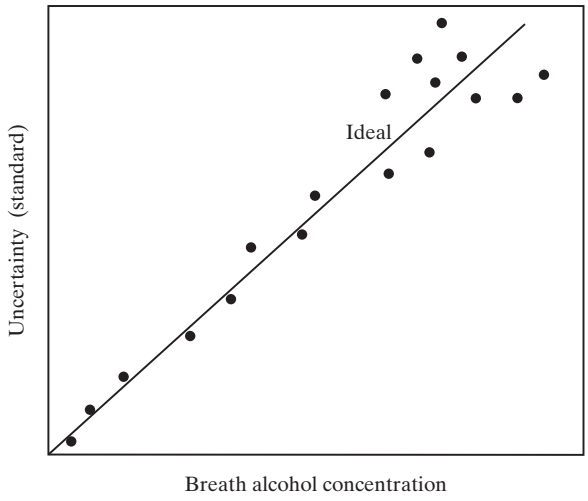

FIGURE 9 The uncertainty estimates of breath alcohol as a function of concentration. Adapted with permission from Gullberg, R. G. 2006. "Estimating the measurement uncertainty in forensic breath-alcohol analysis." *Accreditation and Quality Assurance* no. 11 (11):562–568. doi: 10.1007/s00769-006-0176-y. Copyright 2006 Springer Publications.

2 SAMPLING AND SAMPLING PLANS

The previous section described in detail how we can estimate the uncertainties associated with analytical operations, but as noted at the start of the chapter, the uncertainty of the process also includes a contribution to the uncertainty from sampling. This value is extraordinarily difficult to quantify and varies from case to case. Therefore, sampling must be undertaken with the same thought and care as any estimation of uncertainty, and it starts with development of a sampling plan. Devising a defensible and reasonable sampling plan can be difficult when the number of exhibits is large. In the steroid seizure depicted in Figure 10, there appear to be several different types of evidence within the seizure. In the Ecstasy pill case shown in Figure 11, there are

Oklahoma State Bureau of Investigation

FIGURE 10 A large steroid seizure. Image courtesy of the Oklahoma State Bureau of Investigation.

two different colors of tablets, some light blue and some light green (although the difference is difficult to discern in a grayscale image). Because it is impractical to test every single item in these seizures, the goal becomes to devise a method of obtaining a representative sample of the whole. The methods that have been and currently are being used in forensic science (principally in seized drug analysis) are summarized in Figure 12.

At its most basic, the purpose of sampling is to reduce the bulk mass (total mass) of the material that has to be characterized down to a manageable level for analysis. The parent population containing N items (in Figure 12, $N = 100$) must be selectively sampled to produce n items, and the characteristics of the subset n should be as close a representation of the parent population as possible. This concept is illustrated in Figure 13. In a simple case, where the evidence consists of one small bag of a white powder, all that is needed is a thorough homogenization of the contents of the bag before a subsample is taken. In cases such as those illustrated in Figure 10 and 11, the situation is more complicated. In these instances, the first step would be to sort the evidence according to physical appearance, based on the reasonable assumption that

Oklahoma State Bureau of Investigation

FIGURE 11 A large MDMA (Ecstasy) seizure. Image courtesy of the Oklahoma State Bureau of Investigation.

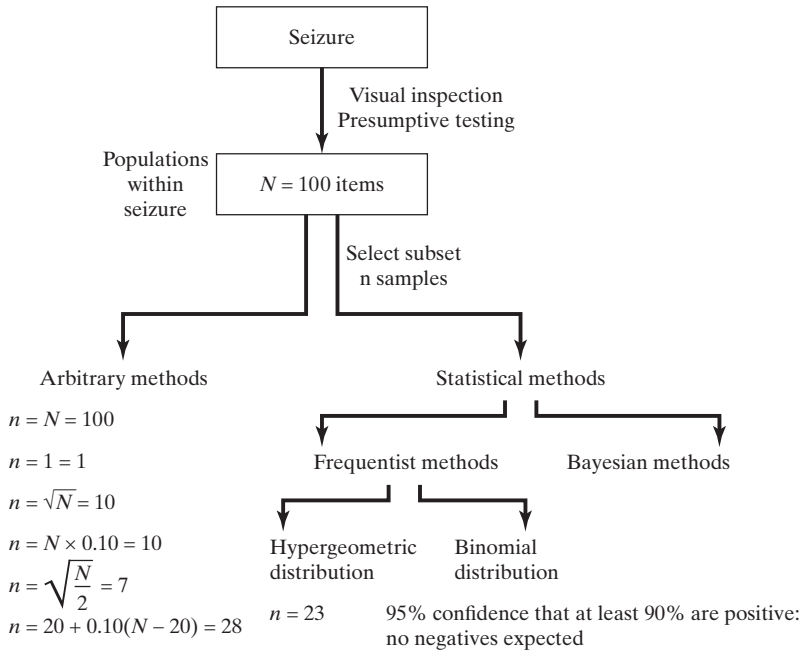

FIGURE 12 A summary of sampling methods in forensic chemistry.

EXHIBIT A

Statistics and Probability

Although the terms ***statistics*** and ***probability*** are often used interchangeably, they refer to different concepts. Statistics are based on an existing data set. Based on knowledge of random populations and the Gaussian distribution, patterns observed in the smaller data set are extrapolated to the overall population. An example is the use of the sample standard deviation *s* as a starting point for determining a confidence interval. Thus, the application of statistics is an inductive process wherein specific knowledge is extrapolated and applied generally. By contrast, probability is deductive and moves from general knowledge to a specific application. Describing the probability that a coin toss will yield three tails starts with the general knowledge that any one coin toss can give either a head or a tail. However, what statistics and probability do have in common is that they both describe uncertainty in an outcome. A coin-toss question can be phrased in terms of the odds; the odds that three coin tosses in a row will produce three tails is 1 in 8, meaning that, given a fair toss, one can expect three tails $(1/2)^3$ or three heads 1 time in 8, or 12.5% of the time. Nonetheless, that does not mean that if one replicates the three-toss experiment eight times, it is assured that one sequence will be three heads or three tails.

Source: Aitken, C. G. G., and F. Taroni. *Statistics and the Evaluation of Evidence for Forensic Scientists,* 2d ed. Chichester, U.K.: John Wiley and Sons, 2004, p. 6.

the substances in dropper bottles (Figure 10) represent a different parent population than the sealed vials. Similarly, it would be reasonable to assume that the light green tablets in Figure 11 are different from the blue tablets. A laboratory might also select to apply presumptive tests to the exhibits to further identify different parent populations. Once the parent populations are assigned, the sampling plan can be designed and implemented. Other factors that must be considered in the forensic setting are threshold weights and jurisdictional requirements. It makes no sense to test 10,000 kg of white powder to identify it as cocaine if the maximum threshold weight for sentencing is 1000 kg.

Until the early 1990s, sampling plans used in forensic settings were rarely based on statistics and were often referred to as arbitrary methods.[10] These are shown at the left

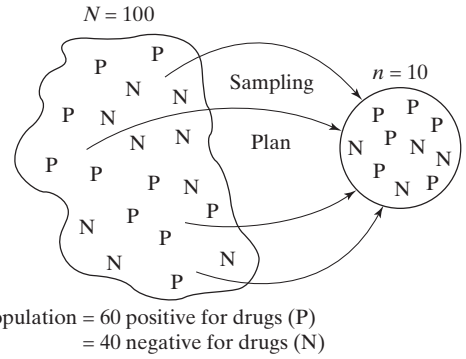

FIGURE 13 Creating a sample is an exercise in mass reduction. The subset should ideally represent the parent population perfectly. Deviations from this ideal generate uncertainty.

of Figure 12, which uses a 100-item parent population as the example. The arbitrary methods ranged from an extreme of $n = 100$ (test everything) to testing only one item, with several variations between. More recently, international working groups such as the European Network of Forensic Science Institutes or ENFSI[10] (also adopted by the United Nations Office of Drug and Crime, UNODC) and SWGDRUG[11] have developed statistically based sampling guidelines for seized drug analysis.† The statistical methods can be further divided into *frequentist* methods (based on the normal distribution and related statistics) and *Bayesian* methods, which invoke prior probabilities and likelihood ratios. We will focus on the frequentist methods, specifically on the hypergeometric model, since it seems to be gaining favor in the forensic community. If you are interested in exploring the other models (Bayesian and binomial), consult the articles and resources listed in the chapter references.

Another critical facet of sampling[12] is collecting **random samples**, or samples that are equally likely to be selected. This is to avoid bias, intentional or otherwise, that could influence the results of the analysis. Suppose, for example, that an analyst decides to sample 28 items from the 100-item parent population used in Figure 12. Which 28 should be selected? To be valid, the sampling must be random, meaning that every item in the 100 population has an equal probability of selection. Manual or arbitrary selection by the analyst is not the best option, because there is no guarantee of unbiased selection. An alternative is to use a random number generation scheme based on Excel or similar software. A recent paper suggested the use of the Excel function INT(NxRAND() +1), where N is the size of the parent population.[13] The function INT rounds the result of the calculation down to the nearest whole number, while the command inside the parentheses generates a single random number between 1 and N. The value of N (here 100) is used, not the symbol. Thus, if the function was executed as =INT(100xRAND() +1), a random number such as 88 might be returned. Sample number 88 would be selected as part of the 28 and the calculation repeated, except with N=99 and so on until 28 random samples were obtained. If the items in the case are not numbered originally, the laboratory could assign numbers and record these in the case file.

EXHIBIT B

The Dreyfus Case

Alphonse Bertillon (French, 1853–1914), a pioneer in forensic science, was involved in an early case that employed probability-based arguments. Alfred Dreyfus was a French officer accused of selling military secrets. A document he admitted writing was seized as evidence against him. Using a grid, Bertillon analyzed the document and determined that 4 polysyllabic words out of 26 were positioned identically relative to the grid lines. Bertillon argued that such coincidence of positioning was highly unlikely with normal handwriting. Arguments were also made concerning the normal frequency of occurrence of letters in typical French versus those found in the questioned document. Subsequent analysis of the case and testimony showed that Bertillon's arguments were incorrect.

Source: Aitken, C. G. G., and F. Taroni. *Statistics and the Evaluation of Evidence for Forensic Scientists,* 2d ed. Chichester, U.K.: Wiley, 2004, sec. 2.

2.1 The Hypergeometric Model

The **hypergeometric probability distribution** is useful for qualitative sampling in which the forensic question has a binary response, such as, does this sample contain a controlled substance or not? The situation would be more complicated if a seizure contained more than one type of controlled substance, but for the sake of this discussion, the simple positive/negative case will be assumed. The hypergeometric distribution provides a method to select a subset of samples based on the number of positives needed or desired along with associated probabilities. In this model, the probability of success is described by

$$\Pr(X = i) = \frac{\binom{M}{i}\binom{N - M}{n - i}}{\binom{M}{n}} \tag{8}$$

where N is the number of objects in the entire population, M is the number of exhibits that contain a controlled substance, and i is the number of items selected from the population (the sample or subset). In this notation, X is the generic symbol for the number of items selected from the population over several iterations (individually, i), and the variable X follows the hypergeometric distribution. For each experiment or collection of i samples, the experiment follows the sampling-without-replacement model, meaning that once an item is taken from the population and placed in the subset, it is not returned to the larger population. Note that in this type of expression, the terms inside of the parentheses are not mathematical expressions but rather expressions of combinations. For example, the denominator in eq. 8 expresses the number of combinations of M items taken n items at a time. Factorials are needed to evaluate these expressions, but as we will see, we can escape this using spreadsheet functions.

We start with a simple and common example, a standard deck of playing cards. If you were to draw 10 cards at random from the shuffled deck, what is the probability that you would get all four of the aces? Not very good, as it turns out:

$$\Pr = \frac{\binom{4}{4}\binom{52 - 10}{10 - 4}}{\binom{52}{10}} \tag{9}$$

In the denominator, the expression indicates how many ways there are to select 10 cards from a deck of 52. Put another way, this term expresses how many different ways there are to sample a deck of 52 cards 10 cards at a time. This value can be calculated using factorials to obtain a value of 1.58×10^{10}, or more than 10 billion possible combinations of 10 cards from a deck of 52. In the numerator, the first term refers to the number of possible combinations of four aces from a deck that contains four aces, or 1, and the second term refers to the number of ways that exist to select 4 cards that are not aces. Although this calculation can be done manually, since it requires the use of factorials, a spreadsheet or similar application is desirable. One of the easiest to use is the "HYPGEOMDIST" function in Excel, which takes as input the number of desired positives, the number of units taken as a sample, the number of positives in the sample, and the size of the population. Using this function here, =HYPGEOMDIST(4,10,4,52) yields a probability of 7.76×10^{-4}, or less than a 0.1% chance that 4 of the 10 cards drawn will

be aces. This could also be stated as odds, here about 1 chance in 1289. If the experiment is repeated with a full shuffled deck and 26 cards are drawn, the probability is 5.5% of getting four aces, or 1 chance in 18. At 50 cards drawn, the probability exceeds 85% or roughly 1 in 1.2 odds.

To extend this calculation to a forensic example, suppose a laboratory receives a seizure consisting of 800 white tablets, all outwardly similar in appearance, meaning that an initial assumption can be made that all are part of the same parent population. Suppose that half of these tablets are pure methamphetamine and half are lactose tablets, although the analyst does not know this. The analyst selects 10% of the tablets (80) at random for testing using one of the arbitrary methods shown in Figure 12. What is the probability that half of these (40) are methamphetamine? The calculation yields a probability of 9.4%. In other words, there is about a 1 in 10 chance that the composition of the sub-sample taken will represent the parent population. This means that the odds of collecting a representative sample from the population are poor if only 10% are tested.

Although such exercises are valuable for illustration and learning, they are not directly applicable to casework in which the analyst often has no idea of the actual composition of the exhibits and no reliable estimate of the number of positives embedded in the population. ENFSI has published a validated Excel workbook (available online) that contains an application of the hypergeometric distribution (among others) to sampling problems associated with large seizures.[14] To apply this approach, the analyst makes an initial assumption about the number of positives or negatives in a seizure and uses data gathered to evaluate the validity of that assumption. Returning to Figure 12, assume that the analyst expects that at least 90% of the 100-item parent population are positive for a controlled substance and want to be able to report findings with a 95% confidence. If those values are inserted into the ENFSI worksheet (with no expected negatives), a sample size of 23 is returned. This result is not dramatically different from many of the values produced by the arbitrary methods; however, this approach allows for quantitative reporting of the confidence of the sample selection and results.

The hypergeometric distribution approach is particularly useful with large seizures. Returning to the 800-tablet example, assume now that the analyst does not know if any, all, or some portion of the seizure contains controlled substances. Based on other similar seizures, the analyst makes an initial assumption that 90% are positive and that no negatives are expected to be found in a subset sample. The analyst wants to know the minimum sample size that must be taken to be 95% confident that the seizure does indeed contain 90% positives. Using the ENFSI spreadsheet and these variables, the analyst obtains a result of 28 samples. If the analyst tests these 28 and obtains positive results for all of them, then he or she can be 95% confident that 90% of the pills in the seizure are positive.

The hypergeometric model is flexible and can be used to respond to data as necessary. Suppose the analyst collects 28 tablets at random and performs a series of color tests on each one. Of these, 26 are positive for a controlled substance, and 2 are negative. This means that the initial assumptions about the population need to be modified. Again, using the ENFSI spreadsheet, the analyst adjusts the number of expected negatives to 2. Now, the confidence level drops to about 55%, and the number of samples required jumps to 59. The analyst has two choices: first, take more samples, or second, adjust the expectation of the number of positives downward until 28 samples provides the desired 95% confidence. This occurs when the population contains 79% positives. Which choice is made will depend on many factors such as threshold weights or numbers, jurisdictional considerations, and laboratory procedures. Whichever path is taken, the sampling plan can now be accompanied with confidence levels, and the number of samples taken can be adjusted based on analytical results. This is clearly a significant improvement over earlier arbitrary approaches.

EXAMPLE PROBLEM 1

A seizure of 369 1-kg bricks of a tan powder is received in a crime laboratory. All the bricks are carefully weighed, and it is determined that all weigh 1.00 kg ± 0.05 kg. The prosecuting attorney explains to the analyst that the penalty is more severe if controlled substances are found in the amount of more than 100.0 kg. Using the hypergeometric mean approach, devise a reasonable and defensible sampling plan.

Answer:

Enough samples must be tested to be sure that the amount of controlled substance found, if any, conclusively exceeds or does not exceed the 100.0-kg threshold. First, take into account the uncertainties of the weights. The worst possible case would be if every brick selected had a low weight. If so, more would have to be sampled to ensure that if all were found to contain the same controlled substance, the weight exceeded 100 kg. The determinative equation is

$$\frac{100\ \text{kg}}{0.995\ \text{kg/brick}} = 100.53\ \text{bricks}$$

Accordingly, to ensure that the weight threshold is exceeded, at least 101 of the bricks must be found to contain the same controlled substance. By contrast, 369 − 101 = 268 bricks must be shown to contain no controlled substance to ensure that the threshold is not exceeded.

The Excel hypergeometric mean function was used to generate the following table:

Initial Random Sample	No. Positives Observed	Population Positives	% Chance	Odds: 1 in …
10	10	10	9E-18	1E + 19
10	10	50	9E-08	1E + 09
10	10	75	7E-06	1E + 07
10	10	101	2E-04	6E + 05
10	10	150	0.01	9478
10	10	250	2	51
10	10	300	13	8
10	10	350	60	2
10	10	368	100	1.0

Here it is assumed that the analyst decided to take 10 samples at random and test them completely. Suppose all test positive for a controlled substance and were the same as far as the analysis showed. What are the odds that at least 101 would then be positive? The first line of the table shows that if there were 10 positives in the entire population of 369 kilos, the odds of selecting those 10 at random are about 1 in 10^{19}. At the other extreme, if 350 of the 369 are positives, there is nearly an 60% chance that the first 10 selected at random will test positive. Assume that 101 are positive. If 10 samples are drawn from the entire 369 at random and are then found positive, that would be expected to occur only once in approximately 60,000 tries.

What does the latter statement mean for the analyst? If he or she samples 10, analyzes them, and finds that all are positive, then there is only 1 chance in 60,000 that fewer than 101 kilos contain the controlled substance. The advantage of using the spreadsheet function is that it allows for a "what if?" approach. Once the formulas are set up, the values for the initial random sample and other variables can be adjusted in accordance with different scenarios.

APPLYING THE SCIENCE 2

Sample Selection for Weighing

The weights of exhibits are an important issue in some cases, and in large seizures it may be impractical to weigh each sample, just as it is impractical to test all the samples. The problem is that total weights must be reported to a reasonable degree of certainty. In Israel, the solution has been both legislative and scientific. The law defines the sampling protocol to be used, assuming that the samples all appear similar: According to this protocol, the sample size (n) from the total population (N) depends on the population size: 5 of a population up to 15 units, 6 between 16 and 50 units, and 7 if the population exceeds 51 units. Each randomly selected sample is weighed on a balance that is linked to a computer that calculates the mean S and a 95%CI for the weights, based on the weights of the random subset. The acceptance criterion is

$$\frac{1.96 \times 0.1}{\overline{X}} \times 100 \approx \ \leq 153$$

where 0.1 is the uncertainty expected for the mean weight. If the calculated value exceeds 15%, the analyst weighs more exhibits until all are weighed or the acceptance criterion is satisfied. As an example, if the mean was 3.0 g and the associated uncertainty at that weight was 0.3 g, the calculation would be $((1.96 \times 0.3)/3.0) \times 100$, or 19.6 %. If this was the case, the examiner would have to weigh more exhibits.

To test the procedure, the laboratory evaluated approximately 1500 seized units in different ways to compare the estimated weights with the actual ones. The findings showed that for about 88% of the exhibits the actual weight was within the 95% CI, and a potential bias in sampling procedure was noted. Original estimates were lower than the true weight by approximately 0.7%, suggesting that the selection of individual exhibits for *n* may not be completely random or representative. The authors offered computerized random selection as a way to eliminate any analyst bias.

Source: Azoury, M., et al. "Evaluation of a Sampling Procedure for Heroin Street Doses." *Journal of Forensic Sciences* 43 (1998): 1202–7.

Summary

Both uncertainty estimation and sampling are evolving concepts in metrology and forensic chemistry. The information presented in this chapter represents current thinking in the community coupled with current practice. However, many aspects remain to be studied and refined. Those interested or working in the field need to stay abreast of developments as they emerge. We have seen that there are multiple valid approaches for both sampling and estimating uncertainty. The goal in all cases is the thoughtful implementation of reasonable and defensible estimations and sampling plans. This mindset must be accompanied by honest reporting with sufficient detail for interpretation.

Key Terms and Concepts

Bottom-up model
Cause-and-effect diagram
Combined standard uncertainty
Coverage factor (k)
Expanded uncertainty (U)
GUM
Joint Committee for Guides in Metrology (JCGM)
Random sample
Readability of a balance
Rectangular distribution
Standard uncertainty (u)
Threshold weight
Top-down model
Triangular distribution
Type A contribution
Type B distribution
Uncertainty budget

Problems

FROM THE CHAPTER

1. Review the propagation of uncertainty calculations. When the uncertainty is derived as the square root of the sum of squares, what implicit assumption is being made regarding the individual contributors and their distributions? What problems might this assumption present?
2. How is the propagation of error method similar to the GUM approach? How is it different?
3. Forensic laboratories that adapt the GUM model tend to favor the top-down concept over the bottom-up for quantitative analyses such as determination of blood alcohol concentrations. Why?
4. In the example presented in Figure 8, which factor would you expect to have the largest contribution to the overall estimated uncertainty and why?
5. A microanalytical balance is purchased by a forensic laboratory. The analyst in charge is asked to develop a SOP and control chart to use as the basis for estimating uncertainty. The working range of the balance is 0.1 mg to 10 g. Based on the control chart, current calibration report, and manufacturer's literature, the analyst identifies three key contributing factors to the uncertainty:

   Drift of the balance reading over time: 0.0002 g (rectangular distribution); repeatability, 0.0009 g (normally distributed) and obtained from a control chart; and readability in the last displayed decimal of 0.0001 g (rectangular distribution). What would be the expanded uncertainty with coverage factors of k=2 and k=3? Show your work using a table or spreadsheet and identify any insignificant contributions.
6. The same lab as in the previous question takes excellent care of the balance, calibrates it regularly, and uses a series of control charts to obtain repeatability values for different mass ranges. One challenge is reporting the uncertainty of small seizures of heroin, which often have weights on the order of 20 mg. This is in the lower working range of the balance. If you have not already done so, create the chart and determine from it the value for the repeatability. Redo the uncertainty budget with this value and comment on what changes. Hint: Watch units.
7. Using the hypergeometric function in Excel, calculate the following probabilities and report as a percentage as a "1 in X" odds
   a. Drawing the four playing cards from a shuffled deck and getting all aces
   b. Dividing a full shuffled deck in half and having all four queens in one pile
   c. Dividing a full shuffled deck into four piles and having one of these piles containing all the cards from one suite.
8. Agents of the Drug Enforcement Administration seize 5000 1-kg "bricks" containing a light tan powder that is suspected to contain heroin. Historically, such seizures are all pure heroin. Outwardly, all the packages appear similar.
   a. Use the ENFSI spreadsheet to select the number of samples that need to be collected and tested for the DEA to be 90% confident that at least 90% of the packages contain heroin. To start with, assume no negatives.
   b. This number of samples is collected and a presumptive test for heroin applied; 2 negatives are identified. If this sampling is representative of the population, what are the predicted percent negatives in the seizure?
   c. What is the confidence level now that 90% of the seizure is positive for heroin?
   d. How many samples must now be taken to be 90% confident that 90% are heroin?

FOOD FOR THOUGHT

1. This chapter focused on uncertainty of quantitative measurements. Do qualitative measurements such as a color test for blood, have an associated uncertainty? How would you estimate it quantitatively?

Suggested resources:

- *Milman, B. L. "Identification of chemical compounds." Trac-Trends in Analytical Chemistry 24, no. 6 (2005): 493–508.*
- *Rios, A., and H. Tellez. "Reliability of binary analytical responses." Trac-Trends in Analytical Chemistry 24, no. 6 (2005): 509–15.*
- *Trullols, E., I. Ruisanchez, F. X. Rius, and J. Huguet. "Validation of qualitative methods of analysis that use control samples." Trac-Trends in Analytical Chemistry 24, no. 6 (2005): 516–24.*

2. For the buoyancy issue in weighing, the statement was made that the conservative approach was appropriate to the forensic laboratory. What does this mean in this case?
3. If you had to design an uncertainty budget term for readability of a bathroom scale, what distribution would you select and why?

INTEGRATIVE

1. How could the GUM method for estimating uncertainty be utilized to improve method validation?
2. A seizure of methamphetamine consists of 100 packets of the powder. The percent purity of each is shown in Table , although the analysts do not know this. After proving that all 100 contain methamphetamine, they select each packet with a number divisible by 10

(#10, #20, etc.) and perform a quantitative analysis. Assuming that the laboratory results closely approximate the true values, answer the following:

a. Is this a random sample?
b. Is the 10-unit subset representative of the whole? Justify your conclusion with 95% confidence and explain your rationale for the approach you selected.
c. Express the bias between μ and the experimental mean as a % error.
d. Assume that rather than selecting 10% of the seizure for testing, they elected to use the hypergeometric mean as a starting point. How many samples are needed for 95% confidence that 90% of the samples are methamphetamine?
e. The laboratory rounds this number upward to a logical value for sampling purposes and collects this number of samples by starting at Exhibit 1 and selected samples evenly spaced analogous to the method used to select 10%. All of these samples test positive for methamphetamine. The laboratory quantitates these samples. Repeat b) and c) with this new sample population (n).
f. Comment on the results. Note that the hypergeometric approach is designed for qualitative sampling only, not quantitative. Incorporate this in your answer.

Table 1: Complete seizure

Exhibit #	%purity	Exhibit #	%purity	Exhibit #	%purity	Exhibit #	%purity
1	24.0	26	10.4	51	23.1	76	29.4
2	17.1	27	21.9	52	11.0	77	25.5
3	27.2	28	29.7	53	22.7	78	11.3
4	19.3	29	13.8	54	15.1	79	28.4
5	11.6	**30**	**25.2**	55	27.7	**80**	**33.7**
6	33.9	31	20.6	56	12.0	81	14.2
7	24.2	32	33.3	57	13.2	82	28.7
8	16.3	33	34.2	58	24.0	83	23.6
9	20.6	34	17.1	59	10.0	84	30.2
10	**33.7**	35	25.6	**60**	**18.0**	85	10.9
11	28.2	36	19.4	61	17.4	86	11.5
12	15.6	37	19.2	62	20.9	87	33.1
13	32.8	38	12.4	63	16.3	88	12.5
14	25.3	39	30.0	64	17.1	89	13.7
15	16.6	**40**	**20.0**	65	16.5	**90**	**16.9**
16	28.3	41	27.8	66	30.0	91	16.0
17	24.5	42	23.1	67	19.4	92	27.9
18	15.7	43	21.1	68	18.6	93	20.8
19	13.0	44	21.3	69	15.4	94	15.0
20	**32.6**	45	25.3	**70**	**21.5**	95	26.3
21	21.2	46	18.9	71	15.6	96	22.1
22	15.3	47	22.5	72	28.7	97	30.6
23	20.6	48	18.1	73	17.0	98	23.9
24	32.2	49	24.0	74	31.1	99	13.0
25	10.3	**50**	**22.8**	75	15.1	**100**	**21.9**

Online Resources

RTI International offers several free online courses for forensic scientists, including a three-part course on estimation of uncertainty. The context is forensic toxicology, but the underlying principles are the same. The URL is http://www.rti.org. Search for "forensic science education." The courses are found under forensic toxicology.

ENFSI. "Validated Software Tool of Qualitative Sampling." European Network of Forensic Science Institutes. http://www.enfsi.eu/page.php?uid=48.

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Chemical Fundamentals: Partitioning, Equilibria, and Acid–Base Chemistry

OVERVIEW AND ORIENTATION

Forensic chemistry is first and foremost chemistry and thus relies on fundamental principles of chemical science. In this chapter, we will examine, review, and extend your knowledge in two foundational areas—partitioning and acid–base character. We will take a different approach to acid–base chemistry, that of viewing acidic or basic sites on a molecule as ionizable centers. The concept takes some getting used to but once grasped, becomes a primary tool in the forensic chemistry toolbox. Before tackling this topic, we will explore some fundamentals of partitioning and equilibria, which will come into play later in the chapter when we discuss acidic and basic drugs as well as thin-layer chromatography. These principles are also key to sample preparation in many forensic applications.

For partitioning to occur, a phase boundary must exist; that is,

$$\text{Analyte}_{p_1} \longleftrightarrow \text{Analyte}_{p_2} \qquad \textbf{(1)}$$

where p_1 and p_2 refer to different phases. The two phases may be insoluble liquids (i.e., water and hexane) or a boundary between a solid and a liquid or a liquid and a gas. Partitioning occurs because the analyte has a greater affinity for one phase over the other, owing to charges, polarity, and other chemical properties. In many instances, **Le Châtelier's principle** is invoked to drive the equilibrium to one side or the other; the more complete this process, the more efficient is the separation. As we will see, the manipulation of equilibrium conditions is a cornerstone of extraction and partitioning.

Partitioning is also at the heart of chromatography, which is used in forensic chemistry for sample preparation, cleanup, screening tests, and as a first step in hyphenated instruments such as GC/MS and LC/MS. Here, we will review chromatographic separations and discuss thin-layer chromatography (TLC) in some detail.

1 GENERAL CONSIDERATIONS

The majority of forensic chemistry focuses on organic analytes such as drugs, poisons, and polymers. Inorganic analytes and metals are less common but still important in areas such as gunshot residue, glass, and heavy-metal poisons. In general, samples are prepared for inorganic analysis through the utilization of acid **digestions**, which are effective at isolating elemental components but which destroy the organic and biological components present in the matrix. Indeed, that is the goal: to attack and destroy everything but the metals or other inorganics of interest. In contrast, organic extractions pluck the analytes from the matrix (Figure 1). Aggressive techniques destroy most molecular compounds and so are rarely used in preparing organics. However, there are types of evidence, such as hair or insects, in which aggressive techniques are needed. Because these matrices are protein-rich, enzymes are incorporated into the sample preparation scheme to digest proteins and other large biomolecules.

For qualitative analysis, simple preparations and cleanups are generally adequate. If quantitative analysis is needed, rigorous techniques, quantitative extractions, and standardization are required. In such cases, the goal is to isolate the analyte from the matrix and to transfer the analyte quantitatively to the final analytical solution. In general, quantitative analysis is the more challenging situation and requires additional quality control procedures to gauge and monitor the efficiency of extraction. Internal standards, surrogate spikes, and matrix spikes are among the tools available, all of which provide analogs for the analyte that can be tracked through the preparation of the sample. The earlier in the process the standards are added, the more realistically they will track the path of the analytes.

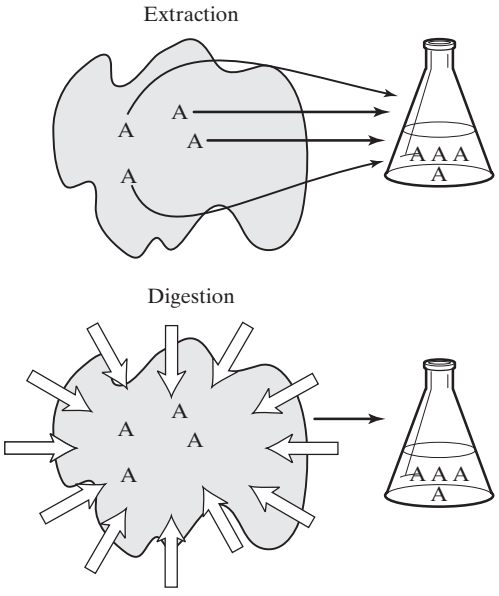


FIGURE 1 The difference between an extraction and a digestion. Most inorganic analyses are based on aggressive digestion, which destroys the matrix in which the durable metal is contained. Preparations targeting organic analytes are designed to remove (extract) the analyte (A) from the matrix. Aggressive digestions such as those with acids, heat, or microwaves would destroy the molecules of interest along with the matrix.

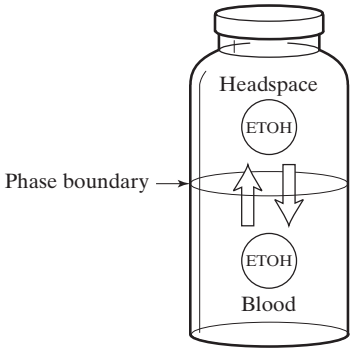


FIGURE 2 Simplified representation of the phase boundary and distribution equilibrium of ethanol in blood.

2 AN INTRODUCTORY EXAMPLE

Two conditions must be met to extract an analyte from a matrix. First, there must be an exploitable difference in a chemical or physical property between the matrix and the analyte. Second, there must be an equilibrium condition that can be manipulated (Equation 1). Consider a sample preparation protocol based on differences in volatility. A headspace method can be used to determine blood alcohol concentration. The premise underlying the extraction is a difference in volatility between ethanol and the aqueous biological matrix of blood. The system is illustrated in Figure 2. The equilibrium at the requisite phase boundary is

$$\text{ethanol(aq)} \longleftrightarrow \text{ethanol(g)} \tag{2}$$

As shown in Table 1, there is an exploitable difference in a physical property: The difference in volatility between ethanol and the matrix is sufficient to effect the desired separation and extract the ethanol from the matrix. Blood is thicker than water (literally as well as figuratively), and the components within blood do have different volatilities than water, but for first approximations, equating the vapor pressure of blood with that of water is reasonable.

A small volume of blood is placed in a sealed container that has headspace above the surface of the sample. In a **headspace** analysis, **Henry's law** is exploited. This law states that the partial pressure of the analyte above a liquid is proportional to its concentration in the liquid:

$$\underbrace{P_{A,g} \overset{K_H}{\Longleftrightarrow} [A]_{aq};}_{\text{equilibrium to exploit}} \qquad K_H = \frac{[A]_{aq}}{P_{A,g}}; \qquad K_H * P_{A,g} = [A]_{aq} \tag{3}$$

The partial pressure of ethanol above the blood, which is determined analytically by gas chromatographic analysis, can be directly related to the concentration of alcohol in the blood as long as the Henry's law constant (the **equilibrium constant** K_H) is known. This law is the basis of many presumptive breath alcohol tests performed in the field. When applied in a portable testing method, the phase boundary between liquid and gas is in the lungs. The concentration of alcohol in the breath can be related through Henry's law to the concentration of alcohol in the blood, which in turn is related to the degree of intoxication. For the blood–air system, K_H is approximately 2300, meaning that the concentration of alcohol in the blood is about 2300 times as great as it is in the headspace above. Field instruments used to estimate alcohol intoxication detect the quantity of alcohol in exhaled breath. Blood alcohol concentration is estimated using equation 3, K_H, and by accounting for temperature. However, laboratory analyses

TABLE 1 Relative Volatility of Ethanol and Water: An Exploitable Difference

	Vapor Pressure (atm)		
Temperature (°C)	Ethanol	Water	Difference
29	0.10	0.04	2.5
78	0.99	0.43	2.3

Source: Handbook of Chemistry and Physics, 84th ed. Boca Raton, FL: CRC Press, 2004.

rely not on K_H directly, but on calibration curves and internal standards.

Like all equilibrium constants, K_H depends on temperature:

$$\text{ethanol(aq)} + \text{energy} \longleftrightarrow \text{ethanol(g)}^{\circ}\Delta H = (+) \quad \textbf{(4)}$$

According to Le Châtelier's principle, if the equilibrium is disturbed, the system will respond. In effect, heating the sample is like adding a reactant, favoring the products. As long as the calibration standards are analyzed under the same experimental conditions, reliable quantitation is possible. The manipulation of equilibrium conditions is at the heart of most organic sample preparations.

Even in this straightforward example, there are caveats. As the blood is heated, water and any other volatile constituents will be driven inevitably into the headspace along with the ethanol. Reducing the heat reduces the water content, concomitantly reducing the ethanol concentration. Even under mild heating, other blood gases and volatiles will be introduced into the headspace, along with other compounds that might be present, such as acetaldehyde (a by-product of alcohol metabolism) and acetone. This situation is illustrated in Figure 4. A trade-off is made, the analytical compromise between sample and matrix that is always necessary and, fortunately, almost always manageable. In our blood alcohol example, it is not necessary that every last molecule

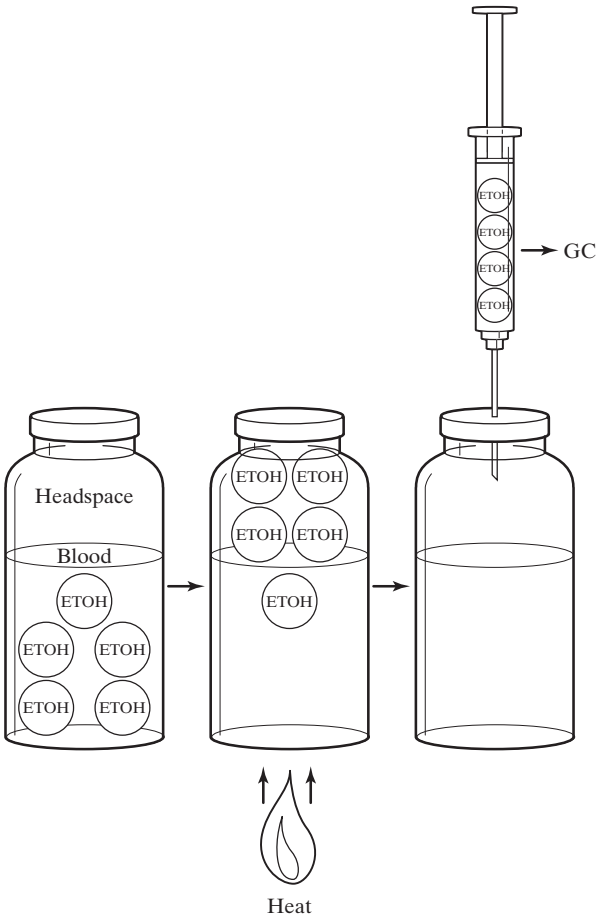


FIGURE 3 The process of driving ethanol out of the aqueous (blood) phase into the gas phase, using gentle heating. The headspace above the blood is sampled, and the air, now enriched with ethanol, is transferred to a GC for quantitative analysis

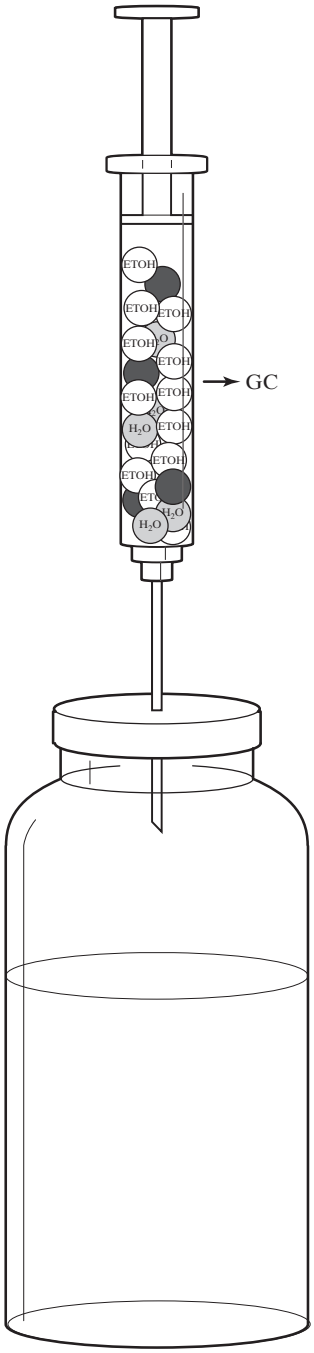


FIGURE 4 A more realistic picture of the headspace sample. The heating will drive other substances—notably, blood gases, water, acetone, and so on—out of the blood sample. The separation is never complete, and these potential interferents must be accounted for and dealt with in subsequent analytical steps.

of ethanol be transferred to the vapor phase. Rather, all that is essential is that sufficient amounts be transferred and that the transfer be reproducible. This need is met using validated analytical methods.

Even under optimal conditions, ethanol will not be the only component of blood that is transferred to the vapor phase. No separation is 100% complete, nor does it have to be. As long as enough of the analyte is separated and the separation is reproducible, it can be used qualitatively and quantitatively. In the foregoing example, the chromatographic column in the gas chromatograph will be capable of sorting out the most common potential interferents from the ethanol. This is another reason to use validated methods, since common interferents are accounted for and their effects understood. The worst-case scenario, in which unexpected interferents coelute with ethanol, can still be mitigated by selecting the proper detector and implementing various types of quality assurance and quality control measures. Regardless, the analytical scheme is still fundamentally dependent on sample preparation and calibration.

3 THE SPECIAL *K*'S

3.1 Equilibrium Constants

The example in the previous section illustrates the importance of equilibrium in partitioning and separation processes. Before delving into specific sample preparation considerations, a review of the more important of these in the forensic context is worthwhile. The generic expression of equilibrium for a reaction $aA + bB \longleftrightarrow cC + dD$ is

$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b} \quad \textbf{(5)}$$

This equation is a ratio of concentrations of products to concentrations of reactants, and some generalizations can be made. First, no matter what type of reaction is being studied, the form of the equation and the underlying equilibrium principles apply. The subscript on *K* denotes the type of reaction, such as acid ($\boldsymbol{K_a}$), base ($\boldsymbol{K_b}$), or dissolution ($\boldsymbol{K_{SP}}$), but equilibrium is equilibrium. This is a good point to keep in mind when facing seemingly complex systems, such as the dissociation of diprotic acids or competing equilibria. The generic equation 5 applies to *any* equilibrium. A second generalization is that relative values of *K* describe the balance of products to reactants. The ratio of the numerator to the denominator in equation 5 determines whether products or reactants predominate when the system is at equilibrium.

For example, a small *K* value results when a small number is divided by a large number:

$$K_{eq} = \frac{1}{10} = \frac{\text{small}}{\text{large}} = 0.1 \rightarrow \text{reactants "win"} \quad \textbf{(6)}$$

In contrast, a large *K* value results when a large number is divided by a small number:

$$K_{eq} = \frac{10}{1} = \frac{\text{large}}{\text{small}} = 10 \rightarrow \text{products "win"} \quad \textbf{(7)}$$

Thus, if a K value is large relative to others in the same series (acid strength, water solubility, etc.), then the reaction favors the products. Certainly, any coefficients and exponents cannot be ignored, but the ability to glance at a K value and translate it to a likely chemical result is helpful.

EXAMPLE PROBLEM 1 Acid and Base Calculations

a. Rate the following acids from strong to weak on the basis of their pK_a values:
Phenylbutazone $pK_a = 4.40$
Ascorbic acid $pK_a = 4.10$
Arsenous acid (H_3AsO_3) $pK_a = 9.29$

Answer:

The pK_a value is defined as the negative of the logarithm of K_a for the reaction $HA \longleftrightarrow H^+ + A^-$. The larger the K_a value, the stronger is the acid, as illustrated in equations 6 and 7. However, because of the negative sign, a large K_a corresponds to a small pK_a. In this example, ascorbic acid (vitamin C) is the strongest acid and arsenous acid is the weakest. The same logic applies to any such constant including K_b and K_{SP}.

b. Calculate the pH of a 2.00 M aqueous solution of methamphetamine (in the free base form), a basic drug with a pK_a of 9.8. (*Note:* This is a bit of a simplification—we will learn more about drugs and solubility later in the chapter).

Answer:

This problem requires that the equilibrium constant be expressed in terms of the base dissociation $B \longleftrightarrow BH^+ + OH^-$. With that value in hand, the calculation is straightforward.

$$pK_a = 9.8\text{: } K_a = 1 \times 10^{-9.8} \longrightarrow K_a = 1.58 \times 10^{-10}$$

$$K_w = K_a \times K_b\text{, so } K_b = \frac{1.00 \times 10^{-14}}{1.58 \times 10^{-10}} = 6.33 \times 10^{-5}$$

Generically:	$B \rightleftharpoons$	$BH^+ +$	OH^-
initially	2.00 M	–	–
equilibrium	$2.00 - x$	x	x

2.00 M $\gg$ than x because the K_b is relatively small (a weak base), so it can be ignored.

$$K_b = \frac{[BH^+][BH^-]}{[B]} = \frac{x^2}{2.00} = 6.33 \times 10^{-5}$$

$$x = 0.0113 \text{ M} = [OH^-]$$

$$pOH = -\log[OH^-] = 1.95 \approx 2$$

$$pH + pOH = 14\text{, so } pH = 12.0$$

c. Calculate the pH of a 0.0025 M solution of methamphetamine (in the free base form).

Answer:

The only change from the previous calculation is that the dissociation can no longer be ignored, and the quadratic equation must be used to calculate $[OH^-]$.

As with the previous calculation:

	B	$\rightleftharpoons$	$BH^+ + OH^-$
initial 0.0025 M			– –
equilibrium 0.0025 – x			x x

A rule of thumb is that if the difference between the initial concentration of a base is within ~ ± 10^3 of the K_b, the loss from the initial concentrate should not be ignored.

$$\frac{x^2}{0.0025 - x} = 6.33 \times 10^{-5}\text{: use the quadratic equation.}$$

$$x^2 = 6.33 \times 10^{-5}\,(0.0025 - x)$$

$$x^2 + 6.33 \times 10^{-5}x - 1.58 \times 10^{-7} \qquad x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

(a, b, c mark the coefficients)

$$x = 3.67 \times 10^{-4} = [OH^-];\ \text{pH} = 10.6$$

Equilibrium constants are often presented as **p*K***, values where p means "–log of", just as pH = $-\log[H^+]$. The negative sign has the effect of turning the relationships presented in equations 6 and 7 upside down; a large p*K* translates to a small *K* and reactants favored, a small p*K* to a large *K* and products favored:

$$K_{eq} = \frac{1}{10} = \frac{\text{small}}{\text{large}} = 0.1 \longrightarrow \text{p}K = -\log[0.1] = -(-1) = 1 \qquad \textbf{(8)}$$

$$K_{eq} = \frac{10}{1} = \frac{\text{large}}{\text{small}} = 10 \longrightarrow \text{p}K = -\log[10] = -1 \qquad \textbf{(9)}$$

Again, these relationships are approximations, but useful ones.

3.2 Solubility Equilibrium Constant K_{SP}

The **solubility** of drugs and other solids determines whether they are stable in the aqueous phase. For drugs, water solubility is critical. The unique aspect of solubility equilibrium is that since one component is a solid, it is not expressed, because there is no aqueous concentration. The solubility is referred to as *S* and is obtained as shown in Example Problem 2.

The solubility of salts is an important criterion in sample preparation, as is the ionic character of drugs and larger molecules. One of the techniques used to increase the solubility of a drug is to convert the drug from an insoluble form to a soluble salt (more on this subject shortly). Common drug salts include sodium, calcium, sulfates, chlorides, tartrates, citrates, and lactates. The pH also plays a critical role in the water solubility of drugs and will be examined in detail momentarily. Soluble compounds are characterized as **hydrophilic** or **lipophobic**, for reasons to be discussed next.

EXAMPLE PROBLEM 2 K_{SP}

Calculate the solubility (S) of the solid Ag_2CO_3 with a K_{SP} of 8.1×10^{-12}.

Answers:

The first step is to write the equation of the dissolution:

$$Ag_2CO_3(s) \longrightarrow 2\,Ag^+(aq) + CO_3^{2-}(aq)\ K_{SP} = [Ag^+]^2[CO_3^{2-}]$$

The solubility (S) of the compound is calculated by setting the carbonate concentration to S and the silver ion concentration to $2S$:

$$S(2S)^2 = 8.1 \times 10^{-12} = 4S^3;\ S = 1.27 \times 10^{-4}\ \text{mole/L}$$

We use the symbol S to specify solubility, rather than the generic x notation used in other types of equilibrium calculations.

EXHIBIT A

Analytical Separations

Without separations chemistry, it is hard to have any meaningful analytical chemistry. Historically, one of the driving forces behind the development of analytical separations was the need to isolate precious metals such as gold and silver from base metals. Separations in turn require dissolution, a task that was not possible until the thirteenth century, when hydrochloric and nitric acids were created. This period also saw the introduction of *aqua regia*, a combination of these two acids that will dissolve gold. The interaction of acids with metals was put to use to detect counterfeiting. For example, when nitric acid reacts with copper, the copper dissolves and forms a distinctive green-colored complex. If a coin of "pure gold" showed a green color when nitric acid was dropped on it, the claim was false. This is an example of an early color-based presumptive test.

Source: Salzberg, H. W. "Chapter 5: Medieval and Renaissance European Artisans," in H. W. Salzberg, *From Caveman to Chemist: Circumstances and Achievements*. Washington, DC: American Chemical Society, 1991.

The K_{SP} relationship can also be used to predict when a solid will begin to form. In the preceding example problem, the equilibrium expression is

$$[Ag^+]^2[CO_3^{2-}] = 8.1 \times 10^{-12} \quad \textbf{(10)}$$

Under nonequilibrium conditions, we can still calculate the quantity $4S^3$, but the quantity is referred to generically as Q. Q is calculated using the same mathematical relationship as in the K_{SP} expression, but Q applies to nonequilibrium conditions.

Suppose you have a solution containing silver at a concentration of 1.0×10^{-5}M and start adding a solution containing carbonate ion. At first, nothing happens, but eventually, the white carbonate solid forms. This occurs when $Q > K_{SP}$. With our silver solution example, we can calculate that this will occur when the concentration of carbonate exceeds 0.081 M:

$$[1.0 \times 10^{-5}]^2[CO_3^{2-}] \geq 8.1 \times 10^{-12} \quad \textbf{(11)}$$

$$[CO_3^{2-}] \geq \frac{8.1 \times 10^{-12}}{[1.0 \times 10^{-5}]^2} \quad \textbf{(12)}$$

$$[CO_3^{2-}] \geq 0.081\ \text{M} \quad \textbf{(13)}$$

Another important factor in solubility and precipitation reactions is the **common ion effect**. In the preceding example with carbonate, we could limit the solubility of the silver salt by adding carbonate anion to the solution. The presence of this common ion will force the equilibrium toward the solid and reduce its solubility. For example, suppose the concentration of carbonate is adjusted to be 0.20 M in solution:

$$[Ag^+]^2[CO_3{}^{2-}] = 4S^3 = 8.1 \times 10^{-12} \quad \textbf{(14)}$$

$$[Ag^+]^2[0.20] = 8.1 \times 10^{-12} \quad \textbf{(15)}$$

$$[Ag^+]^2 = 4S^2 = 4.05 \times 10^{-11} \quad \textbf{(16)}$$

Now, $S = 3.18 \times 10^{-6}$ instead of 1.27×10^{-4}, as calculated in the example problem, where no common ion was present. Solubility is decreased because of the presence of the common ion carbonate. The common ion effect plays an important role in drug solubility, as we will soon see.

EXAMPLE PROBLEM 3

The solubility for the drug codeine hydrochloride $C_{18}H_{21}NO_3$ ¥ HCl is listed as 1 in 20 in water. Estimate the K_{SP} of this salt form of codeine.

To solve this problem, we have to convert the solubility into molarity. If 1 part of the drug is soluble in 20 parts of water, we can restate this as 1 g in 20 g of water, and for a first pass approximation we will assume that the density of water is 1.0 g/mL, and 20 g of water is equivalent to 20 mL. The solubility (S) of the salt can be estimated

$$\frac{1\,\text{g}}{20\ \text{mL}} = \frac{1\,\text{g}}{0.020\ \text{L}} = \frac{1\,\text{g}/(335.8\,\text{g/mol})}{0.020\ \text{L}} \approx 0.15\,\text{M}$$

Generically, we can write the dissolution as

$$\text{B¥HCl} \longrightarrow BH^+ + Cl^-$$

The equilibrium expression is thus

$$K_{SP} = S^2 = 0.15^2 = 2.2 \times 10^{-2}$$

Accordingly, a reasonable estimate for the K_{SP} of the drug salt is 0.02.

3.3 Octanol–Water Partition Coefficient K_{ow} (log P)

For drugs, solubility in fat is as important as solubility in water. Since drugs must cross a lipid membrane to enter a cell, solubility in octanol, a lipophilic alcohol, is often used to estimate fat solubility (**lipophilicity**). K_{ow} is the octanol–water partition coefficient and is calculated exactly as one would expect. This expression can be generalized as

$$P = \frac{[A_{org}]}{[A_{aq}]} \quad \textbf{(17)}$$

where P is the partition coefficient and the organic phase is octanol.[1] The value log P is an important descriptor for drugs.

Compounds with significant solubility in the organic phase are characterized as **hydrophobic** or **lipophilic**.

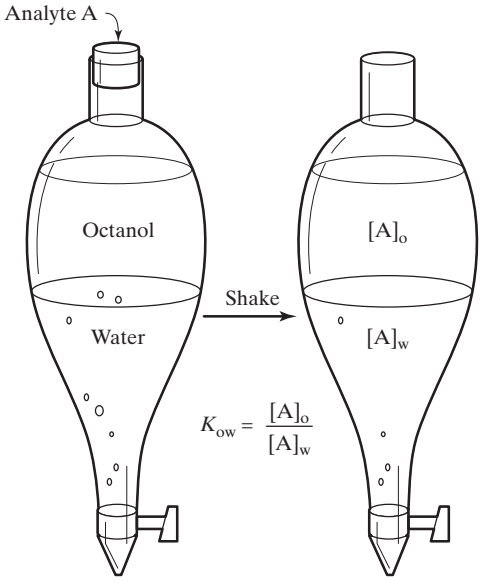


FIGURE 5 Determination of the octanol–water partition coefficient K_{ow} provides an estimate of lipid solubility.

3.4 Partition Coefficients

A partition coefficient K is established between two phases and an analyte that does not undergo any chemical changes when moving between one phase and the other. The use of headspace techniques to extract ethanol from blood is an example of a partition coefficient, defined here as K_H for the Henry's law constant. Ethanol undergoes a phase change, but not a chemical one, an important distinction. A liquid–liquid extraction using water and octanol is a partitioning as well. The analyte does not change chemically during the movement between the aqueous and organic phases. The generic form of the equilibrium constant for a partitioning is K_D, where "D" stands for distribution.

The value of K_D depends on the **relative affinity** of an analyte for each phase. The basis of that affinity may be any of the following properties:

- volatility (as discussed before in the blood alcohol example)
- **polarity (like dissolves like)**
- hydrogen-bonding interactions
- **ion–ion interactions**
- **ion–dipole interactions**
- **dipole–dipole interactions**

The concepts of distribution, partitioning, and relative affinity are central to chromatography and chromatographic separations. This topic will be discussed in detail in Section 5.

3.5 K_a/K_b

Acid–base chemistry plays a central role in drug chemistry and thus in drug analysis, toxicology, and sample preparation. Indeed, drugs are often classified as acidic, basic, or neutral. The functionalities in drugs that define their classes are amino groups (bases), phenolic groups (acids), and carboxyl groups (acids), all of which are weak acids or bases. It is not unusual for a drug molecule to have more than one acid or base group, each of which is called an **ionizable center**. In general, when ionized, a drug

$$HA \rightleftharpoons H^+ + A^-;\ K_a$$

$$K_a = \frac{[H^+][A^-]}{[HA]};\ \text{solve for } [H^+]$$

$$[H^+] = \frac{K_a[HA]}{[A^-]} = K_a\left(\frac{[HA]}{[A^-]}\right)$$

$$-\log[H^+] = -\log K_a + -\log\frac{[HA]}{[A^-]}$$

$$pH = pK_a - \log\frac{[HA]}{[A^-]}$$

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

$$pH = pK_a + \log\frac{[\text{ionized}]}{[\text{un-ionized}]}$$

FIGURE 6 Derivation of relationships used with monoprotic acid sites.

$$B \xrightarrow{H_2O} BH^+ + OH^-;\ K_b$$

$$BH^+ \rightleftharpoons B + H^+;\ K_a$$

$$K_a = \frac{[B][H^+]}{[BH^+]};\ \text{solve for } [H^+]$$

$$[H^+] = \frac{K_a[BH^+]}{[B]} = K_a\left(\frac{[BH^+]}{[B]}\right)$$

$$-\log[H^+] = -\log K_a + -\log\frac{[BH^+]}{[B]}$$

$$pH = pK_a - \log\frac{[BH^+]}{[B]}$$

$$pH = pK_a + \log\frac{[B]}{[BH^+]}$$

$$pH = pK_a + \log\frac{[\text{un-ionized}]}{[\text{ionized}]}$$

FIGURE 7 Derivation for large molecules with a single basic site. These molecules are called **monobasic acids** to indicate that the equilibrium is written as an acid dissociation.

molecule (with one ionizable center) is soluble in water; when un-ionized, it is soluble in solvents such as octanol and chloroform. Accordingly, acid–base character is intimately related to solubility.

The key equations and expressions used in the forensic context are summarized in Figures 6 and 7. For drugs or other large molecules with a single weak-acid site, the relationship between pH and pK_a is derived using the **Henderson–Hasselbalch equation**. For large molecules with a single weak base (B), it is convenient to express the equilibrium in terms of the conjugate acid BH^+. Convenient, yes, but somewhat confusing; it is important to keep this convention in mind as we discuss drug chemistry. A basic drug when added to water acts like a base and will make a solution basic. An acidic drug makes a solution acidic. We characterize both in terms of their ionizable center. Once the equilibrium is stated this way, all basic drugs may be described in terms of K_a. Thus, a drug's K_a value alone is not sufficient to define the drug as an acid or a base; knowledge of the structure and functional groups at the ionization centers is also needed.

The expressions shown in Figures 6 and 7 are useful for designing extractions. In general, equilibrium is considered to lie completely to one side or the other if the concentration of the product substance is at least 100 times greater than that of the reactants, or vice versa.† In terms of the two equations presented, this corresponds to ratios of 100/1 or 1/100, depending on which form is desired. The corresponding logarithmic values are ±2 units. If an analyst desires to extract an acid drug into chloroform, for example, the drug must be in the un-ionized HA form. Suppose the drug has a pK_a of 3.5. To force the equilibrium to the fully protonated form and to a point such that [HA] exceeds $[A^-]$ by a factor of 100, the pH of the solution would need to be

$$pH = 3.5 + \log\frac{[A^-]}{[HA]} = 3.5 + \log\frac{[1]}{[100]} = 3.5 + (-2) = 1.5 \qquad \textbf{(18)}$$

This makes sense; to favor protonation, the concentration of protons in the solution should be high (the common ion effect) in order to drive the reaction toward the reactant HA. The more acidic the solution, the less the HA will dissociate. Analogous arguments and calculations can be made for setting the pH to extract a basic solution.

EXAMPLE PROBLEM 4 Simple Liquid–Liquid Extraction pH and pK_a

Propose a liquid–liquid extraction (LLE) scheme to separate a sample containing naproxen sodium (an analgesic) and codeine as the hydrochloride salt. Assume that both of these drug salts are completely soluble in water.

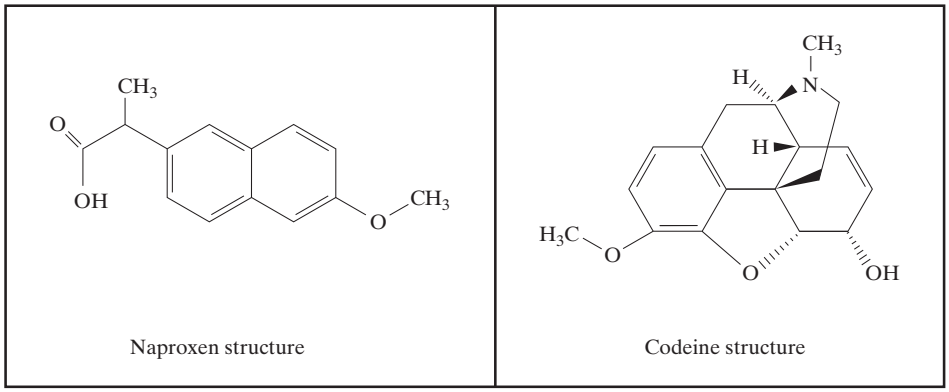
Answer:

The first step in designing a separation is to obtain the necessary solubility data and pK_a values. *Clarke's Handbook* and the *Merck Index* are two possible sources. A check of *Clarke's* Volume 2 provides the following data:

†You may recall this same approach from discussions of indicators used in titrations. Acid–base indicators change color over a range of 2 pH units; the reasoning is the same as described here.

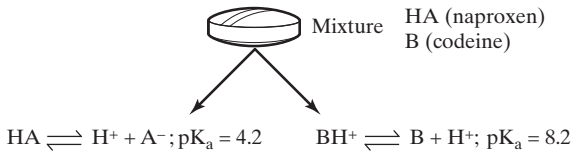
Naproxen: pK_a = 4.2; log P = 3.2 Solubility of naproxen: insoluble in water, soluble 1 in 25 in ethanol, 1 in 15 in chloroform, and 1 in 40 in ether.

Codeine: pK_a = 8.2; log P = 0.6. The HCl salt is soluble 1 in 20 in water, 1 in 180 in ethanol, and 1 in 800 in chloroform.



Naproxen structure | Codeine structure

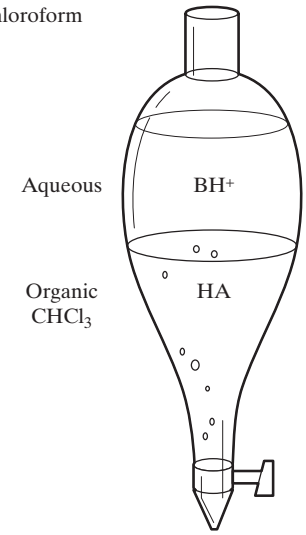
The exploitable differences here are, first, the pK_a values and, second, solubility. Naproxen has an acidic ionization center and codeine has a basic ionizable center, affording relatively clean separation, as shown below:



① Dissolve in dilute phosphoric acid such that the pH is approximately 2. This will drive the acid toward the neutral protonated HA form and the base toward the protonated ionized form (BH^+).

② Add chloroform

③ Isolate the individual layers



Dilute phosphoric acid is a good choice in this case because phosphate salts tend to be less soluble in organic solvents such as chloroform than are the corresponding hydrochloride salts. However, as we are about to see, the solubility of the salt form of the drug can be a critical consideration depending on the context.

In general, the un-ionized form of a drug is lipophilic and will partition preferrentially into the organic phase with relative polarities of the solvent and un-ionized form of the drug dictating the extent of solubility.

4 PARTITIONING

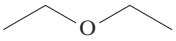
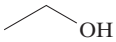
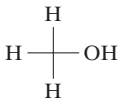
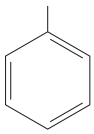
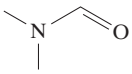
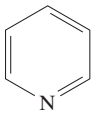
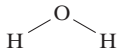
4.1 Partitioning with Liquid Phases: Solvent and Liquid–Liquid Extractions

Solubility is a function of relative polarities and is the basis of many separation techniques. In some cases, the separation is clean and simple, such as when a water-soluble drug like cocaine hydrochloride is cut (diluted) with an insoluble substance like cornstarch. It would be nice if that were the norm, but it rarely is in forensic laboratories. Simple or complex, "like dissolves like" is exploited to great effect in many forensic sample preparations. In the simplest case, an organic solvent such as chloroform is added to white powder to selectively extract drugs from diluents such as sugars. This procedure is referred to as a **dry extraction.** The next level of complexity is a liquid–liquid extraction (LLE) in which an analyte is separated from one liquid and transferred into another by partitioning. It should come as no surprise that the pH of the aqueous phase is an important consideration even in such simple procedures. The solvents used for these tasks in forensic chemistry include the usual suspects, shown in Table 2. From the table, a solvent can be selected that is suitable for dissolution of the target analyte solute. If the analyte is ionic or polar, water is an ideal choice for many reasons; if the analyte is nonionic or nonpolar, a solvent such as hexane or pentane would be a reasonable choice, with a caveat or two.

Other practical considerations are involved in selecting a solvent. Principal among these are safety and exposure concerns. Versatile solvents such as benzene, carbon tetrachloride, and the trichloroethanes are rarely used owing to safety concerns. The use of chloroform and methylene chloride has also been reduced. The cost of disposal, often proportional to the safety risks, must also be weighed in laboratories that may consume liters of solvents weekly. The cost of the solvents is always an issue, and price increases with purity. It is not surprising that water is used whenever feasible. Finally, miscibility must be taken into account. There is no use attempting to separate analytes on the basis of their relative solubilities in ethanol and water, since ethanol itself is soluble in water. Most solvent separations are based on an aqueous phase and on solvents such as hexane, methylene chloride, or chloroform, which are insoluble in water. By comparing miscibility and polarity, an analyst can select candidate solvents for a separation LLE. If the analyte is appreciably more soluble in one liquid than in the other (100 $\times$ or more), partitioning can be successful. The relative densities of the solvents allow the analyst to predict which layer is which in a separatory funnel or similar vessel.

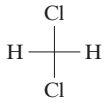
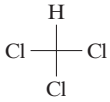
We saw an example of LLE applied to a sample containing drugs in Example Problem 4. Here, pH adjustments of the aqueous phase were exploited to effect an essentially complete separation of the drugs into two phases. There are many variations of LLE methodology. For example, suppose our drug mix, in addition to containing the acidic and basic drugs, also contained a neutral drug such as meprobamate. This drug does not have an ionizable center. It is soluble in water (~1 in 240), ~1 in 7 in ethanol, and about 1 in 80 in chloroform. This would complicate the separation somewhat, but it could still be done. First, we could add an excess of chloroform to the powder containing

TABLE 2 Characteristics of Selected Solvents

Name	Structure	Molecular Weight	Density	Vapor Pressure	Dipole Moment	Polarity	Relative Water Solubility
n-Pentane		72.15	0.626	420	0.00	0.0	0.04
Petroleum ether[a]	NA	NA	0.640	variable	NA	0.1	I
Hexane		86.18	0.659	124	0.08	0.1	0.01
Diethyl ether		74.12	0.713	442	1.15	2.8	6.89
Acetonitrile (methyl cyanide)		41.05	0.782	89	3.44	5.8	M
Isopropanol (2-propanol)		60.10	0.785	32	1.66	3.9	M
Ethanol		46.07	0.789	44	1.66		M
Acetone		58.08	0.790	185	2.69	5.1	M
Methanol		32.04	0.791	97	2.87	5.1	M
Toluene		92.14	0.867	29	0.31	2.4	0.05
N,N-Dimethyl formamide (DMF)		103.12	0.949	3	3.86	6.4	M
Pyridine		79.10	0.983	18	2.37	5.3	M
Water		18.02	1.000	18	1.87	10.2	NA

(*continued*)

TABLE 2 Characteristics of Selected Solvents (*continued*)

Name	Structure	Molecular Weight	Density	Vapor Pressure	Dipole Moment	Polarity	Relative Water Solubility
Methylene chloride (Dichloromethane)		84.93	1.326	350	1.14	3.1	1.60
Chloroform (Trichloromethane)		119.38	1.489	158	1.15	4.1	0.82

Sources: Handbook of Chemistry and Physics, 84th ed. Boca Raton, FL: CRC Press, 2004. *The Merck Index*, 13th ed. Whitehouse Station, NJ: Merck and Co., 2001. "Solvent Information Website: Solvent Properties," Burdick and Jackson. Downloaded January 25, 2004. www.bandj.com/BJSolvents/BJProperties.html. Snyder, L. R. "Classification of the Solvent Properties of Common Liquids." *Journal of Chromatography*, 92 (1974).

Note: NA = not applicable; M = miscible

[a]Petroleum ether ("pet ether") is a mixture of hydrocarbons formulated to have a boiling point between 35 and 80°C. Pet ether contains no ethers. It is also called naptha, benzin, or petroleum benzin. Since the mixture varies, constants depend on the batch and the manufacturer.

all three, enough to exceed the 1:80 limit for meprobamate. All three drugs would be in the organic phase. At this point, water (pH ~ 2) could be added and the process continued as described in the example problem. The neutral drug would stay in the chloroform. If it became necessary to isolate the HA from the neutral drug, it would be necessary to drive the HA (naproxen) to the A− form. Once the chloroform was isolated, it could be transferred to another separatory funnel and water with a pH of > 6.2 could be added. The A− form of naproxen would partition preferentially into the aqueous phase.

It is important to realize that LLE methods, while simple and effective for many applications, are not always the best method for an extraction or sample preparation. For rapid preparations where qualitative data (i.e., identification) is the primary goal, LLE is often a good choice. For more exacting applications, such as quantitation, other methods such as solid phase extraction can be a better choice. However, before delving into this topic, we need to learn a bit more about drugs and drug solubility.

4.2 Water Solubility and Partitioning

With forensic analyses of toxicological and drug evidence, water solubility of the analytes is of paramount concern for sample preparation and extraction. Solubility is also important toxicologically, since it plays a role in determining how, where, and how quickly a drug is absorbed. The like-dissolves-like rule still applies but is broadened to include acid–base character and solubility of salts.

Neutral molecules with no significant acid–base character are soluble in organic solvents on the basis of polarity and according to like dissolves like. Salts such as cocaine hydrochloride are soluble in water according to their K_{SP}. However, once the hydrochloride salt dissolves, solubility of drug (cocaine will be used as an example here) is pH dependent. In general, drugs or their salts must be water soluble, since the body is an aqueous system. However, in most cases, a drug is best absorbed through lipid membranes (such as cell walls) when it is in its most

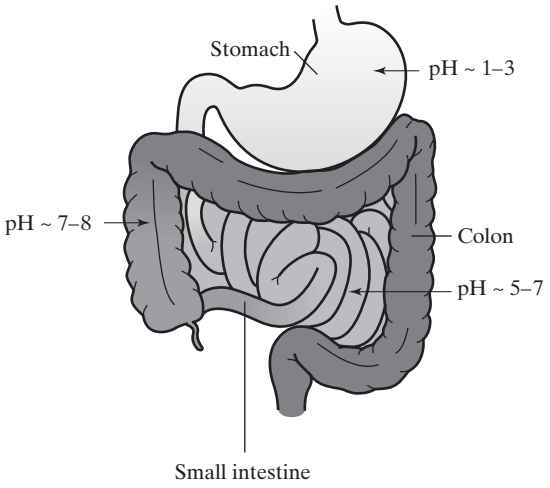
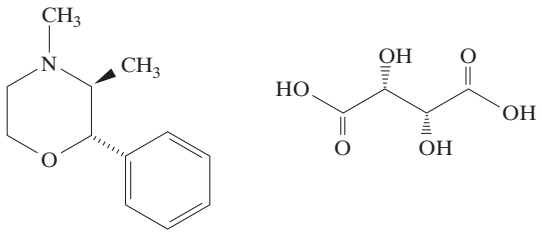


FIGURE 8 Approximate pH values at various locations in the digestive tract.

lipophilic (un-ionized) form. As seen in Figure 8, the pH values in the digestive tract range from acidic to basic, and these differences are important in determining where a drug is likely to be soluble as well as where it is likely to be absorbed. If cocaine hydrochloride is swallowed, it dissolves to form the protonated cation (BH^+ or cocaine-H^+) and chloride ion, a process that is described via K_{SP}. Cocaine is a weak base by virtue of an amine group, so its pH will dictate its further solubility. At more acidic pH values, the amine group will tend to protonate and be charged. If charged, it is an ion and water soluble. Under more basic conditions, the amine group will tend to deprotonate and lose charge. It then becomes a molecular compound with solubility dependent on polarity and related factors. As these characteristics are so fundamental to drug analysis and drug chemistry, we will address them in detail in the following sections.

EXAMPLE PROBLEM 5 Where Drugs Are Absorbed

In what locations of the digestive tract will the drug phendimetrazine tartrate (pK_a of phendimetrazine = 7.6) be most water soluble? Where is it likely to be absorbed?



Phendimetrazine tartrate

Answer:

In general, a drug is most soluble in the digestive tract where it is most likely to be in the ionized form. The tartrate salt indicates that the drug is basic, a fact confirmed by examining its structure. A check of *Clarke's Handbook* reveals that the tartrate is freely soluble in water and thus in the digestive tract. Water solubility will be favored when the drug is in the ionized state, which, for a base, is the BH^+ form. Figure 16 is a useful reference in this type

of problem, as is equation 18. At a pH of 7.6 – 2, or 5.6, and below, the phendimetrazine will exist in the protonated ionized form, favoring dissolution. According to Figure 8, this pH exists in the stomach and portions of the small intestine. As to where the drug is likely to be absorbed, assuming typical behavior, it will be preferentially absorbed when it is in the un-ionized form, which will occur when the pH is at its most basic, here in the small intestine. However, we will see in a later section that the story is more complicated than this approximation might lead you to believe.

4.3 Ionization Centers (ionizable centers)

Many drugs and metabolites are acidic, basic, or amphoteric and can be protonated or deprotonated depending on their pH and pK_a values. An ionization center can produce a charged species and thus a water-soluble ion. Conversely, neutral molecules are soluble in organic solvents such as methylene chloride. Control and manipulation of the pH of an aqueous phase is exploited in LLEs and in **solid phase extractions** (SPEs) to maximize separation efficiency.

A significant number of drugs and metabolites have acid–base character and measurable pK_a values. Many have more than one ionizable center and some, such as morphine, are amphoteric. The ionizable groups in drugs and metabolites include carboxylic acids (COOH), phenolic protons, hydroxyls, and amine groups. A range of drugs and their acid–base characters are illustrated in Figure 9. Meprobamate, an example of a neutral drug, is insoluble in water but soluble in organic solvents according to relative polarities.

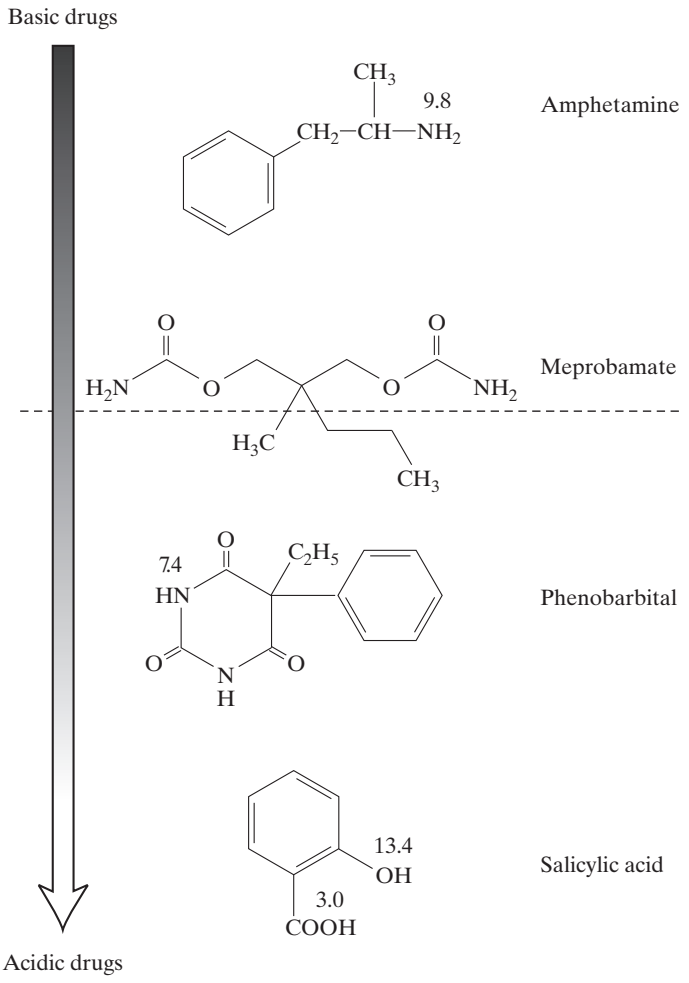


FIGURE 9 Examples of acidic, basic, and neutral drugs. The numbers above the ionization centers are the pK_a values.

For example, when amphetamine is placed in water, the amine group picks up a proton, creating a basic solution:

$$\text{amphet–NH}_2 \overset{\text{H}_2\text{O}}{\Longleftrightarrow} \text{amphet–NH}_3^+ + \text{OH}^- \qquad \textbf{(19)}$$

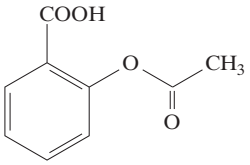


FIGURE 10 Aspirin, an acidic drug with one ionizable center, the carboxylic acid group. The pK_a of this group is 3.5.

Conversely, acetylsalicylic acid (aspirin) is an acidic drug with one ionization center, a carboxylic acid group. When aspirin is dissolved in water, the general acidic dissociation equation HA $\longrightarrow$ $H^+ + A^-$ applies.

The picture is more complicated when a molecule has more than one ionizable center. Some drugs have two, three, and even four such centers,[2] and some are amphoteric. The two acidic drugs shown in Figure 9 have two acidic proton sites each, the model for these types of molecules is H_2SO_4. The relative values of the pK_a's reflect which site will deprotonate first. The larger the K_a, the stronger the acid is; the larger the pK_a the weaker the acid. Consequently, the site with the smaller pK_a value will dissociate first. To ensure that the molecules remain protonated and neutral for extraction, the smaller pK_a is the critical one. To extract phenobarbital, a pH of 5.4 is needed, whereas an extraction of salicylic acid requires a more acidic pH of about 1. Analogous arguments apply for dibasic molecules, but again it is critical to remember that we are describing drugs in terms of ionizable centers and using pK_a. The meaning of a pK_a value in this sense is not the same for an acidic drug and a basic drug. More will be said about this shortly.

Amphoteric drugs present the greatest extraction and partitioning challenge. Consider morphine, an amphoteric drug with two ionizable centers, one an acidic phenol and one a basic amine. Selecting an extraction pH is more challenging than when a single ionization center is present because the two ionization centers, in effect, work against each other. To deprotonate the amino group, a high pH is needed. However, if the solution is too basic, the phenolic group deprotonates. Thus, it is not possible to find a pH such that the molecule will be neutral owing to the lack of charged groups. It is possible, though, to isolate a pH at which the molecule is neutral because the positive and negative charges are balanced. The di-ion is referred to as a *zwitterion* (German *zwitter* (hybrid), from *zwei* (two)). To determine the ideal pH for extraction, the average of the pK_a values is calculated and is referred to as the **isoelectric point** or **isoelectric pH (pI):**

$$\text{pI} = \frac{\text{p}K_{a_1} + \text{p}K_{a_2} \ldots \text{p}K_{a_n}}{n} \qquad \textbf{(20)}$$

Morphine

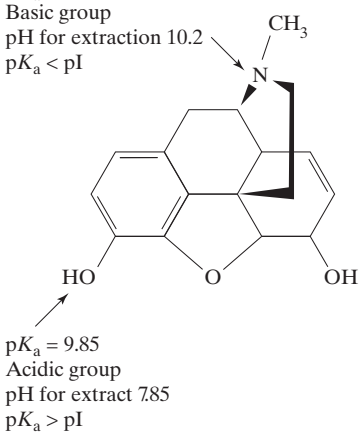


FIGURE 11 Morphine, an amphoteric drug. The pI is the isoelectric pH at which the positive and negative charges are balanced.

For morphine, the isoelectric pH is the average of 9.85 and 8.02, or 8.94. Outside of this narrow pH around 8.9, the molecule is ionized and soluble in water.

Drugs with ionization centers can exist in a variety of forms. Cocaine, with a protonated amine is a cation that can associate with chloride to form what is known as cocaine hydrochloride, a water-soluble sparkling white powder that has lent it the slang term "snow." Crack cocaine ("freebase"), the unprotonated molecule shown in Figure 12 has an oily texture and is much less water soluble than the salt. Other forms of cocaine are a nitrate dihydrate and a sulfate salt, both of which are soluble in water. Morphine can be found as the hydrochloride salt, a monohydrate, an acetate trihydrate, a tartrate trihydrate, a sulfate salt, and a pentahydrate sulfate. Many of the hydrochloride salts of drugs have degrees of solubility in some organic solvents, such as chloroform.[3] The phosphate and sulfate salts tend to be less

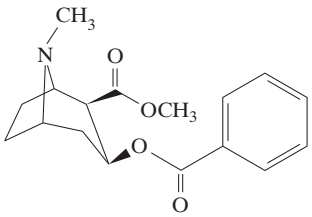


FIGURE 12 The structure of cocaine, which has one ionizable center and a pK_a of 8.6.

soluble in organic solvents, a property that can be exploited in designing separations. The use of alternative solvents is also called for under these circumstances.

4.4 Drug Solubility: More to the Story

Figure 13 provides us with critical information regarding the ionization of drugs, but ionization alone does not tell the whole story of drug solubility. In fact, with drugs that have ionizable centers, there are two solubilities that have to be considered. We have mentioned both, but now we have to clearly distinguish between the two. The **intrinsic solubility (S_0)** of a drug is defined as the solubility (in water) of the un-ionized form, be it HA or B. This in turn is a function of the structure of the molecule.

Consider the case of a basic drug such as methamphetamine (Figure 14). The intrinsic solubility of the drug is that of the base form (B), and this value is small. This is no surprise; we could predict this by the structure. The molecule contains an aromatic ring and is not particularly polar. Thus, S_0 for methamphetamine as the free base (B) is low—it is not very water soluble. However, methamphetamine hydrochloride is a powdery material that dissolves in water according to its K_{SP} value, which is relatively large. It is important to emphasize that the K_{SP} of the salt is *not* the same as the intrinsic solubility S_0. It is possible (indeed common) to have a drug with low intrinsic solubility that is bound in a salt form with high solubility. Once the salt dissolves, intrinsic solubility becomes relevant, as does degree of ionization. The case for a monacidic drug such as naproxen sodium (Figure 15) is analogous. The salt form NaA is water soluble with a large K_{SP} value. Once naproxen sodium is dissolved in an aqueous environment, the intrinsic solubility of HA becomes important. As in the case of a basic drug, this solubility is a function of pH and molecular structure.

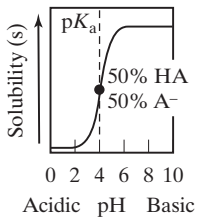
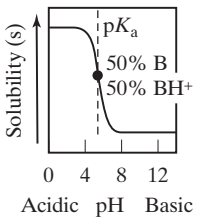
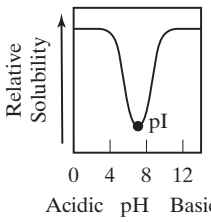
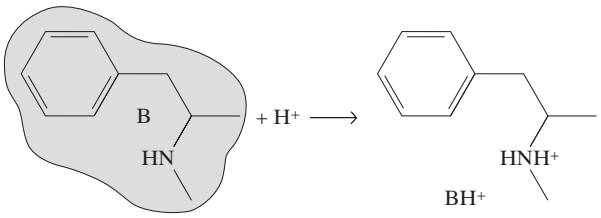
	Acidic	Basic	Amphoteric
Functional groups	– COOH carboxyl – OH hydroxyl/phenolic	—N— amine	Both
Generic equations	$HA \underset{}{\overset{K_a}{\rightleftharpoons}} H^+ + A^-$	$BH^+ \overset{K_a}{\rightleftharpoons} B + H^+$	Both
For extraction	$pH = pK_a + \log \frac{[A^-]_{aq}}{[HA]_{org}}$	$pH = pK_a + \log \frac{[B]_{org}}{[BH^+]_{aq}}$	Both
Water solubility curve			
Examples	**Compound** **pK_{a1}** **pK_{a2}** Salicylic acid 3.0 13.4 Phenobarbital 7.4 Acetaminophen 9.5	**Compound** **pK_{a1}** **pK_{a2}** Cocaine 8.6 Quinine 4.1 8.5 Amphetamine 10.1	**Compound** **pK_{a1}** **pK_{a2}** Morphine 8.02 9.85

FIGURE 13 Summary of acid–base–amphoteric solubility and extraction characteristics. Neutral molecules (not shown) are soluble in organic solvents according to like-dissolves-like characteristics. The solubility curves depict trends in water solubility as a function of pH for the three different categories. In the ionic form (A^- or BH^+), drug molecules are water soluble.

Methamphetamine, basic drug B

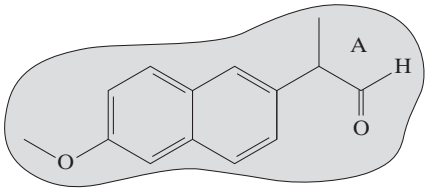


$BH^+ + Cl^- \longrightarrow B \bullet HCl$ methamphetamine hydrochloride

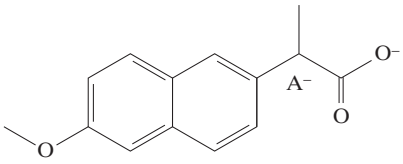
$B{\cdot}HCl(s) \rightleftharpoons BH^+_{(aq)} + Cl^-_{(aq)}$

$K_{SP} = [BH^+][Cl^-] = S^2$

FIGURE 14 Solubility and notations for a basic drug salt.



Naproxen, acidic drug



$A^- + Na^+ \longrightarrow NaA$ (naproxen sodium)

$NaA(s) \rightleftharpoons Na^+_{(aq)} + A^-_{(aq)}$

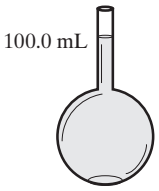
$K_{SP} = [Na^+][A^-] = S^2$

FIGURE 15 Solubility and notations for an acidic drug salt.

Keep in mind that the amount of a drug (HA or B) that is available is not the same as the amount of the salt. Cocaine • HCl is not all cocaine, and the cocaine is usually what we care about; therefore the amount of the salt weighted out must be adjusted upward according to the weight percent of cocaine in the salt. An application of this is shown in Example Problem 5. Of course, the salt must also be soluble in the solvent selected, or the calculations are for naught. If the pH of the final solution is important, there are other considerations, as we will see shortly.

EXAMPLE PROBLEM 6

A forensic toxicologist is asked to prepare 100.0 mL of a methanolic standard containing 1000.0 ppm cocaine. The lab has cocaine hydrochloride as an ultrapure solid standard. How much of the salt is needed?



1000.0 ppm cocaine
$C_{17}H_{21}NO_4$ = 303.4 g/mol

1000.0 ppm = 1000.0 mg/L × 0.100 L = 100.0 mg cocaine needed

Cocaine • HCl
$C_{17}H_{21}NO_4 \bullet HCl$
339.8 g/mol

$$\text{wt\%} = \frac{303.4 \text{ g/mol cocaine}}{339.8 \text{ g/mol cocaine} \cdot HCl} = 89.29\% \text{ cocaine in cocaine} \bullet HCl$$

$$\text{To weigh out: } \frac{100.0 \text{ mg}}{0.8929} = 112.0 \text{ mg cocaine} \bullet HCl$$

Thus, it is clear that the curves shown in Figure 13 are only part of the drug solubility story. Recall that when ionized, a drug can act as an anion (A^-) or cation (BH^+) in a salt. For an acidic drug (Figure 16), at low pH values, the drug is fully protonated (un-ionized), and the intrinsic solubility S_0 dictates how much HA will be dissolved in solution. As the pH increases, the drug deprotonates according to the relationships defined previously. The difference is that now there is a pH value at which the concentration of the ionized form of the drug becomes large enough that the salt form of the drug, here a sodium salt, will begin to precipitate out. At first, this might seem counterintuitive; sodium salts are almost always very soluble. This is true, but recall that K_{SP} is a function of the concentrations of the cation and the anion. Once $Q > K_{SP}$, the solid will form. Here, if the concentration of the sodium ion is high enough, the salt will precipitate out. The **pH_{max}** value is defined as the maximum pH-dependent solubility of that particular drug and salt formulation.

Figure 17 depicts a generic solubility curve of a base drug and the hydrochloride salt. In this case, the intrinsic solubility of the drug controls the solution concentration at high pH values because the drug is in its un-ionized form. As the pH becomes more acidic, the concentration of the ionized form BH^+ becomes more significant until the salt begins to precipitate out, again assuming that there is sufficient counterion (here chloride) present. In both cases, it is possible to derive an expression for the solubility of the drug (S) at any pH as long as the intrinsic solubility and pK_a are known. Figure 18 provides a summary of the regions and influence of different salt forms on the curves for a basic drug; the case for an acidic drug in analogous. Different K_{SP}, K_a, and S_0 values will alter the scale and offset of the curves, but not their general shapes. This type of information can be invaluable in developing analytical methods and understanding drug fate and transport in the body.

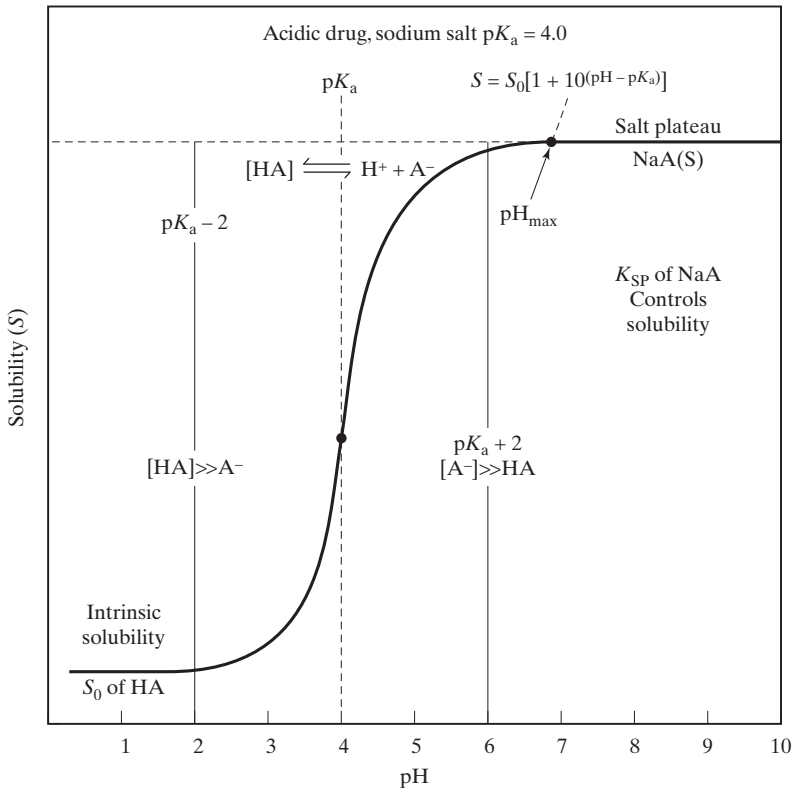


FIGURE 16 A generic solubility curve for an acidic drug. The shape of the curve is simplified for illustration purposes. Note that when the $pH = pK_a$, the ratio of the ionized to un-ionized forms is 50/50.

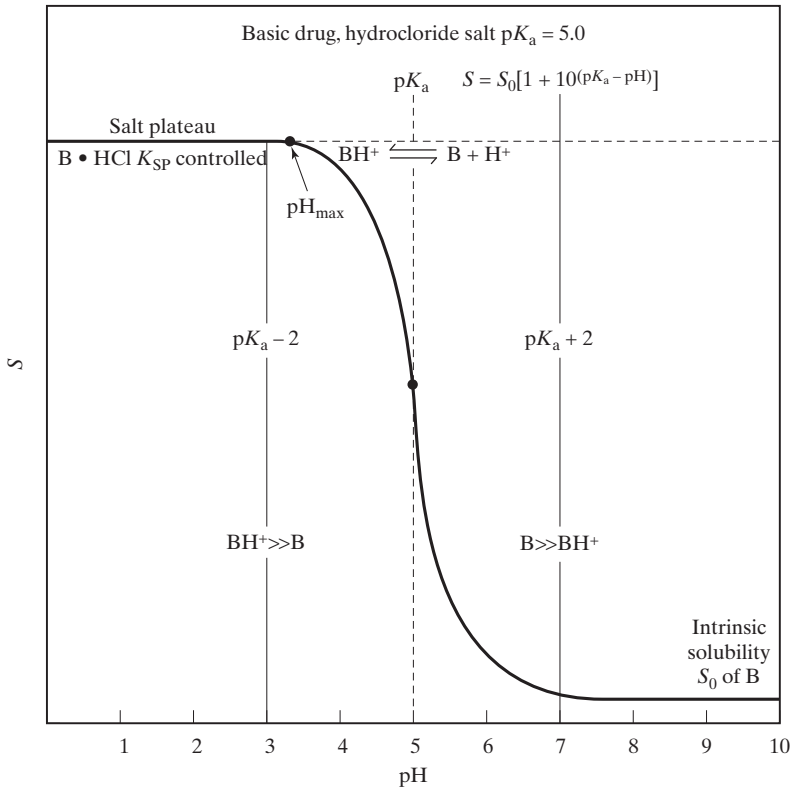


FIGURE 17 A generic solubility curve for a basic drug. The shape of the curve is simplified for illustration purposes. Note that when the pH = pK_a, the ratio of the ionized to un-ionized forms is 50/50.

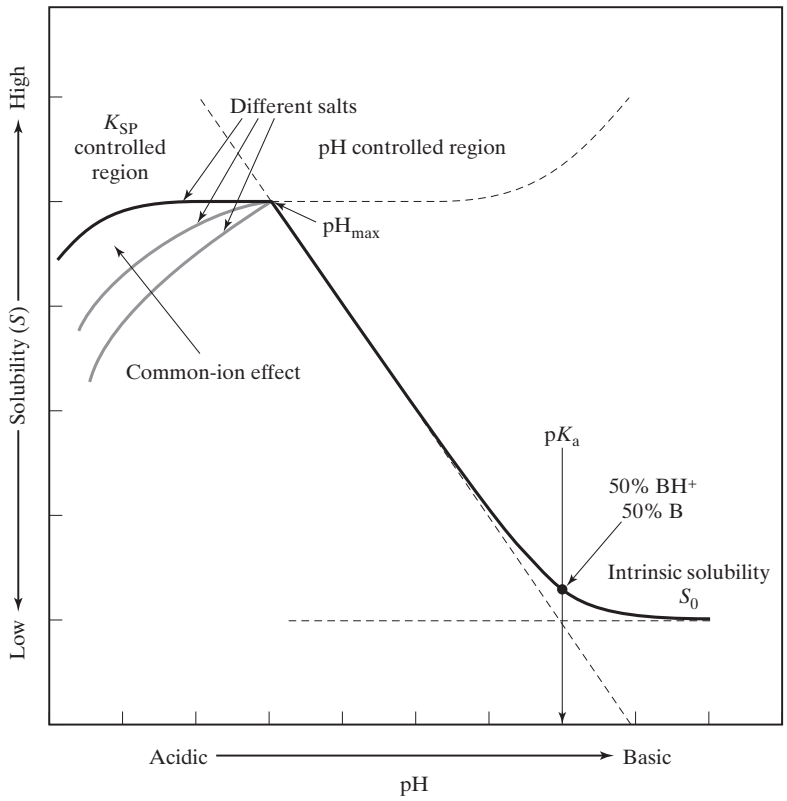


FIGURE 18 Alteration of curve shape as a function of different counter ions.

4.5 Degree of Ionization

In many extraction and separation design problems, it is convenient to be able to calculate the degree of ionization of a drug salt within the region that pH controls solubility. For a basic drug, its pH controlled solubility at a given pH (S_{pH}) is calculated as:

$$S_{pH} = S_0(1 + 10^{[pK_a - pH]}) \quad \textbf{(21)}$$

And for an acidic drug as

$$S_{pH} = S_0(1 + 10^{[pH - pK_a]}) \quad \textbf{(22)}$$

These relationships can also be expressed in terms of % ionization:

$$\% \text{ ionized} = \frac{100}{1 + 10^{(pK_a - pH)}} \quad \textbf{(23)}$$

for an acidic drug, and

$$\% \text{ ionized} = \frac{100}{1 + 10^{(pH - pK_w + pK_b)}} \quad \textbf{(24)}$$

for a basic drug.

Finally, we can derive a more complete and simple expression for describing the pH of a solution of a drug such as we did in Example Problem 1b at the beginning of the chapter. For an acidic drug:

$$pH = \frac{1}{2}(pK_a - \log C) \quad \textbf{(25)}$$

where C is the concentration of the drug (M) in the solution that has a given pH. For a basic drug:

$$pH = \frac{1}{2}(pK_w + pK_a + \log C) \quad \textbf{(26)}$$

The notation C is commonly used in medicinal chemistry and pharmaceutical sciences to represent concentration, so we will adopt that notation as well. The derivation of this equation for a basic drug is shown in Example Problem 8. Once the base dissolution is thought of in terms of degree of dissociation (frequently symbolized as α), the derivation is straightforward. The relationships described in equations 25 and 26 are thankfully easier to apply than to derive; usually the most difficult part is to insure unit consistency. Drug solubilities and concentrations are often provided in units other than molarity, but to employ these equations, molarity is required. An application is shown in Example Problem 9.

It is important to note that these equations apply to the drug itself (HA or B), *not* to a salt form. The latter are more complicated given that a salt such as cocaine • HCl is the salt of a weak base (cocaine) and a strong acid (HCl). Thus, a solution prepared from cocaine alone (B) would be basic, but a solution prepared from cocaine • HCl would be acidic. We can describe these situations using the relationship

$$pH = \frac{1}{2}(pK_a - \log C) \quad \textbf{(27)}$$

for a salt formed from a weak acid and a strong base and

$$pH = \frac{1}{2}(pK_w + pK_a + \log C) \quad \textbf{(28)}$$

for a salt formed from a strong base and a weak acid. These relationships should look familiar (see equations 26 and 27), but now the application is different. The key to using them is the ability to recognize a drug salt and its intrinsic acid–base character. Example Problem 9 illustrates these concepts.

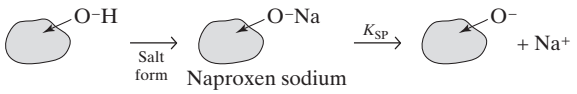
EXAMPLE PROBLEM 7

Derive equation 23 for an acidic drug such as naproxen sodium:

Answer:

$$HA \rightleftharpoons H^+ + A^- \qquad K_a = \frac{[H^+][A^-]}{[HA]}$$

There are <u>two</u> solubilities to be considered:



$$HA \underset{pH}{\overset{S_0}{\rightleftharpoons}} H^+ + A^- \qquad A^- \overset{K_{SP}}{\rightleftharpoons} NaA$$

Mass balance: *At any given pH*

$$S = [A^-] + [HA]$$

Express in terms of pH and S — S_0

Derive $[A^-]$ from acid dissociation:

$$K_a = \frac{[H^+][A^-]}{[HA]} \longrightarrow \frac{K_a[HA]}{[H^+]} = [A^-]$$

$$S = \frac{K_a[HA]}{[H^+]} + [HA]$$

$$S = [HA]\left(\frac{K_a}{[H^+]} + 1\right)$$

$$S = S_0\left(\frac{K_a}{[H^+]} + 1\right)$$

$$\log K_a - \log[H^+]$$

$$-pK_a + pH$$

$$10^{(pH - pK_a)}$$

$$S = S_0[1 + 10^{(pH - pK_a)}]$$

4.6 Acid–Base versus Ionizable Centers: Summary

It is common for students to end a first reading of this section in total confusion, probably given that our approach to acidic and basic drugs is different from that in traditional introductory acid–base chemistry. There are many basic drugs (indeed, proportionally more drugs are basic than acidic), yet in the world of drug chemistry we talk about the pK_a values and rarely mention K_b or pK_b. This can make the unprepared head spin. Keep in mind a few critical points, and the concepts will become

EXAMPLE PROBLEM 8

Answer:

Derive equation 26.

Here, we assume B is a weak base and α is the degree of dissociation, which is small enough to ignore.

$$\begin{array}{ccccc} & \mathrm{B} & \longrightarrow & \mathrm{BH^+} + & \mathrm{OH^-} \\ \text{Final } [\ ] & \mathrm{C}(1-\alpha) & & \alpha\mathrm{C} & \alpha\mathrm{C} \end{array}$$

ignore since small

$$K_b = \frac{\alpha^2 C^2}{C} = \alpha^2 C$$

We also know that $[OH^-] = \alpha C$,

$$\alpha = \left(\frac{K_b}{C}\right)^{\frac{1}{2}} \longrightarrow$$

We want this expression in terms of pH and pK_a for drugs.

$$\text{so } \alpha = \frac{[OH^-]}{C} \qquad \frac{[OH^-]}{C} = \left(\frac{K_b}{C}\right)^{\frac{1}{2}}$$

Take logs:

$$\log[OH^-] - \log C = \frac{1}{2}\log K_b - \frac{1}{2}\log C$$

$$\log[OH^-] = \frac{1}{2}\log K_b + \frac{1}{2}\log C$$

want pH | want K_a

$$[H^+][OH^-] = K_w \qquad K_a K_b = K_w$$

$$\log[H^+] + \log[OH^-] = \log K_w \qquad \log K_a + \log K_b = \log K_w$$

$$\log[OH^-] = \log K_w - \log[H^+] \qquad \log K_b = \log K_w - \log K_a$$

Ready to substitute in | Ready to substitute in

$$\log[OH^-] = \frac{1}{2}\log K_b + \frac{1}{2}\log C$$

$$\log K_w - \log[H^+] = \frac{1}{2}(\log K_w - \log K_a) + \frac{1}{2}\log C$$

isolate pH

$$\mathrm{pH} = \frac{1}{2}\log K_w - \frac{1}{2}\log K_a + \frac{1}{2}\log C - \log K_w$$

$$\mathrm{pH} = -\frac{1}{2}\log K_w - \frac{1}{2}\log K_a + \frac{1}{2}\log C$$

$$\mathrm{pH} = \frac{1}{2}pK_w + \frac{1}{2}pK_a + \frac{1}{2}\log C$$

$$\mathrm{pH} = \frac{1}{2}(pK_w + pK_a + \log C)$$

easier to grasp. First, in this context, you cannot use pK_a to determine whether a drug is acidic or basic; the structure of the drug dictates whether it is an acid or a base. Second, as a result, you also cannot use and compare pK_a values directly as a measurement of relative acid strength. These constraints arise out of the deliberate strategy of viewing acid and base sites on drugs as ionizable centers that can protonate or deprotonate. Why do we use this approach? As we will see when we delve

EXAMPLE PROBLEM 9

Another approach to pH

Recalculate the pH of the 2.00 M solution of methamphetamine from Example Problem 1 using the equations derived in Section 5.1.

Methamphetamine is a basic drug, with a pK_a of 9.8. We are considering the drug alone, so we do not worry about the salt form. The solubility is listed as 1 part in 50 parts of water, so first we must convert this value to a molarity:

$$\frac{1.\,\text{g}}{50.\,\text{mL}} = \frac{1.\,\text{g}}{0.050\,\text{L}} = \frac{1.\,g/(135.2\,\text{g/mol})}{0.050\,\text{L}} \approx 0.14_8\text{M}$$

Now, we plug into equation 26:

$$\text{pH} = \text{pH} = \frac{1}{2}(\text{p}K_\text{w} + \text{p}K_\text{a} + \log C) = \frac{1}{2}(14.0 + 9.8 - 0.830) = 11.5$$

This result compares with a value of 12.0 calculated in the previous example problem.

into forensic toxicology, the ionization state of a drug is one of the key factors in tracking it through the body. The ionization center perspective is not just a forensic convention; it is the standard in medicine, pharmacy, toxicology, and many other applications and disciplines. This does not mean that the normal rules of acid–base chemistry do not apply; it just means we have chosen to discuss this chemistry in terms of protonation and deprotonation—a different perspective on the same chemical behaviors. Another term you may have heard is *proton affinity*; this is yet another viewpoint of the same chemical behavior.

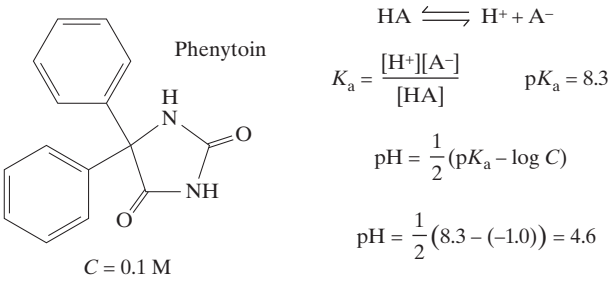
With this explanation in mind, consider the following: two drugs, one acidic and one basic, have similar pK_a values. How do you interpret this similarity? Not in terms of acid strength. Rather, think of the pK_a value as a measure of the propensity of an ionizable center to ionize. Say we have two drugs: phenytoin, an acidic drug supplied as the sodium salt with a pK_a of 8.3, and tetracaine (usually supplied as a hydrochloride salt), a basic drug with a pK_a of 8.2 (and a pK_b of 5.8). Suppose we have two separate solutions, one of each drug, and each solution is at physiological pH (7.4). What is the percent ionization of each? Use equations 23 and 24:

$$\text{Acid drug \% ionized} = \frac{100}{1 + 10^{(\text{p}K_\text{a} - \text{pH})}} = \frac{100}{1 + 10^{(8.3-7.4)}} = \frac{100}{1 + 7.94} = \approx 11\%$$

$$\text{Basic drug \% ionized} = \frac{100}{1 + 10^{(\text{pH} - \text{p}K_w + \text{p}K_b)}} = \frac{100}{1 + 10^{(7.4-14+5.8)}} \approx 86\%$$

This difference is relatively small in terms of a log scale.

Now, we turn the question around and ask: What would be the pH of a 0.1 M solution of the acidic drug HA compared with a 0.1 M solution of the basic drug B? You must recognize this as a different question. These calculations are shown in Figure 19 and utilize equations 25 and 26. The ionization percentages are comparable, but the pH values are not; this is because the *effect* of the ionization on pH depends on whether the drug is an acid or base, which is, in turn, dictated by the structure and the presence or absence of key functional groups.

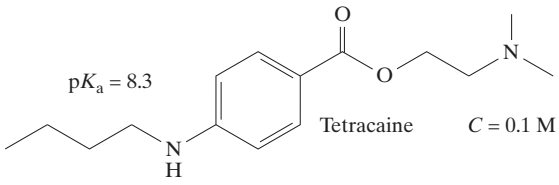


$$HA \rightleftharpoons H^+ + A^-$$

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad pK_a = 8.3$$

$$pH = \frac{1}{2}(pK_a - \log C)$$

$$pH = \frac{1}{2}\big(8.3 - (-1.0)\big) = 4.6$$



Basic drug

$$B \overset{H_2O}{\rightleftharpoons} BH^+ + OH^- \text{; need } \underline{K_b}$$

$$pH = \frac{1}{2}(pK_w + pK_a + \log C)$$

$$= \frac{1}{2}\big(14.0 + 8.2 - 1.0\big)$$

$$pH = 10.6$$

FIGURE 19 Comparison of pH values of two solutions, each prepared with a different drug. Despite nearly identical pK_a values, the resulting solution pHs are significantly different.

5 PARTITIONING WITH A SOLID PHASE

5.1 Overview

Now that we have a handle on the different types of ionizable centers in drugs and the role of pH in determining phase and form of a drug, we can exploit this knowledge to design sample preparation methods. Recall the fundamental requirements of separation that must exist:

1. Equilibrium across a phase boundary.
2. An exploitable difference in physical or chemical properties between analyte and matrix.

If these conditions are met, selective partitioning between the phases is possible. If the difference is large, **solvent extractions**, liquid–liquid extractions, or LLE, pH and solubility-based extractions may be adequate if only qualitative data are needed. To separate a mixture of cocaine • HCl and cornstarch is an easy task, since the drug salt is water-soluble and the cornstarch is not. All that is needed is water with a suitable pH value and a filtration setup; cornstarch will be left in the filter paper and cocaine as the BH^+ form will be in the aqueous solution. There are many cases in forensic chemistry in which this simple approach, or even a LLE, is not an appropriate choice for sample extraction, cleanup, and preparation. Consider a postmortem blood sample that may

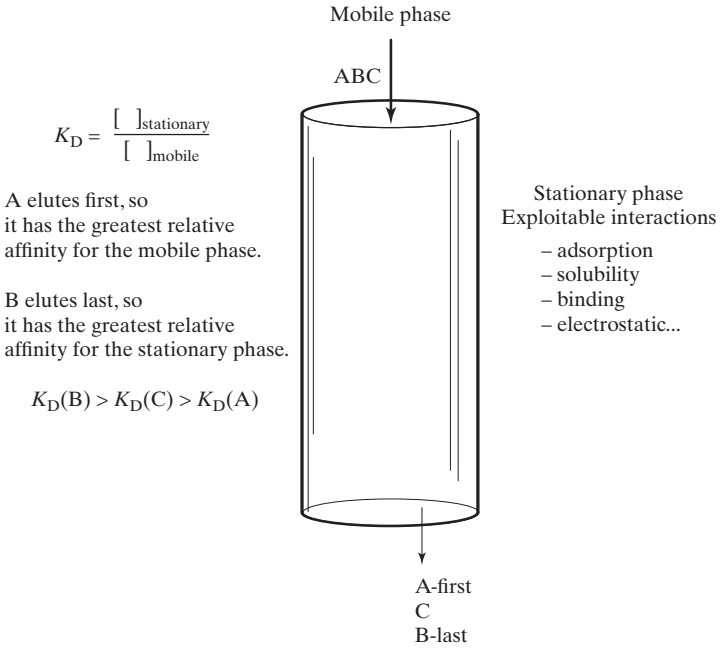


FIGURE 20 Partitioning on a solid phase. A mobile phase flows by or forced past a solid phase on which multiple interactions can occur. The interactions slow the analytes as a function of their degree of interaction.

contain a host of drugs and metabolites, some of which are acidic, some basic, and some neutral. In such matrices, solid phase partitioning methods are often required.

5.2 Solid Phase Partitioning

Solid phase extraction relies on the same fundamentals of partitioning discussed earlier. A general description is presented in Figure 20. The analytes to be separated are dissolved in a solvent (the mobile phase) that moves through a column past a stationary phase on which interactions occur. The more an analyte interacts with the stationary phase, the more its progress through the column is impeded. The less an analyte interacts, the faster it travels through the column. One way to express the degree of interaction is by considering the equilibrium and calculating a distribution coefficient K_D. In chromatography, the distribution coefficient is typically calculated as the ratio of the concentration of the analyte in the **stationary phase** relative to that in the **mobile phase**.

The stationary phase provides a platform on which interactions and partitioning can occur. The interactions may be based on adsorption, solubility, binding, or electrostatic interactions, to name a few types. Fundamentally, most of these can be reduced to polarity-based interactions and even to like dissolves like. The stationary phase may be a solid or a liquid that is bound to a solid support. The selection of mobile and stationary phases can enhance the discrimination.

Solid phase extraction (SPE): The generic structure of the solid phase in SPE is a small silica particle. A silica backbone extends away from the surface and is bonded to an active terminal "R" group. The beads have a porous surface, which increases the surface area available to interact with the analyte. R determines the affinity of the solid phase, while the chemical bonding to the silica ensures that the active groups cannot be stripped from the solid support.

SPE can be classified by the relative polarity of the solid and mobile phases. A nonpolar solid phase that preferentially associates with nonpolar or slightly polar analytes is called **reversed phase**, and the solvents used as **eluant** are polar. **Normal phase** SPE employs a polar solid phase and nonpolar solvents. Highly polar and ionizable solid phases are used in ion exchange, which can also be used as a form of analysis. The continuum of solid phase sorbents runs from reversed-phase nonpolar such as C18 (based on an 18-carbon chain), through moderately polar phases such as cyanopropyl, to strong anion and cation exchangers.

There are four general steps in SPE. After the appropriate column is selected, it is rinsed to condition it. Next, sample is loaded onto the column and flows or is drawn through by vacuum. The analytes of interest will be retained, as will some matrix components, while other matrix components will be eluted. The next step(s) are cleanups that may consist of altering the pH of the system, followed by the introduction of a solvent that will remove matrix components while leaving target analytes adsorbed to the solid phase. The last step is the elution of the target analytes. The cleanups are discarded while the final eluate is retained. Depending on the capacity of the column, large volumes of sample, such as several milliliters of urine or even a liter of water can be introduced; it is only the binding capacity and surface area of the solid phase that limits the volume. The more dilute the sample, the greater is the volume that can be introduced without saturating the active sites on the solid sorbent.

One of the advantages of an SPE preparation over solvent or LLE methods is that multiple solvents can be used to clean the sample before the final eluting solvent is added. The choice of solvents, as with LLEs, depends on the nature of the analyte. SPE can be performed with packed cartridges, disks, and syringe adapters. In addition, SPE can be automated to increase throughput.

TABLE 3 Solvent Strengths Relevant to SPE

Solvent	$e^{\circ\dagger}$	Reversed Phase Eluting Strength	Normal Phase Eluting Strength
Pentane	0.00	Strong	Weak
Petroleum ether	0.01	↓	↓
Isooctane	0.01		
Carbon tetrachloride	0.18		
Ethyl ether	0.38		
Chloroform	0.40	↓	↓
Methylene chloride	0.42		
Ethyl acetate	0.58		
Acetone	0.56		
Acetonitrile	0.65	↓	↓
Isopropanol	0.82		
Methanol	0.95		
Water	large	Weak	Strong

Sources: 1. *A User's Guide to Solid Phase Extraction Reference #M2*, Alltech Associates, Inc., 1998. 2. Rubinson, K. A., and J. F. Rubinson, "Chapter 14: Liquid Chromatography," in K. A. Rubinson and J. F. Rubinson, *Contemporary Instrumental Analysis*. Upper Saddle River, NJ: Prentice Hall, 2000.

$^{\dagger}e^{\circ}$ is a measure of **solvent strength** based on polarity. It is defined as the energy of absorption on a surface of alumina, a polar material.

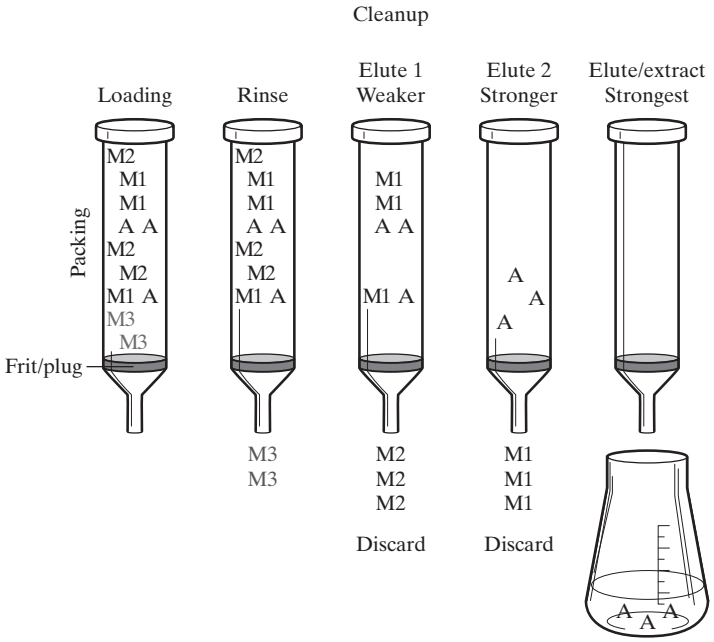


FIGURE 21 Generic SPE using a column. The analyte A is separated and concentrated in the final elution by successive rinsings with weaker solvents. Some components of the matrix (indicated by M1 and M2) may also be left on the column prior to elution with the strongest solvent.

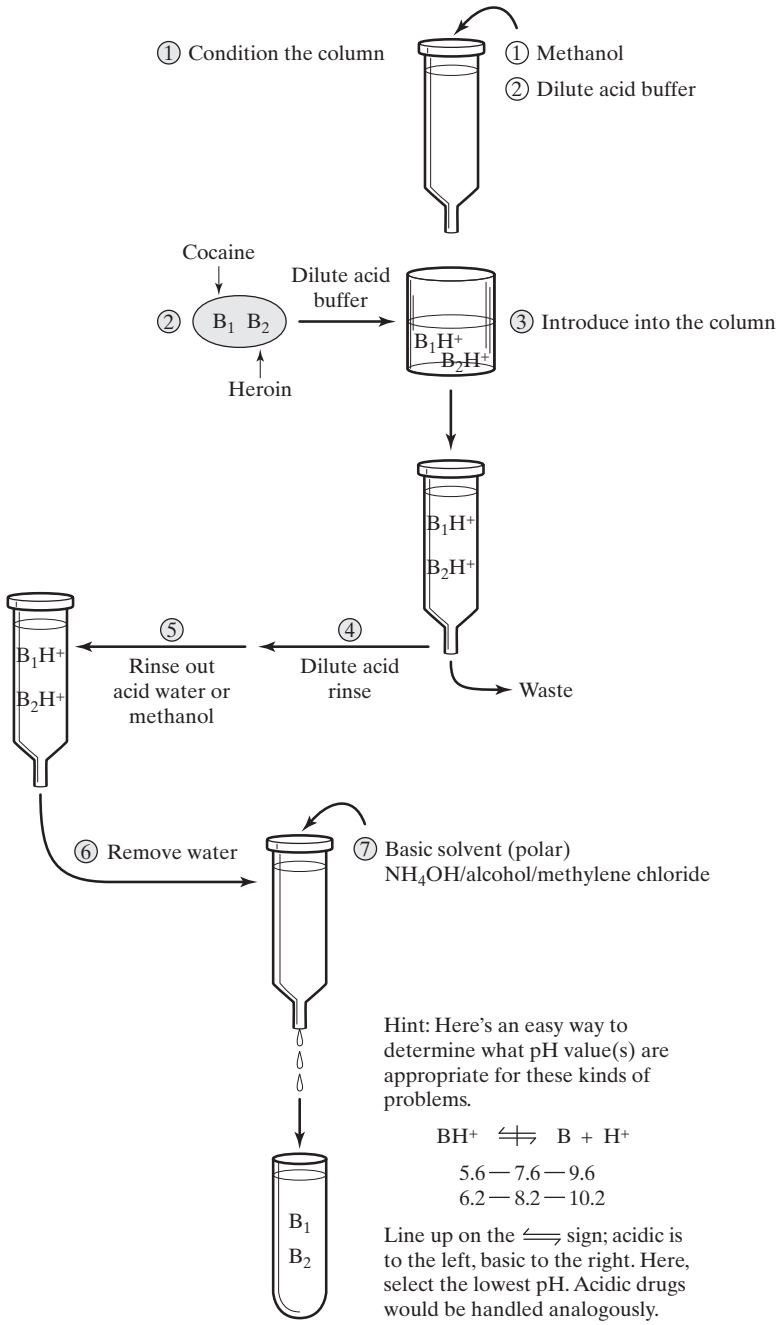
Many multifunctional columns have been developed to allow for the separate extraction of acids, bases, and neutrals that integrate an ion-exchange resin with relatively nonpolar functionality.[4–6] That way, different properties can be exploited sequentially by rinsing the column between elutions. In general, a buffered, slightly basic solution of the sample is introduced into the column. Following a water wash, an acidic solution is introduced. Acidic drugs will protonate and remain uncharged, allowing them to be eluted with an organic solvent, along with neutrals. The column is dried and then flushed with a basic solvent system to elute basic components. The two **eluants** are treated separately in subsequent analyses. When elution solvents are changed, the column must be rinsed and dried in between to avoid miscibility problems .

EXAMPLE PROBLEM 10 Propose an SPE Scheme for Preparing a Sample Containing Heroin and Cocaine.

Answer:

Cocaine has a pK_a of 8.6 and heroin has a pK_a of 7.6. Both are basic drugs that adhere to the generic relationship $BH^+ \longleftrightarrow B + H^+$. Heroin base is soluble in chloroform at a ratio of 1:1.5 but is not appreciably soluble in other organic solvents. Cocaine base is similarly soluble in chloroform, as well as in acetone, ether, and carbon disulfide. This solubility information can be used to select solvents for flushing the column of contaminants and for eluting the drugs at the final stage. For this application, a reversed phase, nonpolar solid phase with cation exchange capability would be a reasonable choice. This selection means that the ionized form of the drug (BH^+) will be preferentially retained over the B form. The first step is to dissolve the sample in an acidic buffer that will drive both cocaine and heroin to ionized form.

(*Continued*)



On the basis of the preceding data, the first step is to dissolve the sample in dilute acid solution with a pH of ~5, which is 7.6 − 2 units, more than enough to ensure that both drugs are in the ionized form. The solution is introduced into the column, where the ionized formwill be retained. An acidic wash of the column will flush out many contaminants. A rinse with methanol will flush out the residual acid solution. To elute the basic drugs into an organic solvent, that solvent must be slightly basic. A moderately polar solvent mixture containing ammonia would be appropriate.

Solid phase microextraction (SPME): An alternative form of SPE is being used in drug and arson analysis. In this form, a small fiber acts as the substrate for the sorbent. **Solid phase microextraction** (**SPME**) is versatile and particularly amenable to the extraction and preconcentration of volatile organics. The active material is bonded to a thin silica needle with dimensions similar to capillary GC columns (fractions of a millimeter). Coating materials are the same or similar to packing used in SPE, so the same types of analytes can be targeted. Although Figure 22 shows the fiber exposed to the headspace, fibers can be also be placed in the liquid.

SPME sample preparation begins with exposure of the fiber. Assuming a headspace application, variables such as height above the liquid, length of fiber exposed, and exposure time must be optimized. The coated fiber is fragile and so is usually kept in a protective housing until exposure. The housing also facilitates setting a constant and thus reproducible depth and height for the exposure. Depending on the volatility of the target analytes, the sample may be heated. Because the sorbent coatings are thin, overloading is a concern that must be addressed in method development and optimization. If used, internal calibration standards are introduced during this absorption phase.

At the end of the exposure period, the fiber is withdrawn into the housing and removed from the sample. Although some methods use a solvent to extract absorbed materials in a way analogous to eluting them from a solid phase cartridge, more often the fiber is introduced directly into the heated injection port of a gas chromatograph or into the injector of a high-pressure liquid chromatograph. As in the absorption phase, the geometry of placement in the injector and time of desorption must be optimized. Automating SPME is more difficult than automating SPE, mainly because of the fragility of the fibers.

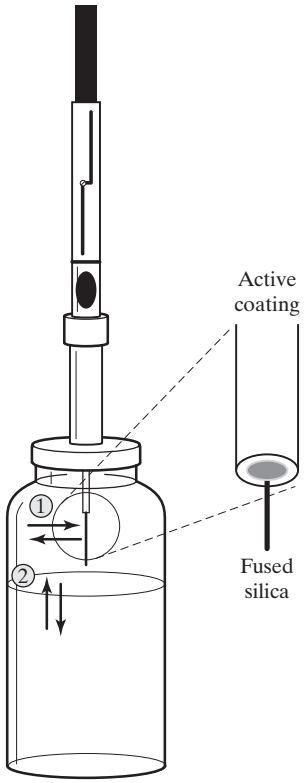


FIGURE 22 SPME using coated fibers. The apparatus above the vial is a holder that protects the fragile fiber. The fiber is kept inside of the housing until it is ready to be exposed to the sample. Components in the headspace partition into the solid support and concentrate there. After the exposure, the fiber is withdrawn into the housing and removed.

Forensic interest in, and applications of, SPME have increased, particularly since 2000. Arson analyses were the initial focus because, due to the nature of the absorption and desorption process, SPME fibers are ideally suited to the analysis of volatile material. Less volatile components are also absorbed, but typically require greater heating and longer desorption times for effective removal from the fiber. In general, the more volatile the analytes, the less of a concern desorption is. Current forensic applications of SPME include toxicology[7–11], inks and dyes[12–13], explosives[14–19], propellants[20–21], and fire debris[22–25].

In arson, the target analytes are volatile hydrocarbons that are ideally suited for SPME. ASTM has published a standard method for screening fire debris for the presence of accelerant residues.[26-27] The specified fiber is coated with polydimethylsiloxane (PDMS), a nonpolar phase, and the sample is heated to 60°C −80°C for 20–30 minutes. Exposure time is between 5 and 15 minutes, depending on sample concentration. This method illustrates one of the limitations of SPME. Because the active coating is thin, it is easily overloaded with analyte. Once the fiber is saturated, no additional analyte can be absorbed. Qualitative results may not suffer, but quantitation becomes unreliable once this breakthrough occurs. Often, as described in the ASTM method (E2154), this limitation necessitates a trial-and-error approach to balance sample heating and desorption times. Furthermore, since the fibers are reused, it is essential that they be thoroughly purged of any residual sample before reuse. In turn, this means that a fiber blank must be analyzed. Consequently, any single use of a fiber generates the need for a second run to ensure that the fiber has been stripped clean and that there will be no carryover.

6 EXTENSION OF PARTITIONING: THIN-LAYER CHROMATOGRAPHY

The leap from solid phase extraction to chromatography is a natural and conceptually easy one. It is also a crucial one, given the central role of chromatography in forensic science, particularly forensic chemistry. **Thin-layer chromatography** (TLC, or **planar chromatography**) is used as a screening technique; however, with the inclusion of multiple solvent systems, standards, and selective developing reagents, TLC can provide tentative identification of many analytes. TLC can be also be used preparatively.

TLC is carried out on a glass plate or other supportive backing coated with a solid phase. Common coatings for TLC are similar or identical to the many types of the phases described for SPE. The types of interactions are analogous (ion-dipole, dipole-dipole, etc.). The solvent systems used vary widely with the type of analyte; standard forensic references are used to select the appropriate system and solid phase for a particular analysis.[28] The most widely used solid phase is silica gel, in which the interaction is adsorption. Drugs associate with the Si–O moieties via ion–dipole interactions. The TLC "plates," as they are usually called, can be purchased impregnated with a compound that fluoresces when exposed to UV light. When the plate has been developed, the final locations of the spots on the plates are visualized as dark areas against the fluorescent background. Sample solvent systems used with silica plates are ethyl acetate:methanol: 30% ammonia (17:2:3) and cyclohexane:toluene:diethylamine (75:15:10). In most applications, the TLC chamber is covered and equilibrium is established between the liquid and vapor phases before use. As illustrated in Figure 23, the sample is dissolved in a small portion of solvent, usually the system selected, and applied in small concentrated areas with the use of capillary tubes. The origin line is a few millimeters above the lower edge of the plate, high enough that the solvent will not cover the origin when the plate is placed in the tank.

EXHIBIT B

How to Talk to a Jury

Because TLC is used extensively in forensic chemistry, analysts are often called on to explain how it works to a jury. The goal is to present the basic concepts in terms that everyone can understand. Here, the forensic chemist can explain capillary action by telling the jury that this is what causes water from a puddle to soak into a paper towel when a corner is dipped into the puddle. Partitioning can be described with reference to two phases everyone is familiar with, such as oil-and-vinegar dressing or sugar in gasoline. The best explanations are visual and familiar; learning how to present them takes practice. Most forensic scientists develop a collection of such explanations.

Once the plate is in the tank, solvent is immediately drawn up the plate by capillary action. When the solvent front reaches the origin, partitioning begins. The process is analogous to what occurs in SPE. The TLC run is complete just before the solvent front reaches the top of the plate. At that point, the plate is removed and the solvent front is marked with a straightedge if retention values are to be calculated; this operation is illustrated in Figure 24. The plate is then allowed to dry or is heated until it is dry.

Aside from the fluorescent background already mentioned, many other options exist for developing the plate to visualize components. Some analytes, such as LSD, fluoresce naturally and are easily visualized under UV light. Most drugs are visualized by the application of one or more developers, and the choice of these reagents can add significantly to the specificity of what an otherwise simple screening test displays.

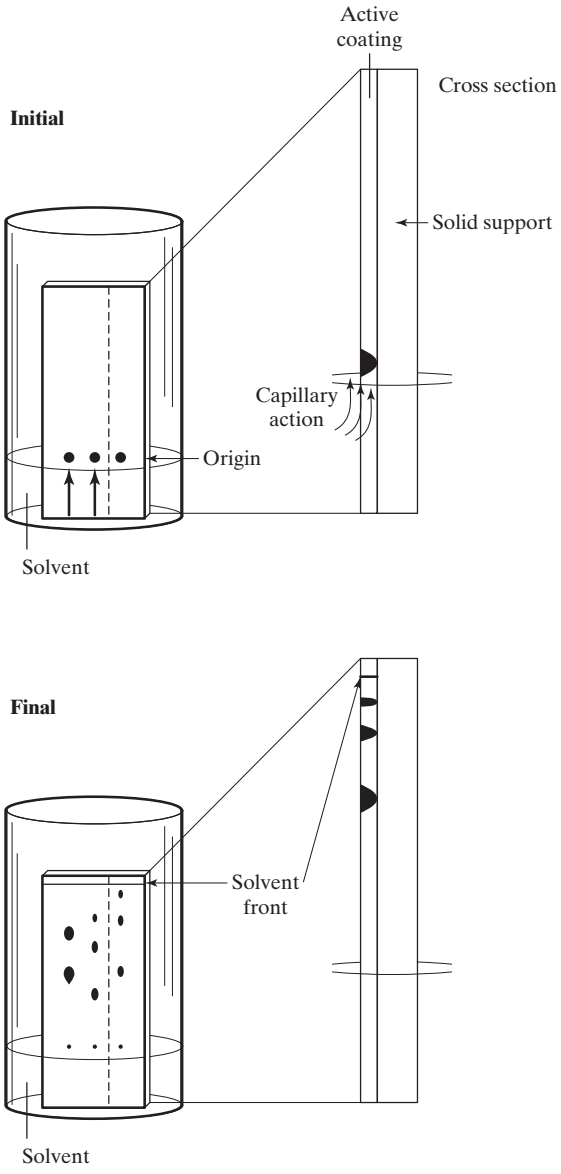


FIGURE 23 Overview of TLC. The active phase is thinly coated on a glass plate with the sample placed at the origin line.

Iodoplatinate (acidified) is a near-universal developer for drugs,[29] reacting with alkaloids and amines to form dark blue or black complexes. The reagent can be purchased ready to use or can be made from platinic chloride or potassium chloroplatinate and potassium iodide. Ehrlich's reagent (also a color test reagent for LSD) is used as a developer for LSD, mescaline, and related compounds. The Marquis and Mandelin presumptive test reagents can be used as developers as well.

Because TLC screening tests follow presumptive tests in the forensic analytical scheme, standards of suspected analytes are easily incorporated. Additional specificity is gained by using multiple solvent systems or different TLC stationary phases.

EXHIBIT C

Chromatography

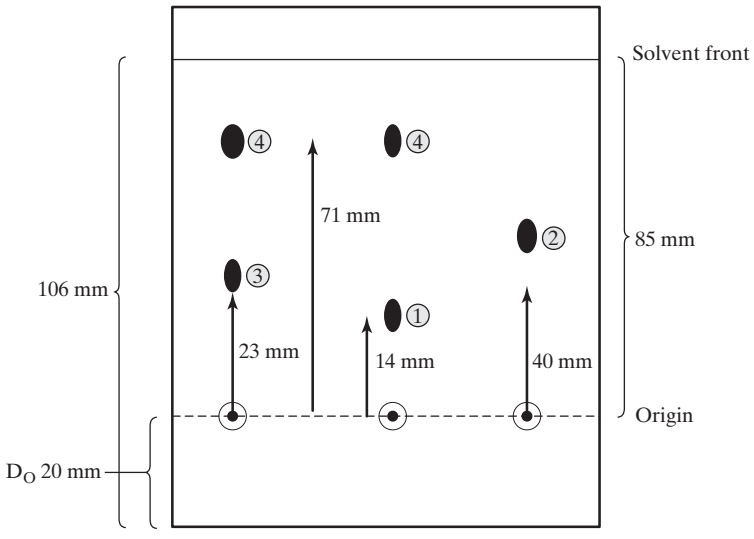
The word *chromatography* means "color writing," and the first chromatographic separations were of colored materials. By the middle of the 1800s, dyes and other colored materials were being separated on filter paper by placing a drop in the center. The components then separated by diffusing radially outward, forming a concentric ring pattern. The name most frequently associated with modern chromatography is that of a Russian, Mikhail Tsvett (1872–1919).[†] His work with pigments paved the way for techniques ranging from column chromatography to thin-layer chromatography.

[†]Interestingly, and appropriately, the word tsvet (ubet), in Russian, means "color."

Spots developed on a TLC plate can be characterized by their **retention factor** (also called the retardation factor), or R_f value, given by

$$R_f = \frac{D_x}{(D_s - D_o)} \tag{29}$$

where D_x is the distance from the spot to the origin, and $D_s - D_o$ is the distance the solvent front travels from the origin. The retention factor is analogous to the adjusted retention-time values reported for liquid and gas chromatography. A compound with an R_f of 1.00 travels with the solvent and does not interact with the bonded coating; conversely, an R_f of 0.00 (no movement) indicates that the analyte has no affinity for the solvent. R_f values are reported to two decimal places or, in some cases, multiplied by 100 and reported as values up to 100.[30] In forensic applications, R_f values are rarely cited to identify compounds, but they are useful for comparison purposes and for selecting



$$R_{f_1} = \frac{14 \text{ mm}}{85 \text{ mm}} = 0.16 \qquad R_{f_3} = \frac{23 \text{ mm}}{85 \text{ mm}} = 0.27$$

$$R_{f_2} = \frac{40 \text{ mm}}{85 \text{ mm}} = 0.47 \qquad R_{f_4} = \frac{71 \text{ mm}}{85 \text{ mm}} = 0.84$$

FIGURE 24 Calculation of retardation (retention) factor R_f for TLC. In diffuse spots, the distance is measured from the line of origin to the center of the spot.

the appropriate solvent system for a given separation. For example, if a separation of cocaine from heroin is required, the TLC system should yield significantly different R_f values for each compound to be of practical use. A sample R_f calculation is shown in Figure 24.

TLC is an extension of sample preparation and shares characteristics with SPE; hence, pH effects can play a role. If ionization is suppressed, the neutral compound will favor the less polar phase, typically the solvent in silica TLC. If ionization is facilitated by pH, the compound will be charged and will interact much more with the charged silica moieties. Careful selection of solvent systems can facilitate separations based on small differences in pK_a values. As shown in Figure 25, the first solvent system incorporates methanol and acetone along with a small amount of ammonium hydroxide. The addition of the base insures that basic drugs will remain un-ionized and thus have little affinity for the polar silica solid phase. Interaction still occurs on the basis of the polarity of the un-ionized molecules, but it is far less than the interaction for an ion. In the second case, where no base is added, the ionized drugs are absorbed onto the polar silica gel and stay near the origin. Consequently, resolution is poor. Further sample TLC analyses are shown in the color insert. Most of the current forensic applications are in drug screening and characterization of inks and dyes[31–44].

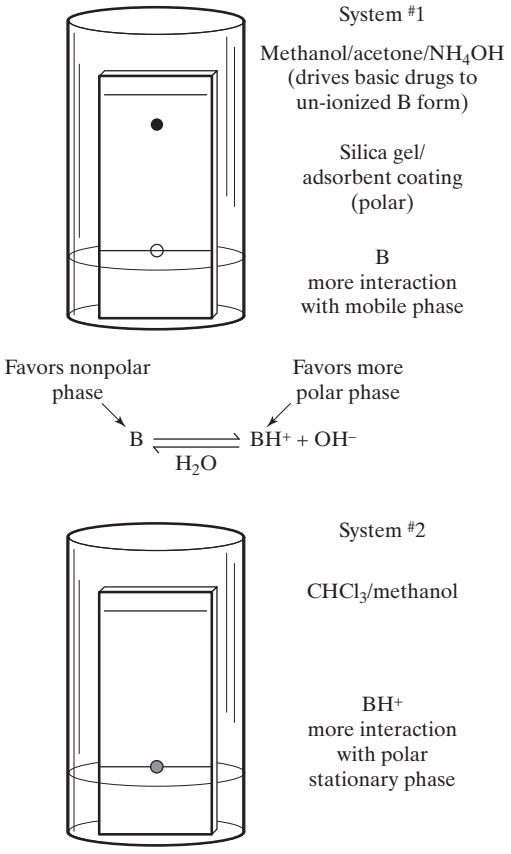


FIGURE 25 The role of pH and ionization in TLC. With a polar stationary phase, nonpolar (un-ionized) forms of bases will partition preferentially into the mobile phase.

Summary

This chapter discussed some of the fundamental chemistry utilized in forensic laboratories every day. The common theme was equilibria, and from there we explored partitioning, solubility, and acid–base chemistry. We also saw how these principles are utilized in sample preparation and thin-layer chromatography. TLC is a method that forms a historical bridge between wet chemical methods and instrumentation.

Key Terms and Concepts

Common-ion effect
Digestion
Dipole–dipole interactions
Dry extraction
Eluant
Eluent
Equilibrium constant
Headspace
Henderson–Hasselbalch equation
Henry's law
Hydrophilic
Hydrophobic
Intrinsic solubility (S_0)
Ion–dipole interactions
Ion–ion interactions
Ionizable center
Isoelectric point (isoelectric pH)
K_a, K_b, K_{sp}
Le Chatelier's principle
Like dissolves like
Lipophilic
Lipophilicity
Lipophobic
Mobile phase
Normal phase
pH_{max}
p*K* value
Planar chromatography
Polarity
Relative affinity
retention factor
Reversed phase
Solid phase extraction
Solid phase microextraction
Solubility
Solvent extraction
Solvent strength
Stationary phase
Thin-layer chromatography

Problems

FROM THE CHAPTER

From now on, you need to be able to find drug structures and formulas on your own. There are numerous sources at your disposal including the *Merck Index*, *Clarke's Analysis of Drugs and Poisons*, and online at the National Library of Medicine (NLM), ChemID search engine.

1. Equation 2 expresses the relationship exploited by breath alcohol testing that is done in the field. Why is this test not considered accurate enough to determine blood alcohol concentration? In other words, why is the test considered to be presumptive rather than conclusive?

2. a. Without resorting to calculations, comment on the relative solubilities of the following compounds: silver bromate, $K_{SP} = 5.5 \times 10^{-5}$; silver sulfide, 6×10^{-51}; magnesium carbonate, 3.5×10^{-8}; Mn $(OH)_2$, 1.6×10^{-13}.
   b. Give the solubility product constant of each compound in part a in the form pK_{SP}.
   c. Calculate the solubility (S) of each compound in part a.
   d. At what pH would manganese hydroxide precipitate out?

3. a. Barium is a toxic metal, yet it is given to patients in large quantities when X-rays of the stomach or intestines are needed. For such imaging, the patient is given a "milkshake" containing barium sulfate. Given the known toxicity of barium, why is this "drink" safe?
   b. Comment on the implications of the barium test in part a for the toxicology of metals such as mercury, cadmium, lead, and antimony.

4. Diazepam tablets are supplied in 2-, 5-, and 10-mg increments. Suppose several tablets are received in a laboratory as evidence, and using the *Physician's Desk Reference*, an analyst is able to tentatively identify them as Valium, 10 mg. Suppose further that you learn that the tablets also contain anhydrous lactose, starches, dyes, and calcium stearate. Describe a method for isolating the active ingredient from fillers, using a LLE scheme. Justify and explain each step of the method.

5. Quinine ($C_{20}H_{24}N_2O_2$) is a dibasic molecule with p*Ka*'s of 4.1 and 8.5. It is encountered as a diluent (cutting agent) for heroin. To extract quinine from an aqueous solution, what pH should be used and why?

6. Devise a solvent extraction method that could be used to separate a mixture of powdered sugar, cornstarch, cocaine, and amphetamine. Justify each step and separation. Repeat, using SPE to effect the separation.
7. A case sample from a suspected arson fire is submitted to the laboratory. The fire was suppressed with large volumes of water. The exhibit submitted consists of approximately 50 mL of this water, which appears dirty and has suspended solids and other visible debris in it. Propose an SPE method for cleaning the sample and isolating any residual accelerants, assuming that gasoline or another hydrocarbon was used.
8. Suppose you have an acidic drug with a pK_a of 5.0 that is in solution held at physiological pH.
   a. What is the % ionization of the drug?
   b. What would the pK_a of a basic drug have to be for the % ionization to be the same as that of the acidic drug?
9. Why is it desirable to provide a drug as a water-soluble salt for oral ingestion?
10. Estimate the K_{SP} of the following drug salts:
    a. Lidocaine • HCl, soluble 1 part in 0.7 part water
    b. Codeine sulfate, $(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O$, soluble 1 part in 30 parts water. *Note:* Hydrates are common in drug formulations.
11. Using data and results from Question 10a, estimate the concentration of chloride ion required for precipitation of the salt to occur in a solution containing lidocaine, fully protonated, at a concentration of 3.82×10^{-4} M.
12. Calculate the solubility, in both mol/L and g/100 mL, of the following drugs at the given pH value:
    a. Papaverine at pH = 4.8, a basic drug, $S_0 = 1.7 \times 10^{-4}$ g/100 mL water; $pK_a = 6.36$
    b. Aspirin at pH 5.1, $S_0 = 1$ g/300 mL water; $pK_a = 3.5$
13. Which proton is ionizable on the drug phenytoin, shown in Figure 19? Explain your reasoning.
14. Determine the pH of the following solutions:
    a. 50.0 mg of naproxen sodium dissolved in 1.00 mL water
    b. 1.0×10^{-3} M (1 mM) cocaine
    c. 1.00 g cocaine • HCl dissolved in 100.0 mL water
15. Determine the % ionization of the following drugs at the specified pH:
    a. A basic drug, pK_a of 8.2, at physiological pH
    b. An acidic drug, pK_a of 5.1, at pH 6.2
16. How much naproxen as the sodium salt would you have to weigh out to prepare a primary standard at a concentration of 5000.0 ppm in 100.0 mL?

INTEGRATIVE

1. Diazepam (Valium) is a member of the benzodiazepine family of drugs. This drug, at one time the most prescribed drug in the country, has a single ionization center, with a pK_a reported as 3.4. Answer the following questions about the drug:
   a. Draw the structure of diazepam or obtain it from a reliable reference source. Indicate the location of the ionizable center.
   b. Is diazepam acidic, basic, or neutral? Justify your answer.
   c. What would be the pH of a 0.01 M solution of diazepam? Show your work.
   d. What would be an optimal pH for extracting diazepam, using a simple solvent extraction?
2. For drugs supplied as salts, it is usually possible to determine whether the drug is acidic or basic by the chemical name. Explain and provide a list of three examples not in the chapter. (*Hint: PDR.*)
3. TLC plates can be made in the laboratory, but it is recommended that they be purchased to ensure the uniformity of the solid phase thickness. Comment on the role that thickness would play, and illustrate how an uneven surface would affect the plates' performance and appearance.
4. A certain drug has a K_b of 3.2×10^{-6}. What are the corresponding K_a and pK_a?
5. When SPE or related techniques are used to extract samples, internal standards are added before the sample is placed on the column. Why?
6. According to the *Merck Index*, a 1% solution (wt/vol) of caffeine in water produces a pH of 6.9. Calculate the K_a and pK_a of caffeine.
7. Derive equation 21.
8. Derive equation 25.

FOOD FOR THOUGHT

1. If TLC with two different solid phases and several solvent systems is used to analyze a sample, will the results be considered conclusive? What about including standards and several developing reagents? At what point does a string of presumptive and screening tests become conclusive?
2. In relation to question 1, comment on the analogy between the described scenario and the situation of combining circumstantial evidence to infer a fact.

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Instrumentation

OVERVIEW AND ORIENTATION

In this chapter, we delve into the instrumental tools, techniques, and procedures utilized in forensic chemistry. The chapter is best thought of as akin to a *CliffsNotes*® of that enormous topic, a supplement to and summary of the many fine works listed in the References and Further Reading sections at the end of the chapter. Your instructor may elect to skip this chapter and have you come back to sections as they become relevant, or you may just dive in. If so, you would do well to review information from your previous courses as it relates to instrumentation. For those who have recently taken an instrumental analysis course, much will be review; for those who have not, enough information is provided to understand how and why the instruments are used. Mass spectrometry and infrared spectrometry often are covered in an organic chemistry course, at least to the level of detail assumed here. The depth and breadth of each treatment corresponds to its widespread application in forensic chemistry. The presentation of each method is necessarily concise and is meant to provide information requisite to an understanding of later topics; it is not meant as a replacement for an instrumental analysis course.

One of the first forensic science laboratories was founded in 1910 by Edmund Locard, who reportedly had two instruments: a microscope and a spectrophotometer. The more things change, the more things remain the same: Forensic chemists have many procedures and devices at their disposal, but their core instruments are still spectrophotometers (hereafter, spectrometers), microscopes, and now, combinations of the two. Recently joining the arsenal are hyphenated systems incorporating chromatographic separation and advanced detectors, most notably mass spectrometers.

The microscope has been associated with forensic science ever since Locard and Sherlock Holmes. The study of microscopy provides a foundation for study of spectroscopy. Simple microscopy is based on the interaction of visible light with matter, whereas spectroscopy is broadly defined as the interaction of electromagnetic energy with matter. Once visible light interacts with a sample, that light carries information about the

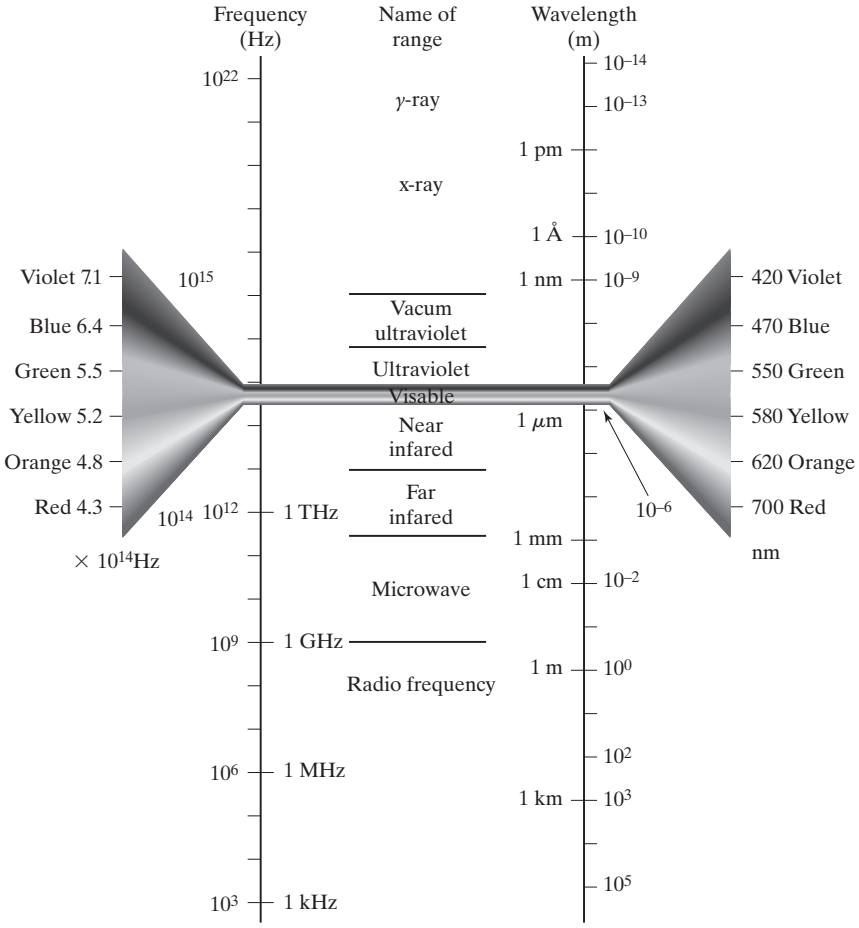


FIGURE 1 The electromagnetic spectrum. Simple microscopy and colorimetry exploit the visible range. X-ray spectroscopy probes elements and crystal structure; UV spectroscopy focuses on molecular structure, while IR probes bonding through vibrational interactions.

physical and chemical characteristics of the sample. The same is true in all modes of spectroscopy. The detector in a microscope is the human eye, and the characteristic that is most studied is color, but color is an expression of frequency and wavelength, characteristics exploited across the electromagnetic spectrum.

In forensic science, UV/VIS and IR spectroscopy are the most widely used types of spectroscopy. X-ray techniques are employed for elemental and structural analysis, often as a complement to **scanning electron microscopy (SEM)**. **X-ray diffraction (XRD),** used to examine crystal structures, is much less common. On the other end of the spectrum, nuclear magnetic resonance (NMR) is occasionally used, but an NMR instrument is not a common sight in forensic laboratories. However, every forensic laboratory has at least one microscope, and it is with microscopy that we begin our exploration of instrumentation.

EXAMPLE PROBLEM 1

Calculate the following:
Green light has a wavelength of 550 nm.

(1) What is the frequency?

$$\nu = \frac{c}{\lambda} = \frac{3.0 \times 10^{10} \cancel{\text{cm}}/\text{s}}{550 \cancel{\text{nm}} \times \dfrac{10^{-7} \cancel{\text{cm}}}{\cancel{\text{nm}}}} = 5.5 \times 10^{14}\,\text{s}^{-1} = 5.5 \times 10^{14}\text{Hz}$$

(2) What is the frequency in cm^{-1}, a unit used for IR spectroscopy?

$$\nu(cm^{-1}) = \frac{1}{\lambda(cm)} = \frac{10^7 \cancel{nm}/cm}{\lambda(\cancel{nm})}$$

$$\nu(cm^{-1}) = \frac{10^7 \cancel{nm}/cm}{550\ \cancel{nm}} = 18{,}200\ cm^{-1}$$

(3) What is the energy of the photon?

$$E = h\nu = \frac{hc}{\lambda}$$

$$E_{550} = \frac{(6.62 \times 10^{-37} KJ.\cancel{s})(3.0 \times 10^{10} \cancel{cm}/\cancel{s})}{550\ \cancel{nm} \times \frac{10^{-7} \cancel{cm}}{\cancel{nm}}}$$

$$E_{550} = 3.6 \times 10^{-22} KJ(\text{per photon})$$

1 MICROSCOPES AND CHEMICAL MICROSCOPY

Microscopy was an established forensic tool by the 1890s.[1] In toxicology, microscopy was being used to evaluate crystals characteristic of poisons, not unlike the way microcrystal tests in modern drug analysis are used. Microscopes were also employed in the analysis of fibers.[2] To explore the principles of microscopy, the best place to begin is with the one instrument that symbolizes forensic science. A magnifying glass (Figure 2) consists of a single lens that creates a magnified image of the specimen on the retina of the person using it. The image is called a **virtual image,** because it exists only in the eye of the viewer (Figure 3). This kind of image cannot be projected onto a screen in the way a movie projector creates an image on the plane of the movie screen. The effect of using a magnifying glass is to make the object appear as it would if it were placed much closer to eye, but the image disappears when one stops viewing the sample through the lens; the image is not in real space.

The lens in the magnifying glass is made of glass, a substance that refracts or bends light. The refractive index (RI) of any material is given by

$$N_r = \nu_{vacuum}/\nu_{material} \quad \textbf{(1)}$$

where ν represents the speed of light in a given medium. The greater the difference in speed, the greater the refraction. Because a lens has two interfaces, two refraction events occur. The refraction angles θ_{air} and θ_{glass} are related through **Snell's law** (Figure 4) which is often expressed as:

$$N_{r,air} \sin\theta_{air} = N_{r,glass} \sin\theta_{glass} \quad \textbf{(2)}$$

FIGURE 2

This relationship is also important in some types of spectroscopy, such as attenuated total reflectance (ATR), which is discussed in Section 2.

Recall that refraction is a function of wavelength as well, and applications of this phenomenon are referred to as **dispersion** techniques. A familiar example is the glass prism that disperses visible light into component wavelengths by exploiting the differences in their refractive indices. Gratings also disperse light by creating zones of constructive and destructive interference. Dispersion may play a role in microscope optics and is the basis of a technique called *dispersion staining*, which is useful in determining refractive indices of small particles.[3]

Lenses exploit the air–glass interface to magnify and focus light at predictable points, as shown in Figure 5. When light illuminates an object so that the light rays are

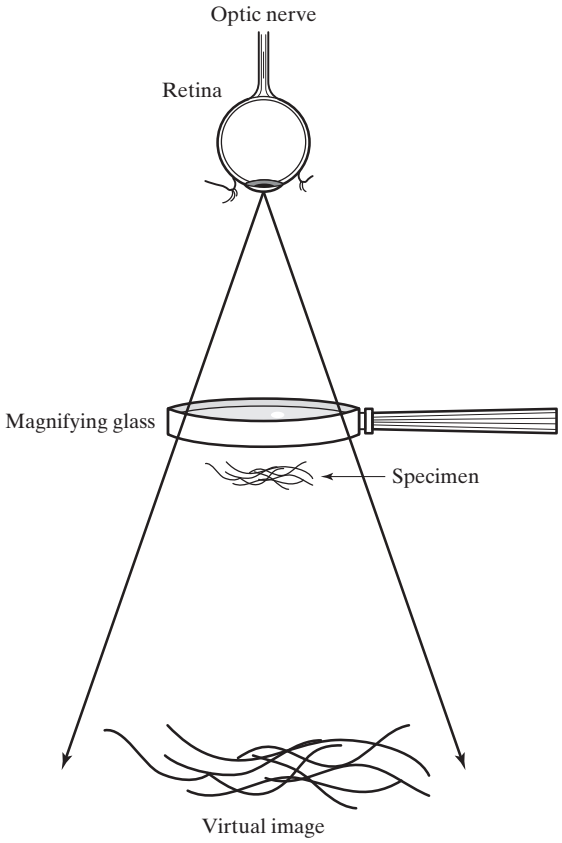


FIGURE 3 A simple magnifying lens.

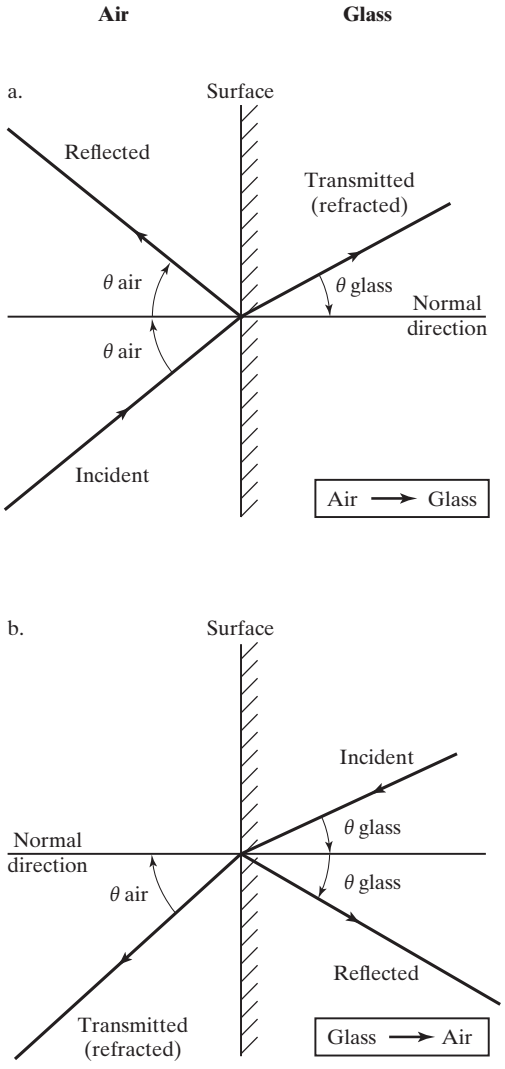


FIGURE 4 Refractive index at an air–glass–air interface such as a lens. Differences in relative speed cause the light to bend when it is (a) reflected and (b) transmitted. In (a), the light enters the lens; in (b), it exits. Hence, there are two changes in speed and two refraction events.

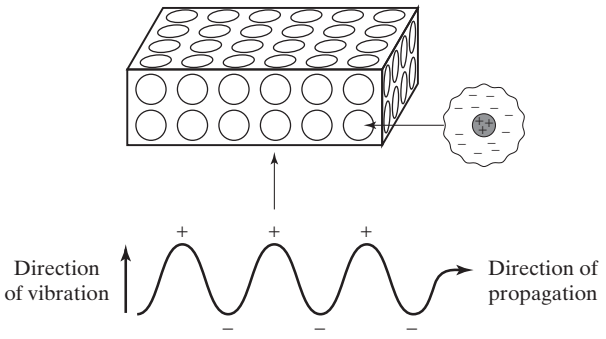
parallel (rather than converging or diverging), the **principal focus** *(p)* is the point at which the light rays traveling out of the lens converge. The line that passes through the center of curvature of the lens is called the **optic axis,** and the distance on that line to the principal focus is the **focal length** *(f)*. For the viewer to see an image of the object in focus, the principal focus must correlate with a point on the viewer's retina. Similarly, if the lens were in a movie projector, the image would be in focus at the focal length, and the screen would correspond to the focal plane.

In cases where the light rays are not parallel and the object is not at an infinite distance, as in a microscope, the situation changes. The relationship of the points, as shown in Figure 6, can be summarized in the formula[3]

$$\frac{1}{f} = \frac{1}{p} + \frac{1}{q} \qquad \textbf{(3)}$$

EXHIBIT A

Why RI?



What is responsible for the refraction of light in a material? Light is electromagnetic energy, which is an oscillating electromagnetic wave with positive and negative regions. These regions interact with atoms, which can be visualized as tiny concentrations of positive charges surrounded by a negatively charged electron cloud. When the oscillating electromagnetic wave interacts with these charges, it can distort them, resulting in an attenuation or modulation of the original electromagnetic wave. The refractive index (RI) is a measure of the ability of the material—or, more specifically, the electron clouds of atoms and molecules within the material—to alter incoming electromagnetic energy. One analogy is to imagine what happens when a small magnet is dragged through a sea of other magnets. Anyone holding the moving magnet would feel the forces asserted by the larger magnets and would sense the smaller magnet being moved and directed by those forces.

The RI of a material depends on the types and sizes of the atoms in the material, the nature of the bonds and how the atoms are arranged, the number of interactions between atoms (which depends on the density and crystal structure of the material), the thickness of the material, and the closeness of the wavelength of light in size to the size of the particles in the material. All these factors influence the magnetic field environment encountered by light, which itself consists of oscillating electric and magnetic waves.

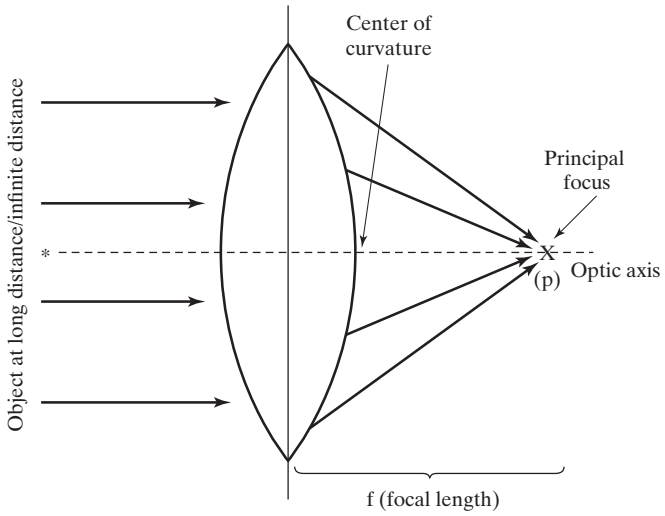


FIGURE 5 Lens focusing light passing by an object far enough from the lens that the light illuminating it is travelling in parallel and not diverging rays, as shown in the next figure.

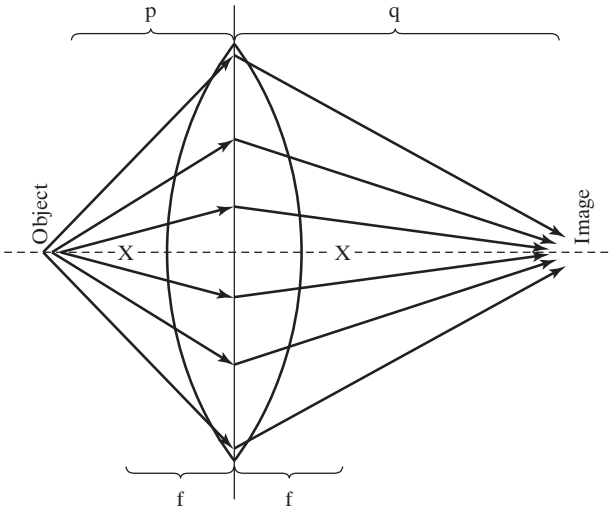


FIGURE 6 Lens with closer object. The focal point moves away from the lens as the object moves closer.

As the object moves closer to the lens (i.e., as p decreases), the point where the image is in focus moves away from the lens (i.e., q increases). The value of f, intrinsic to the lens, does not change. This relationship allows for calculation of the magnification as

$$m = \frac{-q}{p} \tag{4}$$

where the negative sign indicates inversion of the image. If an object is placed such that p is twice the focal length, then q will also be twice the focal length, and the magnification of the image that focuses at point q will be $2f/2f = 1$ (no magnification).[3] At any other combination, the image is enlarged or reduced.

For example, consider a lens that has a focal length f of 25 mm, as shown in Figure 7. If an object is placed on the optic axis at a point 75 mm away, the image that comes into focus at q (37.5 mm) appears twice as large as the object itself:

$$\frac{1}{f} = \frac{1}{p} + \frac{1}{q}$$

$$\text{so } \frac{1}{25} = \frac{1}{37.5} + \frac{1}{q} \text{ and } q = 75$$

$$m = \frac{-q}{p} = \frac{-75}{37.5} = -2$$

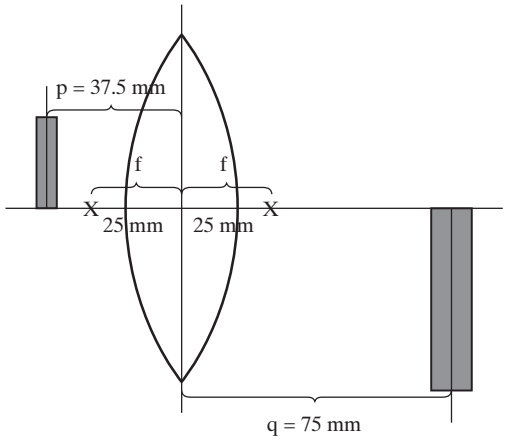
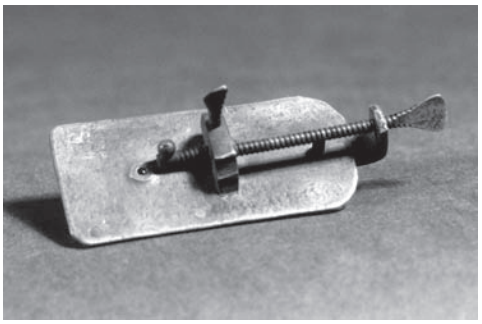


FIGURE 7 Magnification of an image by a lens. The object comes into focus at point q and is magnified to appear twice as large as the real object. Note that the image is inverted.

EXHIBIT B

History of the Microscope

The microscope was conceived in the 1600s by Anton von Leeuwenhoek, who built the first workable magnifying device. Peering into a drop of pond water, he was able to see a variety of microscopic life-forms. Others, including Galileo and Robert Hooke, were also working on optics and magnification, leading to the production of serviceable, if primitive, devices. In the 1700s, microscopists learned to combine lenses to improve their performance, leading to the first compound microscopes. Modern designs emerged in the mid-1900s, and while components and designs continue to improve, the basic principles have changed little in over a century.



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Source: Molecular Expressions™ website, "Optical Microscopy Primer." Available at http://micro.magnet.fsu.edu/primer/anatomy/introduction.html. Downloaded November 2004.

The image formed at q is called a **real image,** because it exists at a definable point in space and can be captured by placing a screen at the focal plane. Its existence does not depend on looking through a lens, as does that of a virtual image. The distance from the object to the lens and the distance from the lens to the focused image are critical in microscope and spectrometer design.

A compound, or binocular, microscope is constructed by creating a magnified real image and magnifying this image again to produce an image that is focused on the retina of the viewer. As shown in Figure 8, the sample is placed on a stage, and light is transmitted upward through it and into the first lens, or **objective lens.** The image comes into focus some distance away, inside the body of the microscope. This is a real image that can be viewed directly if desired. This real image is magnified again by the **eyepiece,** or **ocular lens,** to produce a virtual image seen by the viewer. The total magnification of the object is the product of the objective and ocular lens magnifications, so if the ocular is 10× and the objective lens is 40×, the virtual image appears 400 times larger than the sample.

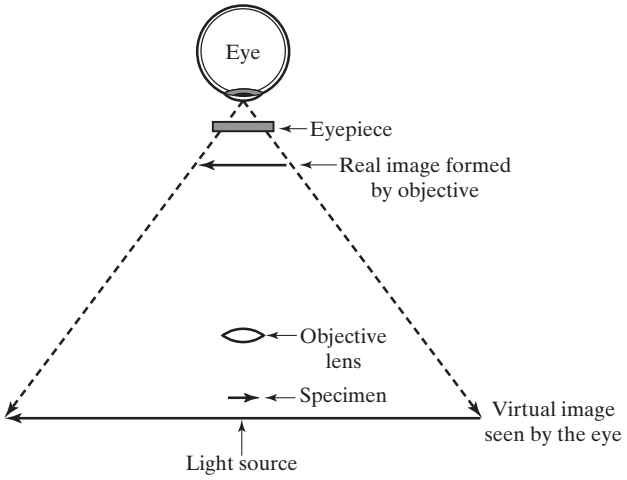


FIGURE 8 A simple microscope. The viewer sees an image such as that produced by a magnifying lens; the difference is that what is magnified is not the object, but a magnified image of it. The dark arrows indicate the orientation of the sample and its images at different points in the light path.

A compound microscope contains more optical components and lenses than just two simple lenses. In fact, the objective and the ocular are not single lenses, but rather a system of lenses, as shown in Figure 9. However, in the interests of clarity, we will treat them as if each were a single lens. There are two additional lenses, one that focuses light emerging from the source of illumination and one that condenses this light into a tight cone that passes through the sample. A simplified optical path of a compound microscope is illustrated in Figure 10. Light from the source lamp is focused into convergent rays by the lamp **condenser** (a) and is then condensed again into a tight cone of light by the substage condenser (b). Light passes through the sample (c) and into the objective lens (d), which forms a magnified real image in the body tube of the microscope (e). The ocular lens (f) creates the virtual image seen by the viewer (g). The spiral object represents the image of the lamp filament. Note that at three points a real image of the lamp filament is created. Although the filament is not viewed when a specimen is examined, its image is used to align the components of the microscope.

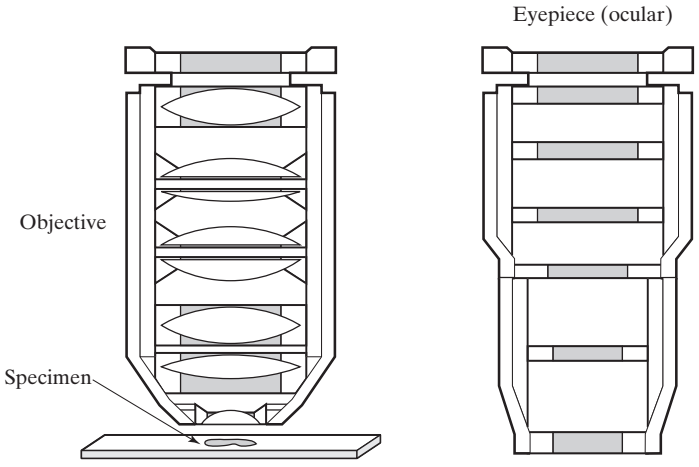


FIGURE 9 The objective and eyepiece lenses of a microscope are actually a series of lenses encased in a single housing.

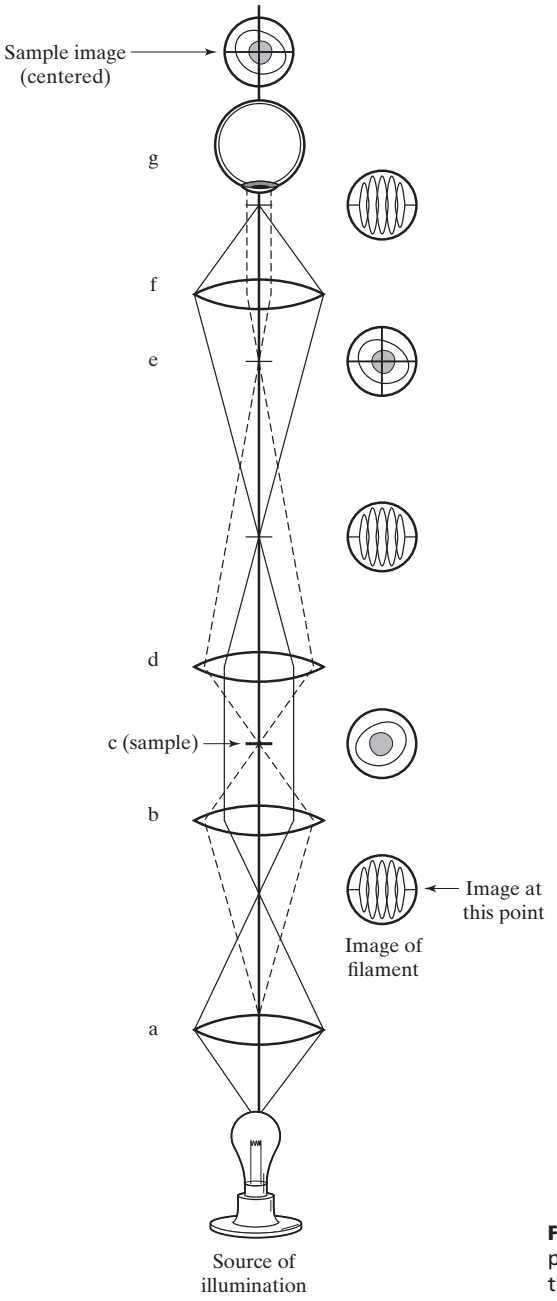


FIGURE 10 The simplified optical path found in a compound microscope, transmission mode.

EXAMPLE PROBLEM 2

Why does the working distance decrease with increasing magnification?

Answer:

Magnification, numerical aperture, resolution, and working distance are all related, and optimizing any one of them involves compromises with the others. To increase the magnification of a lens, the curvature must increase. The more curved a lens is, however, the less light it can collect. Yet a high numerical aperture is desirable in order to collect the largest cone of light emerging from the sample and to provide maximum resolution. The only way to compensate for the curvature of high-magnification lenses is to move the lenses closer to the sample, decreasing the working distance.

Figure 10 illustrates **Köhler illumination,** the most common type of illumination utilized in forensic chemistry. To establish Köhler illumination, the image of the filament is used to align the lenses and to set **apertures** such that the field of view (the portion of the sample being examined) receives maximum illumination. The cone of light directed through the sample is adjusted to illuminate only the field of view and nothing more. The image of the filament is centered and focused with the **Bertrand lens,** which is inserted either inside the body of the microscope or near the ocular lens. Establishing Köhler illumination is analogous to aligning the optics in a spectrometer and setting slit widths.

Important measurements and considerations for microscopy are shown in Figure 11. Optimal imaging is attained when the objective collects the maximum possible amount of light passing through the sample. Ideally, the **angle aperture** (AA, or angle of acceptance) should match the ability of the objective lens to collect light transmitted through the sample. Objective lenses are characterized by their **numerical aperture (NA),** expressed as $N \sin(AA/2)$, where N is the refractive index of any material between the condenser and the specimen. This calculation assumes that the sample is mounted on a glass slide.[3] The larger the NA, the more light is collected and the better is the image quality. As magnification increases, the NA of the objective lens increases also. The ability to resolve fine structure and detail in a sample depends on the quantity of light passing through it and is directly related to the NA of the objective lens. **Resolving power** is expressed as $0.6\lambda/NA$, where λ is the wavelength of illuminating light. Wavelength matters because, as mentioned previously, dispersion is a function of wavelength. This property is the basis of light dispersion by a prism.

At low magnification, more of the sample area (the **field of view**) can be seen, and focus can be maintained throughout a deeper portion of the sample (**depth of focus**). As the magnification increases, both the field of view and the depth of focus decrease. Also, as the magnification increases, the working distance decreases. If a sample has significant depth, such as a sample containing fibers or a sample under a coverslip, short **working distances** can result in physical limitations. In such cases, the sample may not fit under the objective.

Forensic chemists use two other kinds of microscopes. The first is a stereomicroscope with magnification in the range of 40×. Stereoscopes are used for preliminary investigations and the sorting of fibers, soil, paints, glass, and for particles. The second kind of microscope is an adjunct to the compound microscope that incorporates

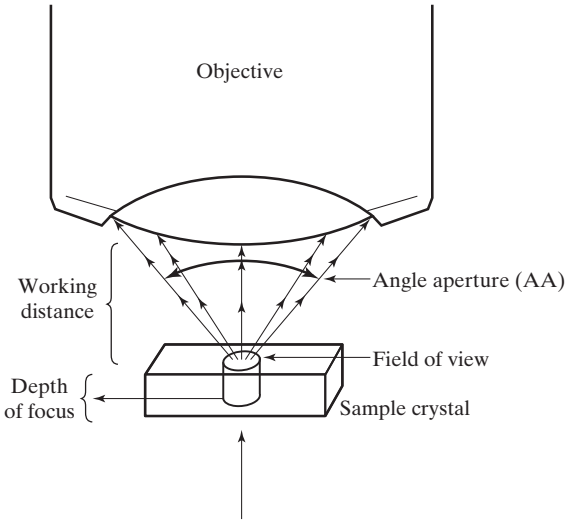
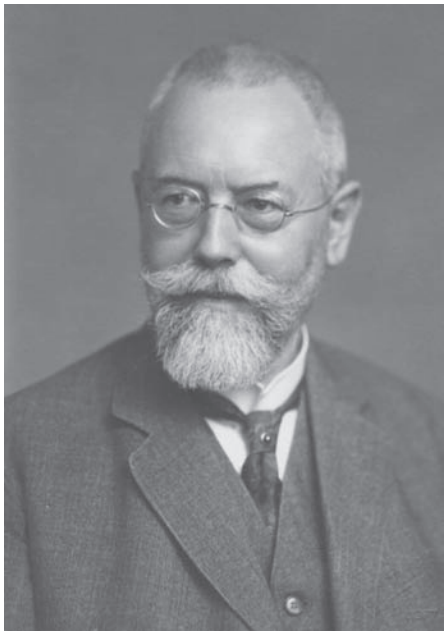


FIGURE 11 Important characteristics and descriptors used in microscopy.

EXHIBIT C

August Köhler (1866–1948)

Köhler developed the illumination pathway that is standard today in forensic microscopy. He worked extensively in the field of photomicrography, an infant science in the late 1800s. Köhler used the method of illumination now named after him to obtain full, even, and bright lighting of specimens that was essential for early photography.



Carl Zeiss Archives

Source: Murphy, D. B. *Fundamentals of Light Microscopy and Electronic Imaging. Danvers,* MA: Wiley-Liss, 2001, p. 9.

polarized light. **Polarized light microscopy (PLM)** is central to forensic science and is used extensively in forensic chemistry and trace evidence analysis. Probing samples with polarized light reveals information about their crystal structure and organization and thus about their chemical structure. An overview of PLM is illustrated in Figure 12.

Visible light such as that from the sun or a lamp vibrates in all directions. When sunlight reflects off of a window, glare results. Polarized sunglasses reduce glare by blocking all vibrational directions except one. In a microscope, inserting the polarizer in the light path does not produce a color change. It is only when the analyzer is placed in the light path ("cross polars") that things get interesting. If the sample does not change the direction of vibration, the field of view appears black, because the analyzer blocks out the light. However, many samples alter the light path. These samples under crossed polars appear colored against a black background. Many materials that are crystalline or **pseudocrystalline** interact with polarized light, and the observation and measurements of the interactions that take place reveal information about the chemical structure of the sample.

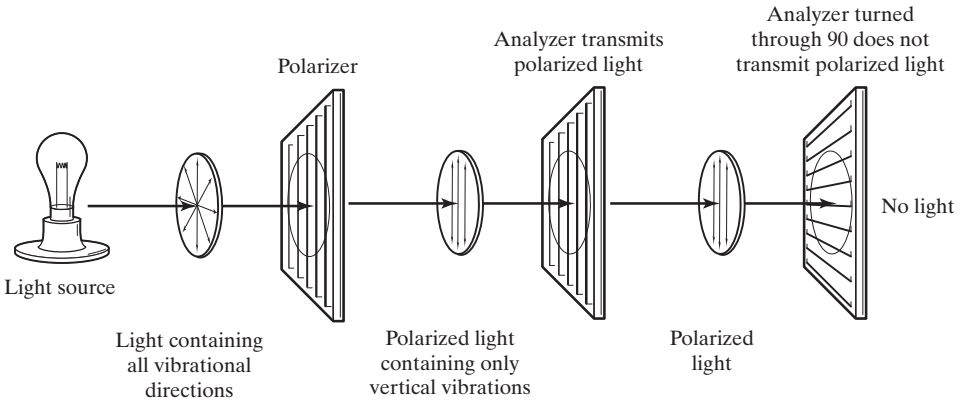
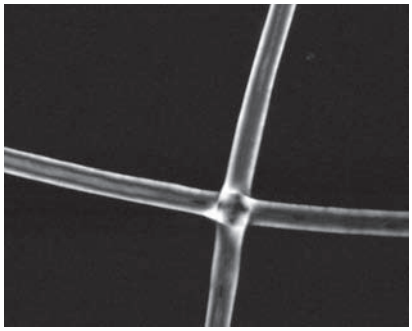


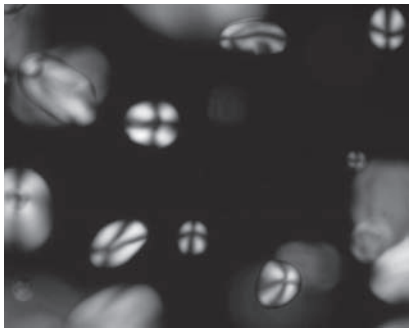
FIGURE 12 The basis of PLM. A polarizing filter called the polarizer is positioned between the light source and the sample. The light emerging from the filter vibrates in only one plane. A second filter positioned perpendicular to the polarizer is placed in the light path, above the sample but before the ocular lens. This filter is called the analyzer. If the sample does not change the direction of vibration of the light, the analyzer will block the light, and the field of view will appear black.

EXAMPLE PROBLEM 3

Under polarized light, starch grains have a distinctive appearance that is similar to the pattern produced when two synthetic fibers overlap. What does this pattern indicate?



Suzanne Bell



Answer:

In both cases, what is observed is an interference pattern. Because the "crosses" appear only in polarized light, the pattern indicates that both starch and the fiber have an ordered, or pseudo-crystalline, structure. Starch is a glucose polymer, and the fiber is a synthetic polymer, so the appearance of interference is not surprising. Black areas are portions of the sample that completely block the polarized light (via destructive interference); the brighter locations are where constructive interference occurs.

Materials such as glass are not crystalline, and there is no order (in the sense of repeated and organized crystals) to the way in which their atoms are organized. Since there is no directionality to the internal organization, light that is polarized when it enters the glass remains polarized when it exits the glass. It does not matter how the light enters or exits, nor does it matter how the glass is oriented: polarized light remains

polarized after traversing the glass. This characteristic is not to be confused with a material's refractive index. Because its speed changes when light enters and exits the glass, the light is refracted, even if it is polarized. There is no difference in refraction characteristics between polarized and unpolarized light. Glass and other such substances are referred to as **isotropic**, meaning that they have only one refractive index; the same is true of cubic crystals.

Non-cubic crystals behave differently when polarized light passes through them, because they have a repeated structure, internal organization, and directionality. As shown in Figure 13, 14, and Applying the Science 1, light entering from one direction encounters a different organization of atoms than does light entering from the perpendicular direction. If upon entering the crystal, polarized light vibrates in a plane parallel to a crystal axis, the light is unaffected, and the field of view remains dark. However, at orientations other than parallel, the polarized light is split into two components that vibrate perpendicular to each other along the axes. The two components are called *fast* and *slow*, as shown in Figures 14 and 15. In one direction, the light encounters more atoms than in the other and thus is slowed to a greater extent than light that encounters fewer atoms in the other direction. Other factors, such as size of the atoms, also influence the degree of difference. In effect, the crystal acts as a beam splitter.[4] The two rays, one fast and one slow, emerge from the crystal out of phase by a distance called the **retardation distance**. When the two vectors emerge from the crystal, the interfere to form a series of waves, one for each color. These waves have been rotated by different angle; those rotated by 90 or 270 degrees are able to pass through the analyzer and are visible. In other words, as soon as the beams emerge from the crystal, they interfere. Adding the two vectors of the two components produced a rotation of the angle of polarization; it is this angle that varies with wavelength and thus produces different colors. The observed colors correlate to the crystal structure and thickness of the sample because the thicker the material, the greater is the retardation. (We will encounter this principle again later in the chapter in a discussion of interferometry.)

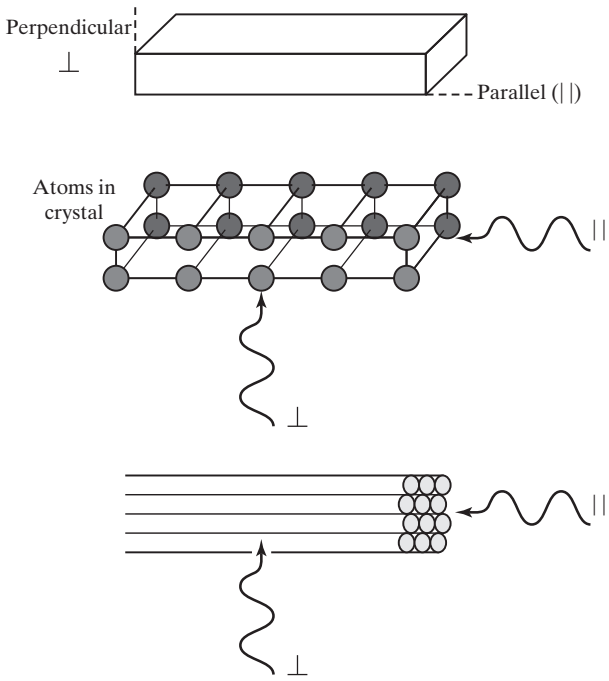


FIGURE 13 Crystalline and pseudocrystalline materials. The ordered structure of the crystal means that light will "see" a different environment, depending on the orientation. Synthetic fibers (lower frame) show similar behavior, since their structure is ordered (pseudocrystalline).

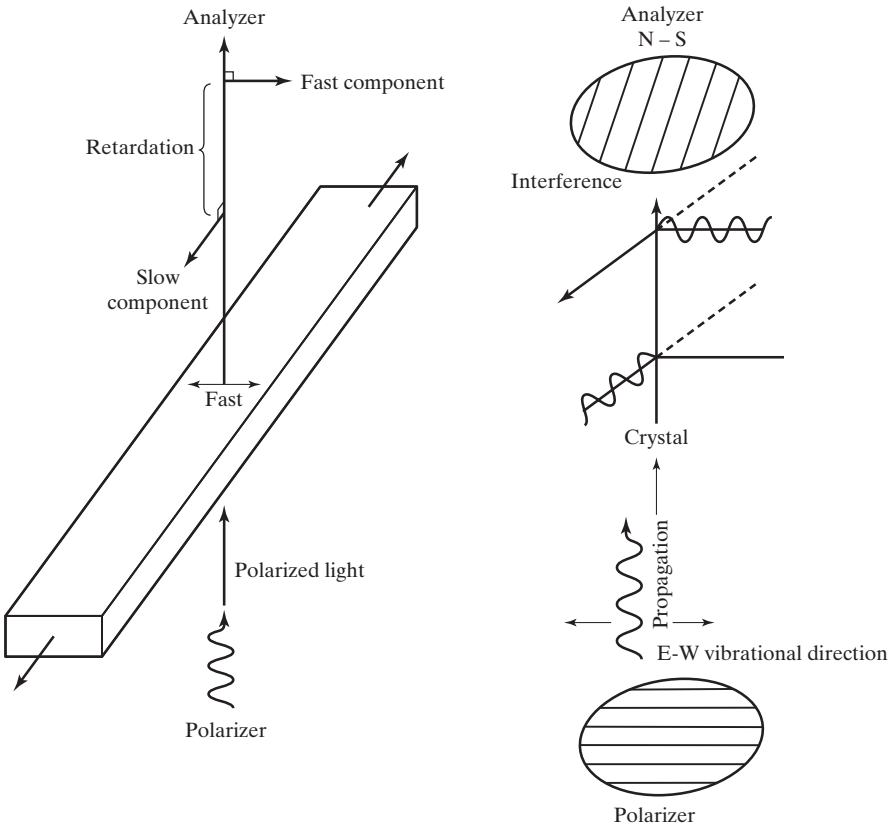


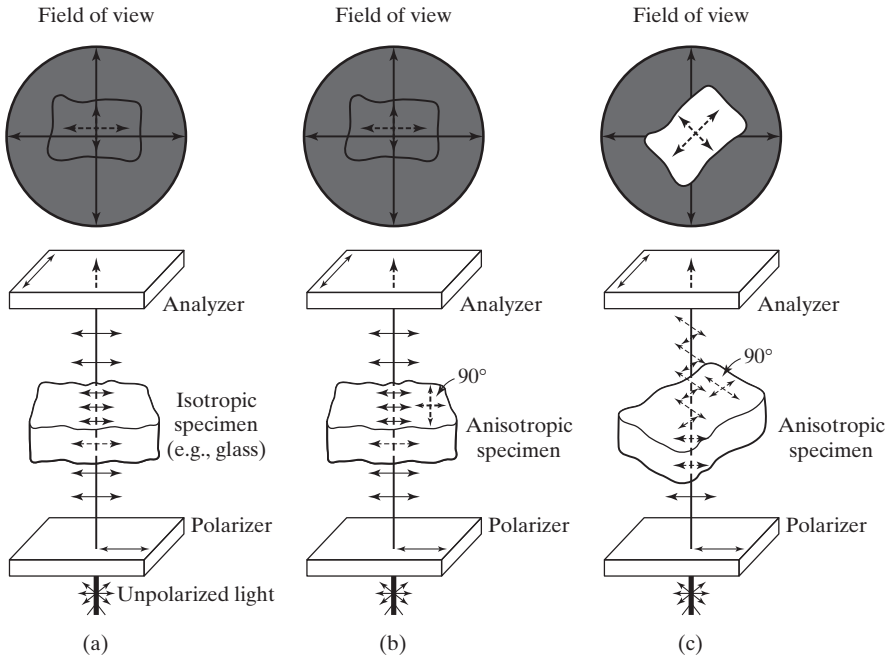
FIGURE 14 Splitting of polarized light into two components by a birefringent sample. As light propagates upward through the crystal, its interaction with the sample alters the vibrational direction of the wave, which divides into two components, one that emerges quickly and one that lags owing to the different interactions encountered. These two components vibrate perpendicular to each other. Once they leave the sample, they recombine producing colors as described on the previous page. The notations N-S and E-W stand for directions and are used to indicate when the analyzer and polarizer are at 90° to each other.

Materials that display **retardation** are **anisotropic,** meaning that they have more than one refractive index. If an anisotropic fiber or crystal is placed on a microscope stage under crossed polars and is rotated, there are two positions from which the fiber disappears from view. This phenomenon is called **extinction**, and it occurs when the light is propagating along a direction parallel to a crystal axis. At 45° between these extremes, maximum brightness or intensity occurs, because that is the point where the difference between the relative magnitude of the fast and slow rays is the greatest. Because the sample is illuminated by white light and because dispersion is a function of wavelength, a range of vivid colors is observed, depending on the thickness and the degree of retardation of the material. Accordingly, PLM can be used to determine the thickness of anisotropic materials.

The numerical difference of the two refractive indices (parallel and perpendicular) is called **birefringence** (abbreviated B or Bi). Materials with low birefringence show small differences, whereas highly birefringent materials have large numerical differences. In addition, light entering a thicker portion of a sample interacts more and undergoes a greater degree of retardation than light entering a thinner portion of the sample. As a result, a birefringent material often shows a banding pattern of repeating colors. This pattern is related to the thickness of the sample and can be thought of as a topographical map. The colors are correlated with thickness by means of a Michel-Levy chart, after Auguste Michel Levy, French geologist and crystallographer.

APPLYING THE SCIENCE 1 Glass Analysis and Refractive Index

(a) An isotropic specimen, such as glass, remains dark in any orientation when it is placed between crossed polarizers. (b) An anisotropic substance appears dark when it has been positioned in its extinction position between crossed polarizers. If the specimen is rotated 45°, it will be at a position of maximum brightness, as shown in (c). The object now appears bright in a dark background.



One of best illustrations of how polarized light interacts with solid samples is found in the forensic analysis of glass. As shown in the figure, polarized light is not altered by an isotropic substance such as glass, and under crossed polars, the field of view appears black, since the analyzer effectively blocks all light that has passed through the glass. In contrast, anisotropic materials will appear black only when the optical axes in the samples are aligned with the polarizer or analyzer.

Related to, but distinct from, birefringence is **pleochroism:** variations in a material's absorbance of light, as opposed to variations in its refractive index. To determine if a sample is pleochroic, it is placed on the stage and illuminated as with polarized light, but not under crossed polars. Pleochroism can be visualized by rotating the stage and seeing if the sample changes color. If the color changes only at 90° angles of rotation and only two colors are visible, the sample is **dichroic.** Dichroism is common in fibers: when the fiber is oriented perpendicular, the color is different than when the fiber is oriented parallel.

Many other optical and crystalline properties are measured and explored with PLM. The interested reader is directed to any of the references listed at the end of the chapter for more information. One website deserves special mention: Nikon's

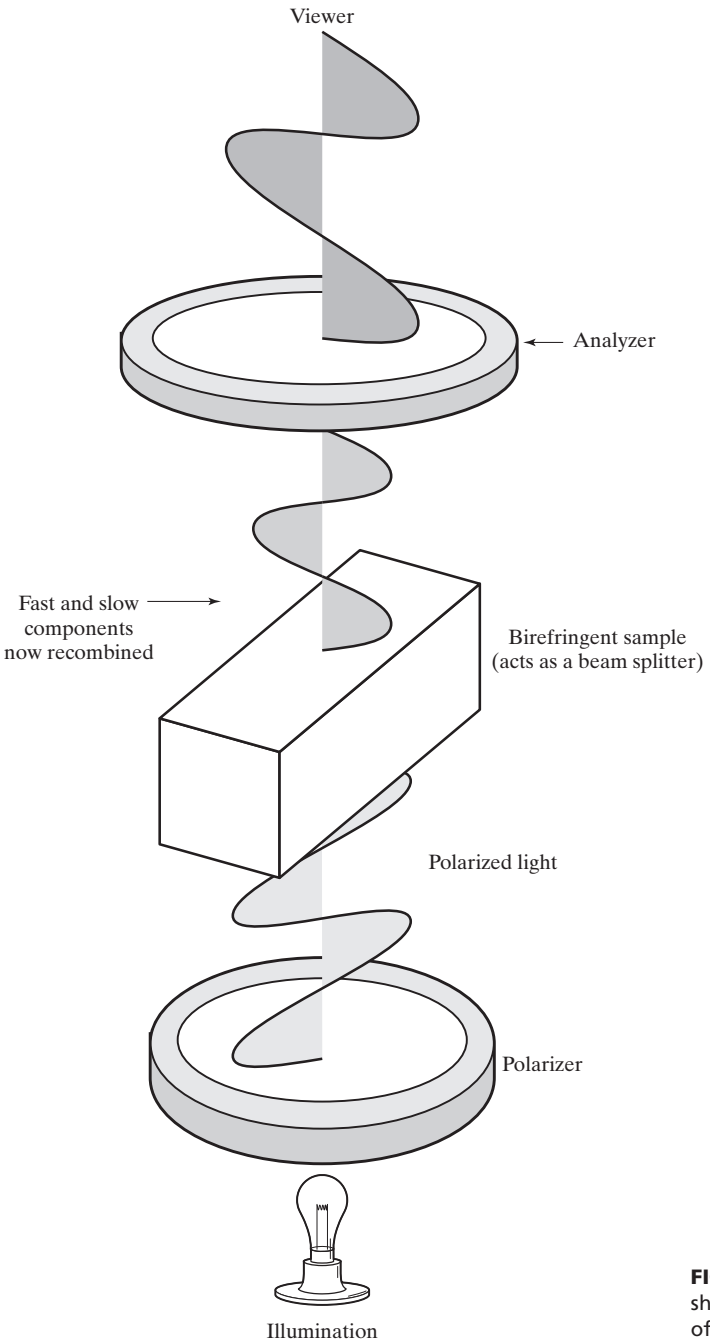


FIGURE 15 Another version showing the implementation of polarized light microscopy.

"Microscopy University" (*http://www.microscopyu.com/*). This site is a wonderful resource for learning about microscopes and microscopy and includes excellent figures and tutorials.

2 SPECTROSCOPY

Spectroscopy is the use of electromagnetic energy to probe matter and interpret the results to characterize chemical structure (Figure 16). When energy is absorbed by an atom, an ion, or a molecule, the energy is converted in accordance with the first law

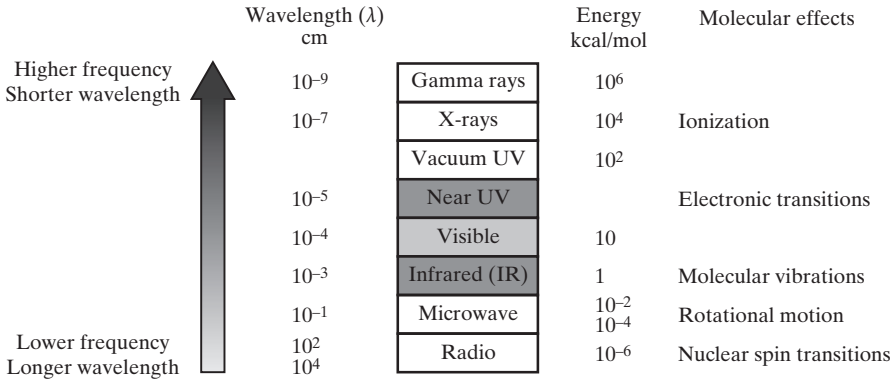


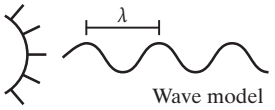
FIGURE 16 The result of probing energy with matter depends on the energy involved. High-energy X-rays remove electrons from an atom, causing ionization; less energetic UV/VIS energy promotes electrons but does not eject them from the atom or molecule; infrared energy and weaker forms are not energetic enough to promote electrons. Rather, absorption in these regions affects the kinetic energy of molecules by causing bonds to stretch (vibrational spectroscopy) or to rotate faster (microwave spectroscopy). At low energy, only the spin of the nucleus itself is affected (NMR spectroscopy).

of thermodynamics. Absorption promotes the sample into an excited state, the exact form of which depends on the type of electromagnetic energy absorbed. Broadly speaking, spectroscopy can be divided into *atomic* (elemental) and *molecular* (having to do with compounds) spectroscopy, based on which transitions occur and where they occur. Regardless of the mode of energy conversion, the wavelength of light absorbed and the intensity of the absorption can be used to extract qualitative and quantitative chemical information. In forensic analytical chemistry, UV/VIS/IR and elemental spectroscopy are routinely used, whereas other kinds, such as nuclear magnetic resonance (NMR) spectroscopy and rotational spectroscopy are rarely employed.

EXHIBIT D

A Light Review

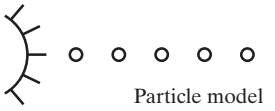
Electromagnetic energy is modeled as both a wave and a particle. In the wave model, frequency and wavelength are the descriptors and are related to each other through the speed of light, c. In the particle model, electromagnetic energy is visualized as a discrete massless particle carrying a discrete amount of energy. The two can be related through the frequency, ν.



Wave model

$C = \lambda\nu$

$C = 3.0 \times 10^{10} \frac{\text{cm}}{\text{sec}}$



Particle model

$E = h\nu$

$h = 6.63 \times 10^{-34}$ J sec or 10^{-37} KJ sec

Planck's constant

EXHIBIT E

Newton and Bunsen: The Road to Spectroscopy

None other than Sir Isaac Newton is credited with the crucial first steps toward spectroscopy, with his use of the prism to disperse sunlight. He also worked with different slits placed in the path of the dispersed light and observed that the more "pure" (monochromatic) the light, the lower was its intensity—a trade-off spectroscopists continue to grapple with. The first primitive flame emission experiments occurred in 1752, but infrared energy was not recognized as a form of energy until the early 1800s. The first device similar to spectrometers was reported during the same period, and the first true spectrometer was credited to two famous chemists: Bunsen (of burner fame) and Kirchhoff, who reported his findings in a paper published in 1860. Their device was a simple flame emission device. IR techniques were first developed in the 1880s, followed by X-ray analysis in the early part of the twentieth century. Instrumentation for work across the spectrum improved continually, with the next big breakthrough coming in the 1950s with the development of atomic absorption spectrophotometry. By that time, forensic chemists were using X-ray diffraction, UV/VIS, and IR spectroscopy. The next advance was the development of Fourier transform techniques, made possible by computers and lasers, both of which became widely available by the 1990s.

Source: Szabadváry, F. "Chapter XI: Optical Methods," in F. Szabadváry, History of Analytical Chemistry, tr. Gyula Svehla. Oxford, U.K.: Pergamon Press, 1966.

2.1 The Basics

Microscopy can be thought of as spectroscopy that uses visible light as the probing radiation and the human eye as a detector. There are many other parallels between the two techniques. As in microscopy, in spectroscopy light can be scattered, reflected, absorbed, and transmitted. The energy can be polarized or not; interference and scattering occurs and can be exploited even if it cannot be seen. It is not surprising that the first implementation of instrumental spectroscopy was based on color, which is the visible manifestation of absorption of light in the visible range. As mentioned in the previous section, the absorption of a photon can trigger several events, depending on the energy of the photon. However, absorption is one of many phenomena that can be monitored to extract qualitative and quantitative information. As shown in Figure 17, a sample (denoted M) may absorb energy and consequently be promoted to some type of excited state M*. As a result of the promotion, the transmitted signal is attenuated and reduced in proportion to the sample's concentration, the path length of the signal, and the molar absorptivity (in accordance with Beer's law) of the sample. The absorbance of the sample is given by the formula

$$A = \varepsilon l c \qquad \textbf{(5)}$$

where b is the path length, and c is the concentration. Spectrometers are typically designed with a constant path length. For a given absorber at a given wavelength, the molar absorbtion ε is a constant; therefore,

$$A = kc \qquad \textbf{(6)}$$

which is a linear relationship of the form $y = mx + b$, where $b = 0$. Equation 6 represents a way of linking instrument response to the concentration of the sample. Note that with most calibration curves, b (the intercept) is usually not zero.

Some relaxations $M^* \longrightarrow M$ result in the immediate or delayed emission of photons. Such emission is in all directions, not just in the optical path from the source to the detector. This is an important observation and is exploited to differentiate signals due to

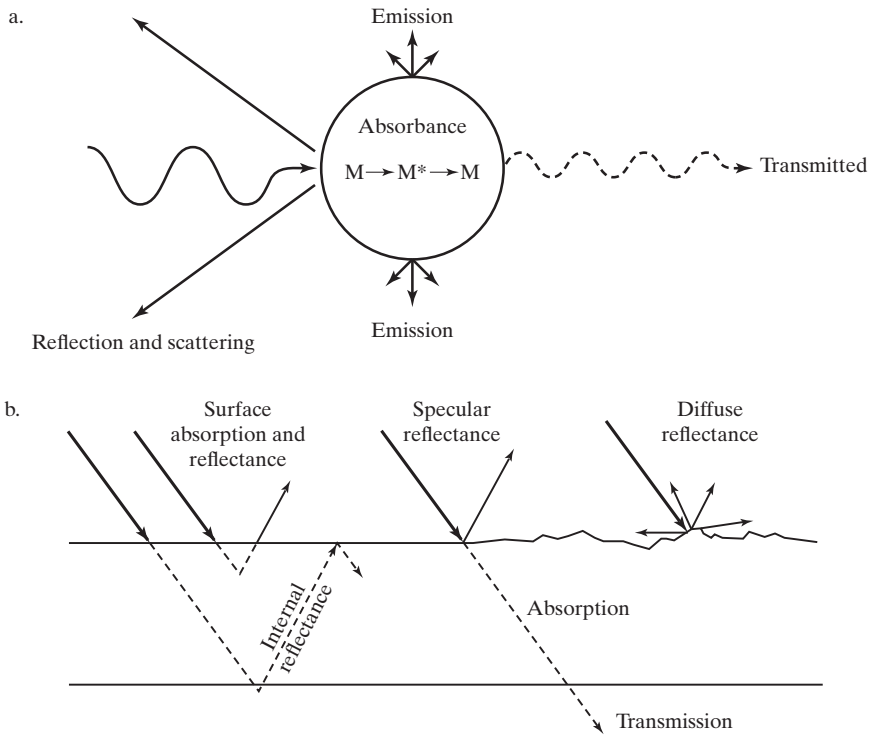


FIGURE 17 (a) Some potential outcomes of the interaction of energy with matter. If emission results from the relaxation of $M^* \longrightarrow M$, it will be in all directions. (b) Potential outcomes of interactions at a surface, particularly reflections.

emission from signals due to absorption and transmittance. Regardless, emission spectroscopy is governed by Beer's law, and the linear calibration applies.

Other types of energy–matter interactions, such as reflection and scattering, can also occur. Both phenomena can be exploited to extract chemical information. **Specular reflection** is simple reflection from a surface, with no interaction between light and the surface and where the angle of reflection equals the angle of incidence. On rougher surfaces, such as powders, the reflection is diffuse because the angles of reflection are randomized. The difference is the same as that between looking in a shiny, polished mirror and looking in one with a scratched and scarred surface. The energy may penetrate a few microns into the material, undergo some absorptive interactions, and then reflect back, a phenomenon referred to as **surface absorption–reflection (SAR).** Finally, certain materials can undergo internal reflections, a property that is used in attenuated total reflectance techniques in the infrared region. Fiber optics works on the basis of internal reflection that, in effect, traps the energy within the fiber.

All absorptive processes are governed by the first law of thermodynamics (energy is neither created nor destroyed; it only changes form), and all involve the excitation of a sample M in the ground state to a higher energy excited state M*. The energy absorbed, ΔE, must be sufficient to bridge the gap in energy between the two levels. The excited state could be the result of the ejection or promotion of an electron or a change in the electron's vibrational or rotational state. Regardless, the excited state is unstable and the system decays back to the more stable state. The process is shown in Figure 18. Excess energy can be dissipated by one of three generic processes. First, molecules in the excited state can collide and convert their excess energy to kinetic energy, a process favored in solutions, where molecular collisions are numerous and frequent. Because no electromagnetic radiation is emitted, these conversions are

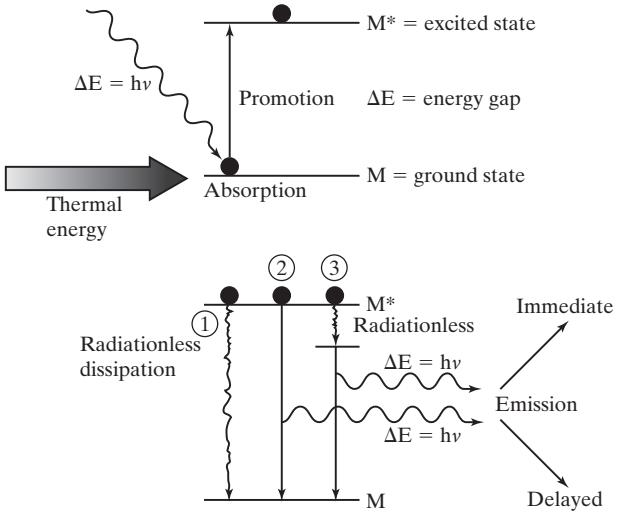


FIGURE 18 The promotion of an atom, an ion, or a molecule to an excited state can be driven by the absorption of electromagnetic energy, thermal energy, or another form of energy, as long as the absorbed energy equals or exceeds the energy gap. Once excitation occurs, the system is unstable and tends to dissipate the excess energy. The small wavy line represents dissipation by conversion to kinetic energy (i.e., heat), while other relaxations involve the emission of a photon, the energy of which corresponds to the energy gap traversed.

referred to as **radiationless transitions,** and this is how excited-state energy is dissipated in UV/VIS interactions. Second, the $M^* \longrightarrow M$ transition can result in the emission of a photon with energy equal to the energy of the original absorbed photon if that is how the excited state was generated. This process is known as emission. Third, a combination of radiationless transmission and emission can occur, resulting in the emission of a photon of lower energy that corresponds to the smaller energy gap traversed in the relaxation. The emission of the photon may be immediate or delayed. In most instruments, the excitation $M \longrightarrow M^*$ is instigated by electromagnetic energy ($E = h\nu$) or by thermal energy, such as that of a flame, a furnace, or plasma.

When a delayed emission of a photon occurs, either directly or with intermediate steps, the process is referred to as fluorescence or phosphorescence (Figure 19). The difference between fluorescence and phosphorescence is time: phosphorescence is delayed relative to fluorescence, and phosphorescence generally lasts longer. If the levels are vibrational levels exploited in infrared methods, the relaxation (dissipation by heat) is nearly instantaneous—on the order of a trillionth of a second (or a trillion relaxations per second). Fluorescence is exploited in many areas of forensic science, including DNA typing instrumentation and fingerprint visualization.

A generic absorption spectrum is a plot of the wavelength of electromagnetic energy interacting with the sample versus the absorbance at that wavelength. Absorbance is governed by Beer's law and depends on the molar extinction coefficient of each transition. In an idealized transition (Figure 20), the absorbance would be reflected in a single sharp peak at the wavelength corresponding to the energy gap. This situation can be approximated in some gas-phase techniques, such as atomic absorption spectrophotometry, but in other techniques and solution environments, the transitions are not singular or sharp. For any one lower energy level, there may be several possible excitations, although some are more likely than others. The more transitions possible, the broader the absorption peak becomes. In a solution matrix, interactions between analyte and matrix components further alter energy gaps such that even a simple transition is recorded as a broadened Gaussian peak, as shown in Figure 21. If more than one transition is allowed, the picture is more complex, but such complexity is not inherently bad from an analytical point of view. The intricacy of infrared spectra facilitates the definitive identification of compounds. With other techniques, such as UV/VIS, the spectra are so general as to be nearly useless for identification of specific compounds.

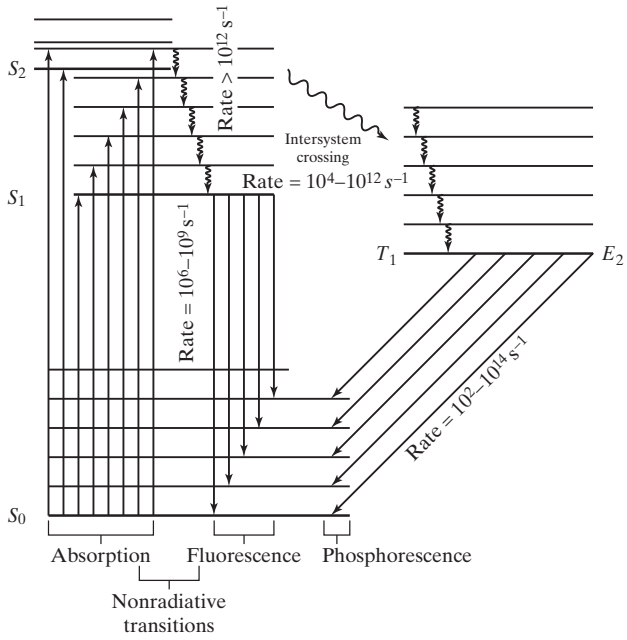


FIGURE 19 Absorption and relaxation modes in emission spectroscopy. "S" refers to singlet states, or those states, in which all electrons are paired with opposing spins (↑ ↓) whereas *T* refers to a triplet state, in which two electrons are paired with the same spin (↑ ↑). The longer-lived states are referred to as metastable and are exploited in lasers.

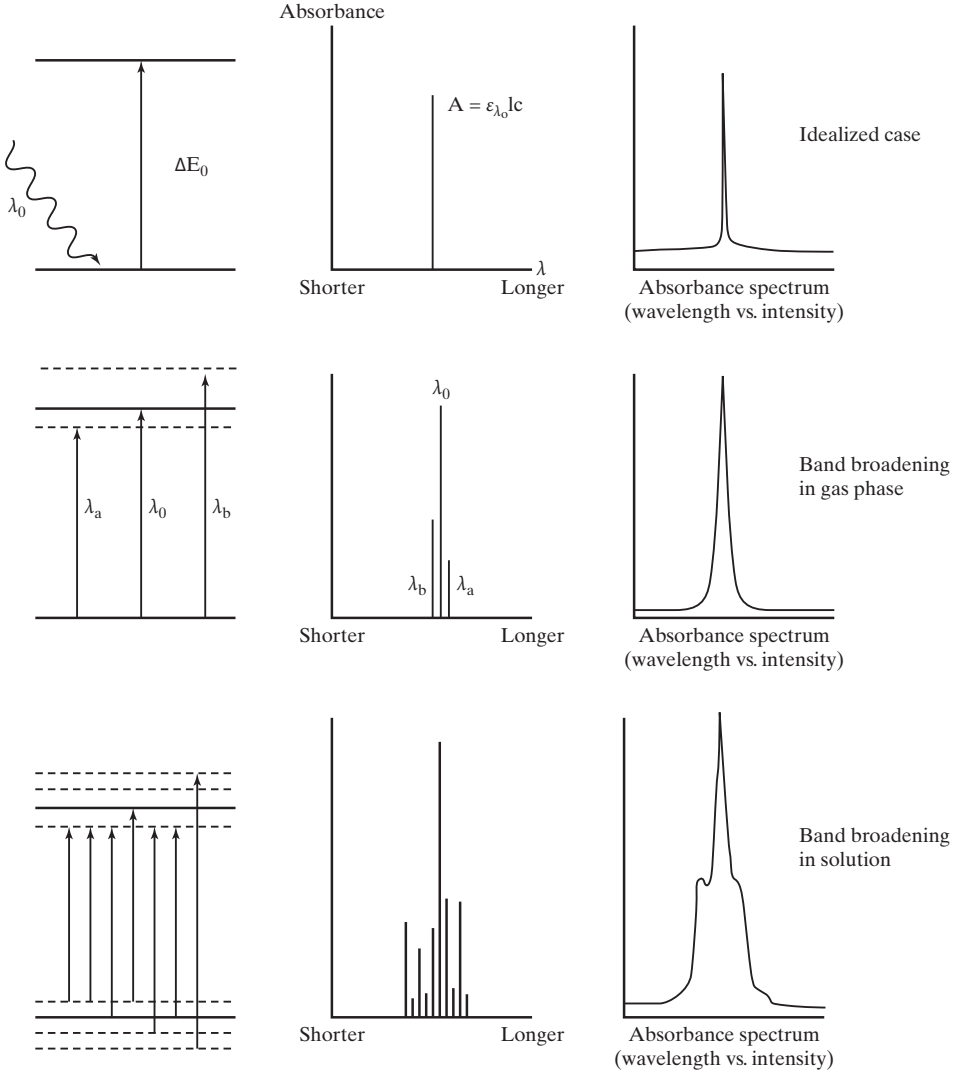


FIGURE 20 Simplified depiction of band broadening. In the top series, there is one possible transition so only one wavelength of light can be absorbed. The absorbance peak would be a single line. The second series down could represent the gas phase where interactions can lead to slight changes in the energy gap amongst the atoms or molecules. As a result, the absorbance band is slightly broader. The third series down represents a more complex matrix, such as a solution, in which a range of interactions lead to even more possible transitions and band broadening.

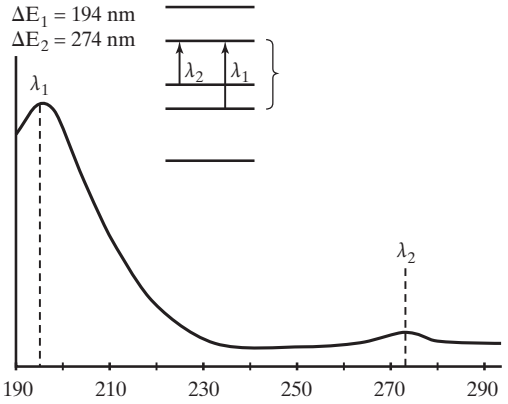


FIGURE 21 When more than one transition can occur, the peak broadens even further. This example is a depiction of the UV spectrum of acetone, with two allowed transitions correlating with two broad absorption peaks.

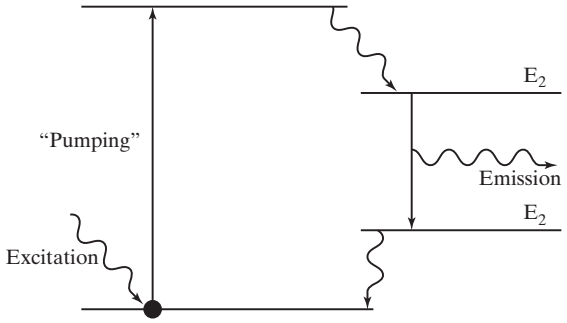
2.2 Instrument Design

Most spectroscopic instruments contain the same generic components but are designed according to the type of electromagnetic energy being probed and whether the energy is absorbed or emitted. This concept is illustrated in Figure 22. Both types of instruments use wavelength filters (**monochromators**) to obtain or isolate monochromatic energy (energy of a single wavelength, which translates into the best resolution possible) from a polychromatic source. Older colorimeters used prisms to break visible light into its component wavelengths, but modern instruments utilize dispersive gratings, which rely on geometry to establish predictable patterns of constructive and destructive interference. One or more slits further limit the range of wavelengths allowed to pass a given point at any given time. The smaller the slit widths, the lower is the intensity of the source, just as decreasing the size of an aperture in a microscope decreases the brightness of the viewed image. Because energy is physically separated by a grating,

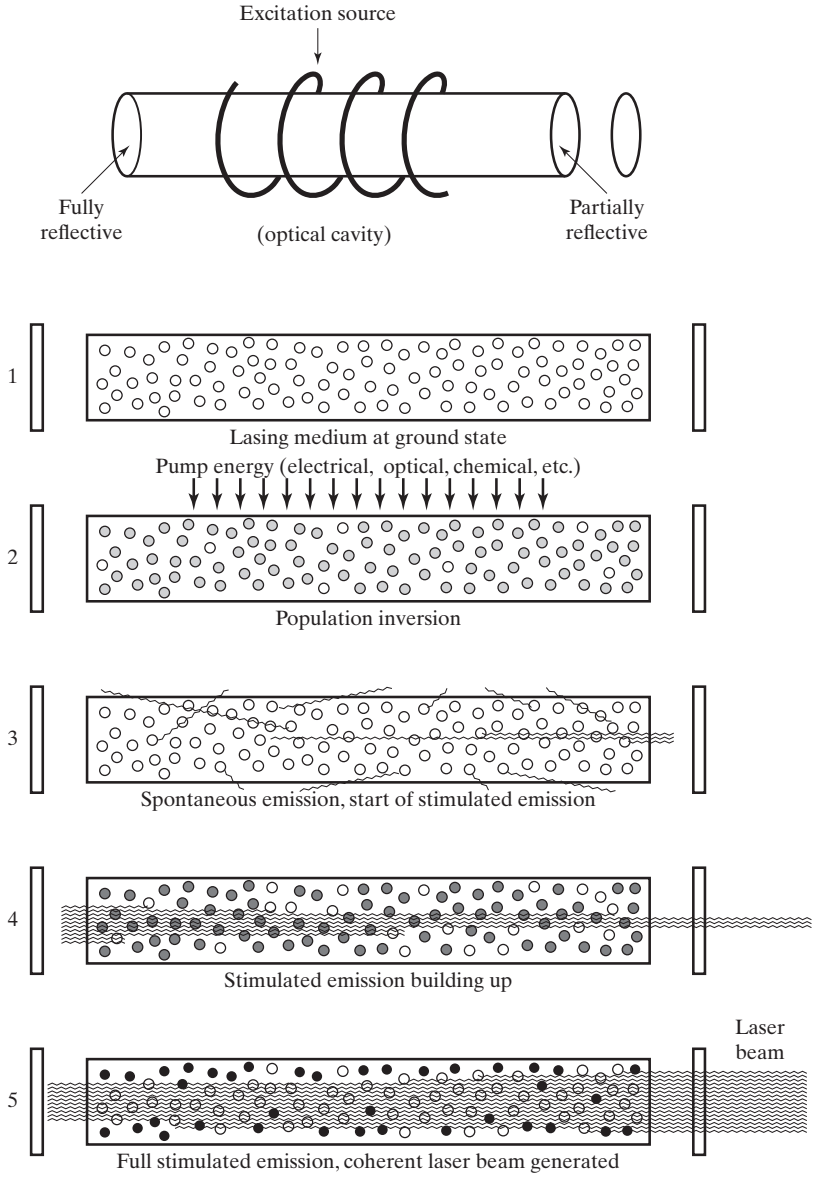
EXHIBIT F

Lasers

Lasers are commonly used as sources of intense monochromatic light for many types of spectroscopy. The word laser is derived from Light Amplification by Stimulated Emission of Radiation. Lasers can be based on **electronic transitions** in molecular systems (e.g., a CO_2 laser) or on an atomic transition such as that in a neodymium yttrium aluminum garnet (Nd:YAG). Laser light is monochromatic, coherent (oscillating in phase), plane polarized, and intense, properties that together make lasers ideal sources for spectroscopy. The beam can be tightly focused in small areas, making lasers ideal for microspectrophotometry as well. The drawbacks of lasers are their cost and somewhat limited lifetimes. Replacing a lamp as a source is cheap, whereas replacing a laser is a several-thousand-dollar proposition.



A laser works by stimulation of the medium to a higher energy state through the input of electromagnetic or other forms of energy, such as thermal or even chemical energy. The system undergoes a radiationless transition to a metastable state (Figure 19, E_2) from which the photon-producing relaxation occurs. A population inversion, in which the population $E_2 > E_1$, is necessary for lasing to begin. Lasing is then perpetuated by a series of internal reflections of the photons emitted by this decay. A laser has four basic components: a medium that can be stimulated to emit light, a source of excitation, a feedback mechanism to perpetuate the excitation, and a means of directing the light out of the source (an output coupler). Different lasers have different mechanisms, but all are based on the foregoing principles. The Nd:YAG is a solid-state laser that emits in the near-infrared region (1064 nm), while a CO_2 laser is a gas laser that emits in the far infrared ($10.6\ \mu m$). Helium–neon lasers emit red light at 632.8 nm.



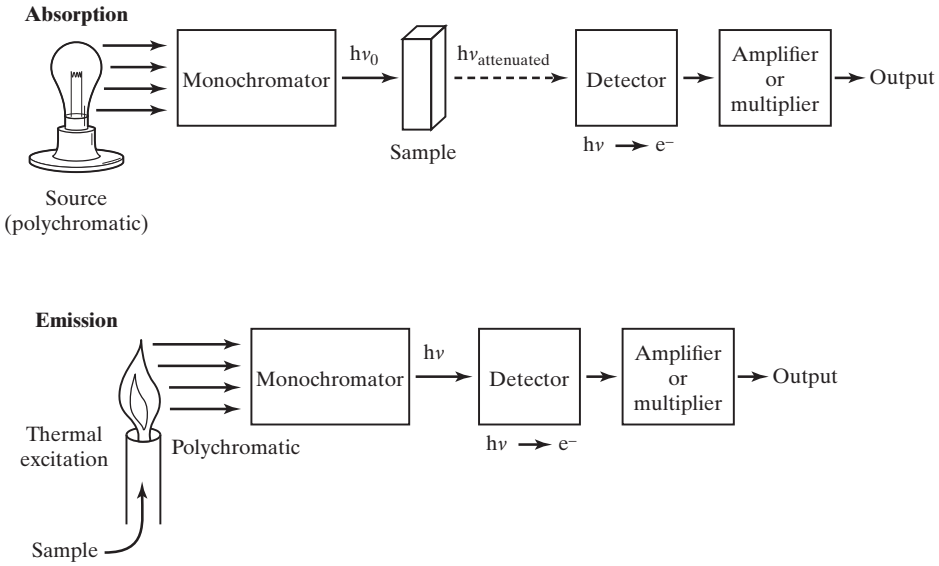


FIGURE 22 Box diagram of an absorption–transmission spectrophotometer (top) and an emission spectrometer (bottom). Shared components are monochromators (typically gratings) and detectors (transducers) that convert photons into electrons and electrical signals.

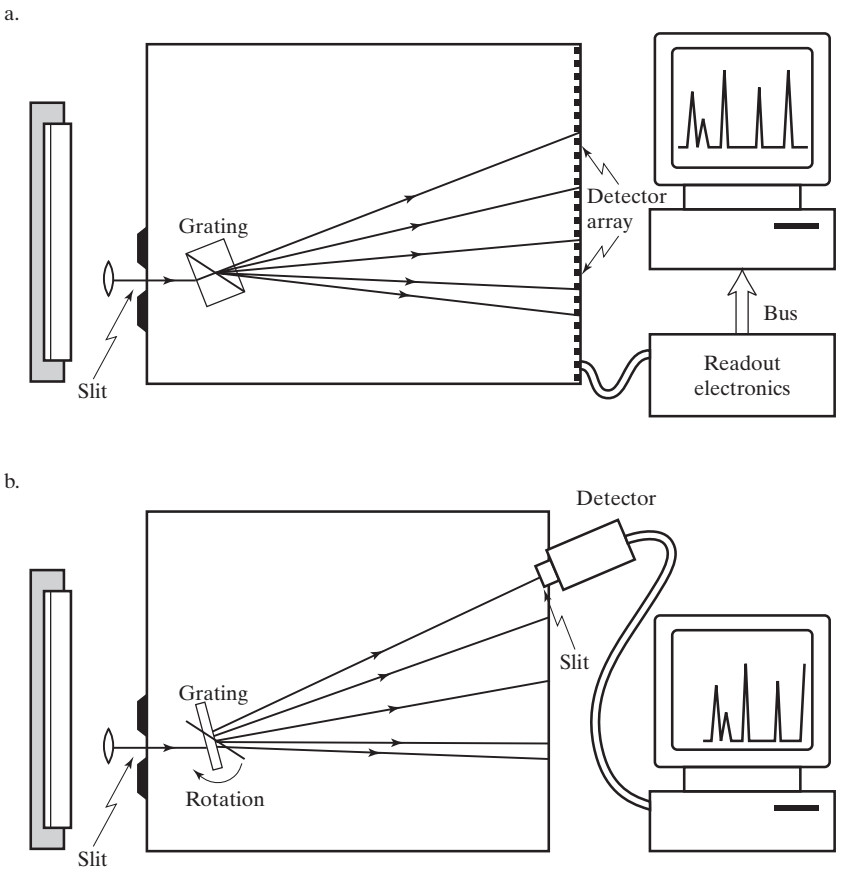


FIGURE 23 (a) Use of a grating to disperse and detect all wavelengths of interest at once. This design is referred to as *simultaneous*. (b) In this approach *(sequential)*, the grating is incrementally moved to direct one wavelength at a time on a fixed detector position. Such a design is also referred to as *scanning*.

it is possible to simultaneously detect many wavelengths. Dispersive gratings can be divided into simultaneous and sequential types, as shown in Figure 23. The other generic category of instruments is based not on dispersion but on interferometry and the Fourier transform. Finally, some instruments, such as atomic absorption spectrophotometers, use monochromatic light, but as is discussed shortly, a monochromator is still required, though in a different place along the optical path.

The source of energy in a spectrometer is thermal or electromagnetic and depends on the type of interactions being probed. Increasingly, lasers are being used either as sources or as part of an interferometer that is coupled to the source. Regardless of the source or region of the electromagnetic spectrum being utilized, the role of the detector is the same across the spectrum—to convert photons into electrons as efficiently as possible. This device is sometimes referred to as a **transducer**, and it can consist of a semiconductor, a charged-coupled device, or some other design. In instruments in which the signal is not strong, such as microspectrophotometers, the detector must be cooled to minimize electronic noise.

2.3 Bandwidth and Resolving Power

All spectrometers are designed to optimize the **bandwidth** of the source relative to the bandwidth of the transition that is targeted while maintaining maximum intensity of the source. Because electromagnetic radiation is a continuum, no light source is truly monochromatic. The *spectral bandwidth* is defined as the width of the radiation band from the source. The bandwidth depends on the source (if monochromatic) or on the slit width of the monochromator. The narrower the slit, the closer the energy is to the idealized monochromatic form; the trade-off is intensity. As the slit narrows, so does the strength of the signal. Figures 24 and 25 illustrate these concepts.

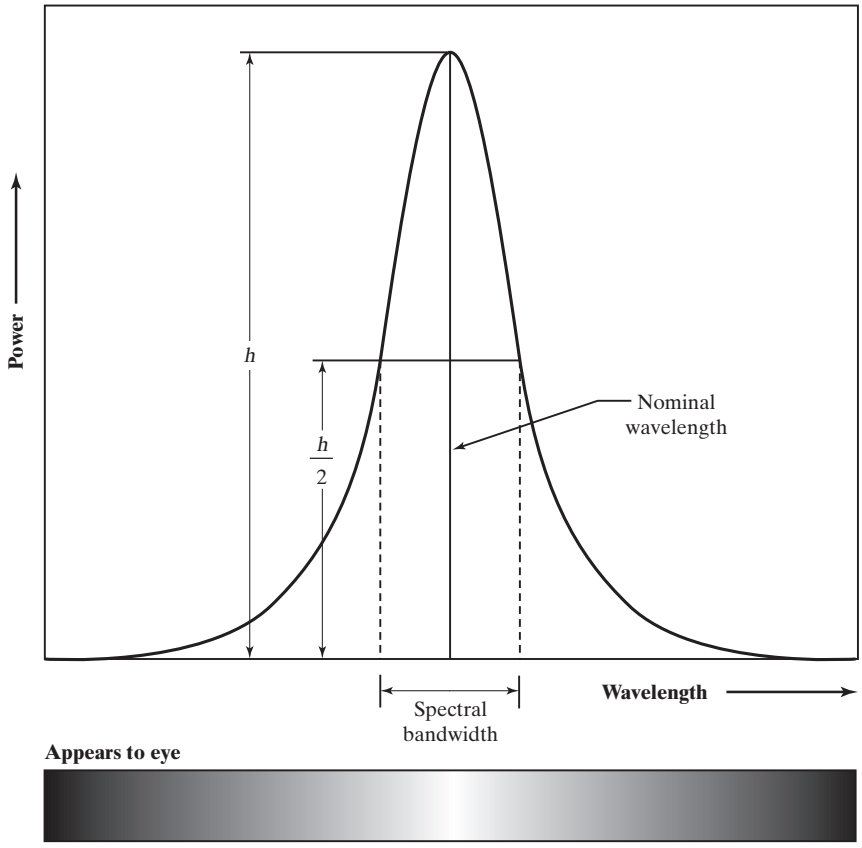


FIGURE 24 Spectral bandwidth of a monochromatic source. Peak power is seen at the nominal wavelength, with detectable intensity on either side. The bandwidth is the full width at half maximum (FWHM) of this peak.

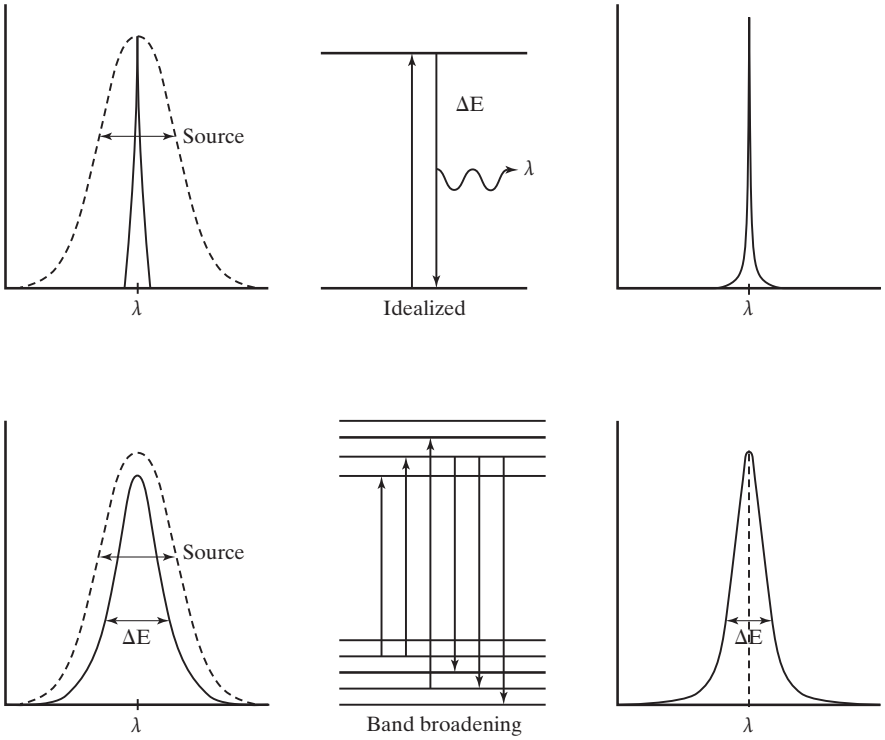
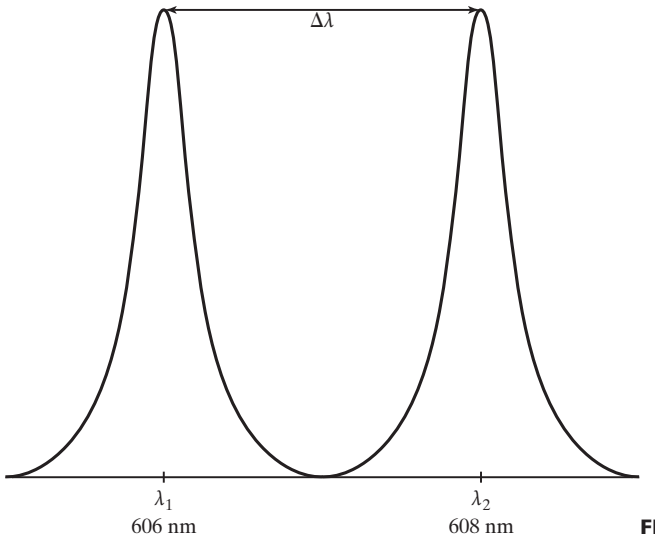


FIGURE 25 Source emission bandwidth compared with absorption bandwidth. Ideally, the emission peak bandwidth is contained within the absorption peak bandwidth; if not, poor resolution results. Monochromator bandwidth is also a critical consideration.

The ability of an instrument to differentiate between adjacent absorption or emission peaks is the resolving power of the instrument. Usually, this is defined as

$$\frac{\lambda_{average}}{\Delta\lambda} \qquad \textbf{(7)}$$

where λ is the average wavelength of the peaks, and $\Delta\lambda$ is the difference between that peak and an adjacent one. The term **baseline resolution** refers to the situation in which both peaks are at the baseline, as shown in Figure 26. The larger the resolving power,



$$R_1 = \frac{607}{2} = \text{large}$$

FIGURE 26 Baseline resolution of two spectral peaks. In this type of application, the average wavelength is used in the calculation.

the more complete is the separation of the peaks; the resolving power in turn depends on the slit width, source bandwidth, and bandwidth of the transition.

2.4 UV/VIS Spectroscopy

A UV/VIS spectrometer is a classic but not the indispensable tool of forensic chemistry it once was. UV absorption is all but relegated to use as part of a detector system in instruments, whereas visible interactions are used as part of color analysis and characterization. Color is an important descriptor of presumptive tests, fibers, dyes, inks, and paints, all of which can be explored spectroscopically. However, traditional UV/VIS spectrophotometry and colorimetry are rarely used in forensic settings. A major reason for this neglect is the kind of chemical information the instrument provides and the need to isolate pure materials prior to recording a spectrum. However, recent advances in **microspectrophotometry** are leading to a revival of interest in this oldest of spectrophotometric techniques. We address the topic in a later section.

Absorptions in the ultraviolet and the visible ranges of the spectrum correlate with electronic transitions between molecular orbitals. Only two types of transitions can occur, as shown in Figure 27. As a result, only compounds that have π electrons have a UV/VIS spectrum. A UV spectrum provides information about double bonds and conjugation. Whereas this information is useful in many contexts, many UV spectra look similar to or even indistinguishable from one another and consequently cannot be used to definitively identify a given compound. UV/VIS instrumentation has been successfully adapted to liquid chromatography and capillary electrophoresis instrumentation as both fixed-wavelength and scanning detectors.

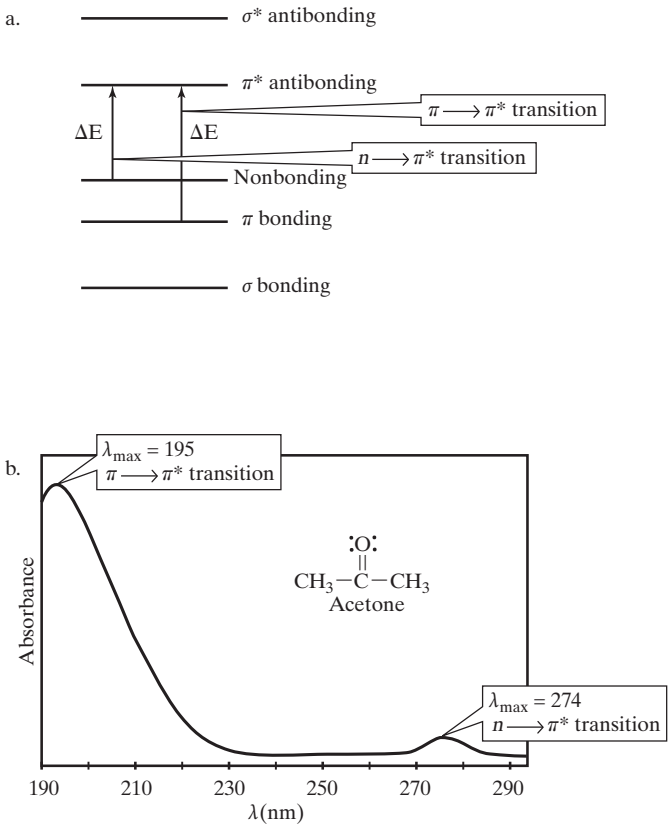


FIGURE 27 (a) The only two types of transitions possible with the absorption of UV/VIS radiation. If a compound lacks π electrons, no absorption in that electromagnetic region is possible. (b) UV/VIS spectrum of acetone, showing examples of each.

2.5 Infrared Spectroscopy

Unlike UV/VIS techniques, IR spectroscopy can provide unambiguous identification of isolated compounds, albeit after meticulous sample preparation. IR techniques are valued for drug analysis, fiber characterization, and other applications in which such identifications are essential. Applications of IR techniques in forensic chemistry are exclusively qualitative; when quantitation is required, other techniques, such as GC/MS, are used.

The infrared method most students are familiar with is absorption spectroscopy spanning the mid-IR range. The infrared region starts just below the visible one (~780 nm) and extends to approximately 1,000,000 nm, which, by convention, is reported in frequency in units of wavenumbers (reciprocal centimeters) (cm^{-1}) rather than in wavelength units of nanometers. The total IR range extends from about 12,800 to 10 cm^{-1} and can be divided into the near IR, from 780 nm to 2500 nm (12,800–4000 cm^{-1}); the mid-IR (4000 to 400 cm^{-1}); and the far IR (400–10 cm^{-1}). All three regions can be exploited in forensic work, but midrange techniques dominate.

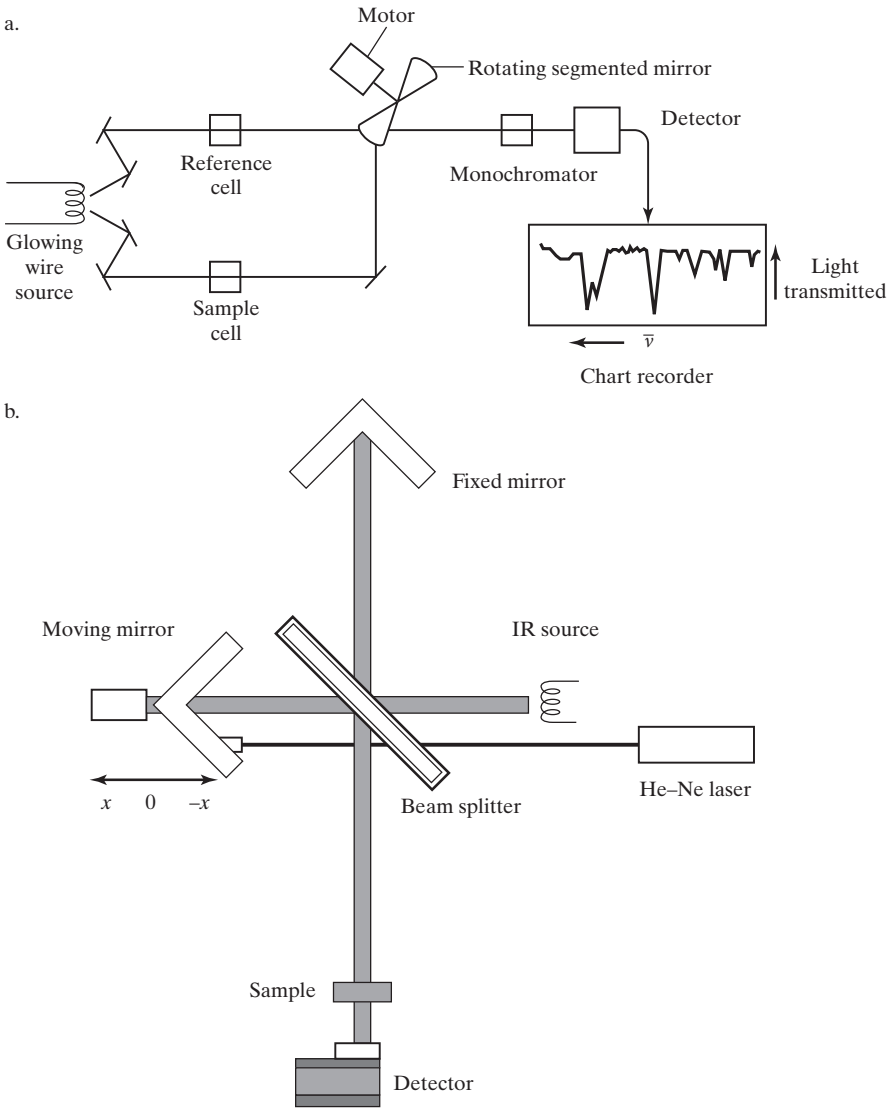


FIGURE 28 (a) Box diagram of a dispersive IR instrument. (b) Box diagram of an FTIR instrument and the interferometer. The front surface of the beam splitter creates two components that recombine at the back surface of the beam splitter; the two waves interfere.

Several recent advances in instrument design have enhanced the applicability of mid-IR techniques. The first and most important was the development of Fourier transform spectrometers (FTIR) (see Figure 28). Dispersive instruments utilized an infrared energy source and a monochromator such as a grating to separate the wavelengths. As mentioned before, isolation and the presentation of single wavelengths to a sample in sequence ("scanning") together cause an inevitable loss of intensity that limits the sensitivity of the spectrometer. To deal with this loss, FTIR instruments have a design that uses a "mathematical dispersion"[5] rather than a physical one. This approach is called **interferometry.**

Interferometry (Figure 29) may at first seem a difficult concept to grasp, but the general principles are the same as some we have already encountered in discussing polarizing light microscopy. Recall that in PLM light enters a birefringent sample that acts as a beam splitter, breaking the light into two components. The fast component emerges from the sample ahead of the slow component, which has undergone retardation. The thicker the sample, the greater is the retardation. As soon as the beams emerge from the crystal, they interfere. Adding the two vectors of the two components produced a rotation of the angle of polarization; it is this angle that varies with wavelength. An interferometer accomplishes the same thing in the infrared region.

Starting with a simple case of a single infrared wavelength, interference patterns are created by recombining beams split into two paths by the beam splitter. At the beginning of the cycle (Figure 30), the mirrors are equidistant from each other, so the two beams travel the same distance and are not shifted out of phase. When the mirror moves away to a point equivalent to one-fourth the value of the wavelength, the beam taking that path now travels the equivalent of $\frac{1}{2}\lambda$ farther, taking into account the trip to the mirror and back. In terms of the PLM analogy, this is the slow beam, retarded by a factor of $\lambda/2$. Retardation is given the symbol δ. Because the slow beam is half a wavelength out of phase, when the beams are recombined, the

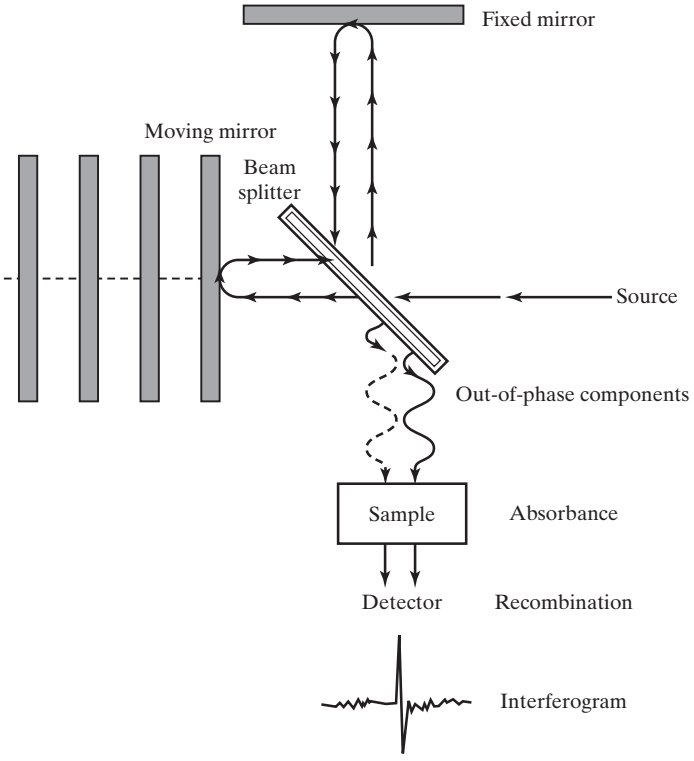


FIGURE 29 An interferometer. Beams are split into two components that when recombined, will be offset from each other an amount that depends on the different distances the beams travel.

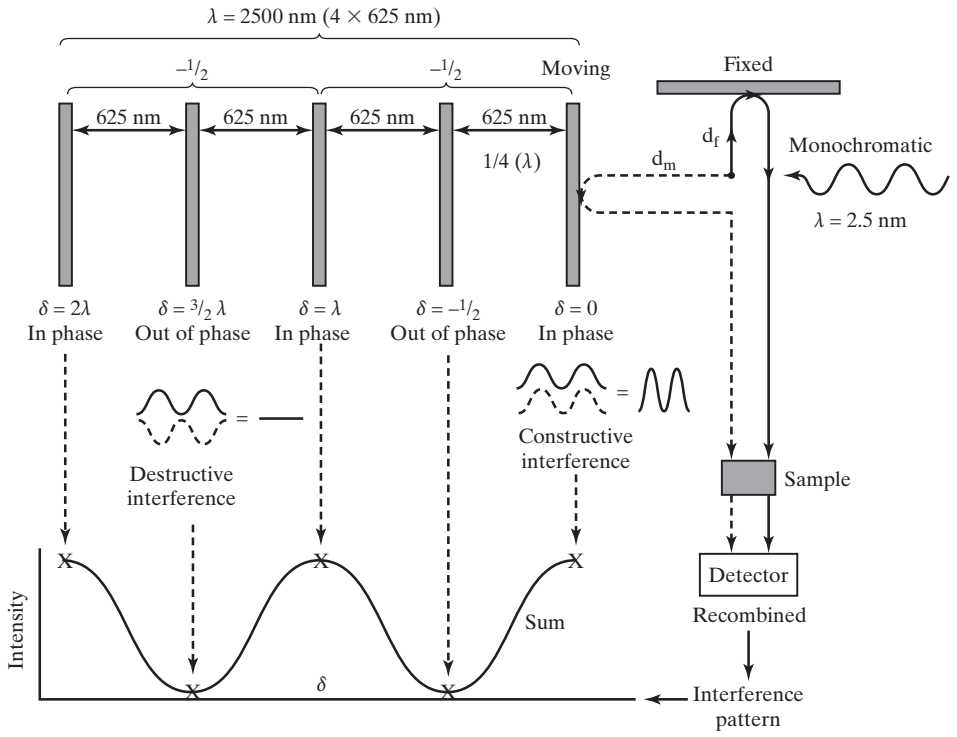


FIGURE 30 The creation of an interferogram with a monochromatic source at 2500 nm, which corresponds to 4000 cm^{-1} in the infrared range. The sum of the two beams is plotted as the interferogram.

interference is destructive. When the offset is a full wavelength, constructive interference is restored. The distance moved by the mirror correlates with the thickness of the sample, in PLM terms. Assuming no interaction with the sample, the interference pattern for a monochromatic source is in the form of a cosine wave. In an instrument, the signal is recorded over the range of the mirror's motion, producing the cosine curve represented by the formula

$$I(\partial) = \frac{1}{2} I_0 \left(1 + \cos \frac{2\pi\partial}{\lambda} \right) \quad \textbf{(8)}$$

The intensity is halved because half the light gets reflected back toward the source and does not contribute to the intensity of light reaching the detector. An interferogram represents a combination of these functions for each wavelength of interest; the Fourier transform separates the signal into the contributions from individual wavelengths.

In an FTIR, the source is not monochromatic but spans the mid-infrared region. A He–Ne laser is used to measure distances and for calibration purposes. An interferogram is thus the combination of as many pairs of recombined wavelengths as are in

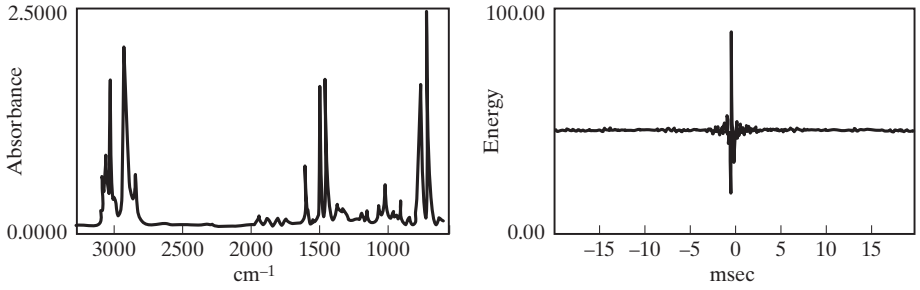


FIGURE 31 An example of an interferogram and the corresponding IR spectrum displayed as absorbance.

the source. These signals are separated mathematically with the Fourier transform. The **interferogram** is a plot of mirror distance versus intensity and may be cast in terms of time because the mirror moves at a controlled speed. Such data are said to be in the time domain, and the Fourier transform is used to convert the data to the frequency domain, producing the familiar absorbance plot of intensity as a function of frequency (or wavelength in colorimetry). An example is shown in Figure 31. The spectral resolution of the instrument depends on the quality of the optics and other factors, whereas the digital resolution depends on the maximum mirror movement. Data points on the interferogram are separated by a value of $1/\Delta$, the inverse of the maximum travel distance. If the mirror moves 1.0 cm, the optimal data point resolution is 1 cm^{-1}. Note that the signal that is recombined at the detector and deconstructed with the Fourier transform is a result of the source signal minus the sample signal. A background spectrum is still required (Figure 32). Because there is no scanning of wavelengths in FTIR, multiple spectra can be quickly collected and averaged, resulting in significantly improved signal-to-noise ratios and sensitivity.

Advances in FTIR and in computer-processing power have fundamentally changed infrared spectroscopy and its forensic applications. Infrared microscopy and microspectrophotometry, all but impossible with dispersive instruments, are becoming common in forensic laboratories. Finally, simplified sample preparation techniques allow for quicker analyses, always an advantage in busy forensic laboratories. Several sampling accessories and interfaces have been developed for FTIR; of particular interest in forensic chemistry is attenuated total reflectance (ATR).

ATR takes advantage of internal reflections within a crystal that is in direct contact with a sample to create the infrared absorbance spectrum of the sample. An ATR accessory (Figure 33) consists of a crystal (sometimes called a prism) made of diamond, germanium, ZnS, TlBr, or ZnSe, all materials with high refractive indexes. The combination of the high refractive index and the geometry of the crystal facilitates a series of internal reflections, typically 10–50, and collectively referred to as an **evanescent wave** or multiple internal reflections (MIRs). Specifically, the evanescent wave is that portion of the wave that penetrates the sample as it is internally reflected. When the crystal is pressed into tight contact with the sample, a tiny fraction of each reflection penetrates the sample, allowing interaction and absorption to occur. The more reflections, the more opportunities there are for absorption, the longer the effective pathlength is, and the stronger are the resulting signals.

ATR offers many advantages. The evanescent wave penetrates only about a micrometer or so of the sample, allowing very thin films and liquids, in addition to solids and powders, to be analyzed. There is no need to purge the sample area, because the IR signal never propagates through the air. In fact, the only destruction of note is

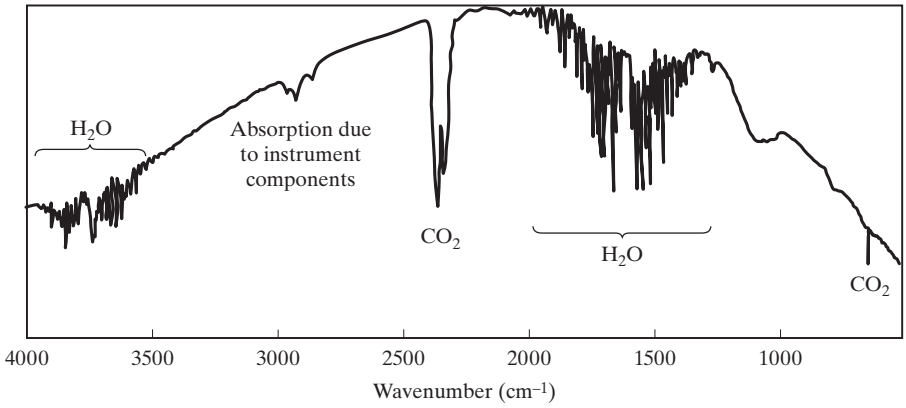


FIGURE 32 A typical background of an FTIR instrument. Sample spectra are ratioed against the background to produce the final spectrum. FTIR spectra can be displayed in either the absorbance or the transmittance mode.

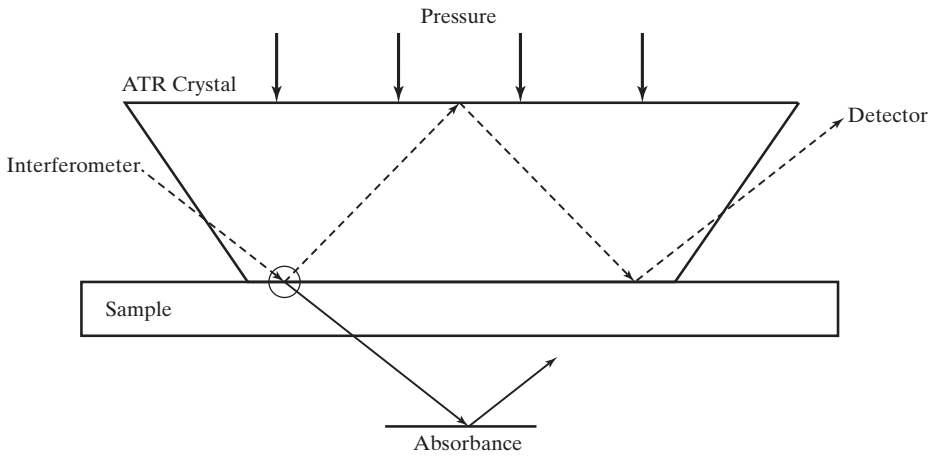


FIGURE 33 Attenuated total reflection (ATR) as used as a sampling accessory for FTIR. A series of internal reflections provides multiple absorptive events similar to the multiple absorptions that occur off different particles in DRIFTS.

the area of compression, which is very small. Sample preparation is minimal and nearly nondestructive. This is an issue only with samples such as fibers or tapes, in which the compression leaves a distinctive mark. ATR can be used for very small samples and is a common accessory to infrared microscopes. ATR may be performed in situ on very large objects such as soda cans and even car doors. As long as firm contact can be made between the crystal and the surface, the analysis is feasible. Several companies now offer micro-ATR as well as accessories for large samples. ATR accessories are now equipped with cameras to record the exact sampling location. Other designs allow viewing of the sample through a diamond element.

Selection of the type of crystal depends on the application. Diamond prisms are ideal for applying high pressures and ensuring good contact between the sample and the crystal. Diamond is also resistant to corrosive and other chemically aggressive samples. However, because diamond is so hard, excessive applied pressure may damage the sample before damaging the crystal. Diamond prisms are also expensive, and for many applications Ge, ZnS, or ZnSe crystals are acceptable. Because the crystal is an optical component in the instrument just like a lens or filter, selecting a crystal depends on what region of the IR spectrum is of interest. A list of crystal types is presented in Figure 34.

ATR is becoming a favorite tool in forensic chemistry, given its versatility, simplicity, small sample requirements, and nondestructive nature. However, it is important to note that spectra obtained by ATR are not directly comparable with transmission or reflection spectra. Although the energy–matter interaction is the same (absorption due to vibrational interactions), the locations of bands are shifted relative to transmission and reflection. Also, in transmission and reflection, the path length is fixed and is controlled by the thickness of the sample. In ATR, the depth of penetration, and thus the path length, is a function of wavelength, yet another manifestation of refractive index effects. The ATR crystal must have a high refractive index to establish the evanescent wave (Figure 33). However, the refractive index of the sample varies with wavelength; consequently, the depth of penetration varies across the spectral range. As the wavelength increases, so does the penetration, which in turn increases the path length and the resulting absorption signal. The influence of the refractive index on the depth of penetration also results in a generalized shift in the absorption to lower frequencies. Thus, correction algorithms are required to correct ATR spectra for these effects and to allow comparison with transmission IR spectra. Preferably, ATR spectra should be compared and evaluated against libraries and databases collected with ATR methods.

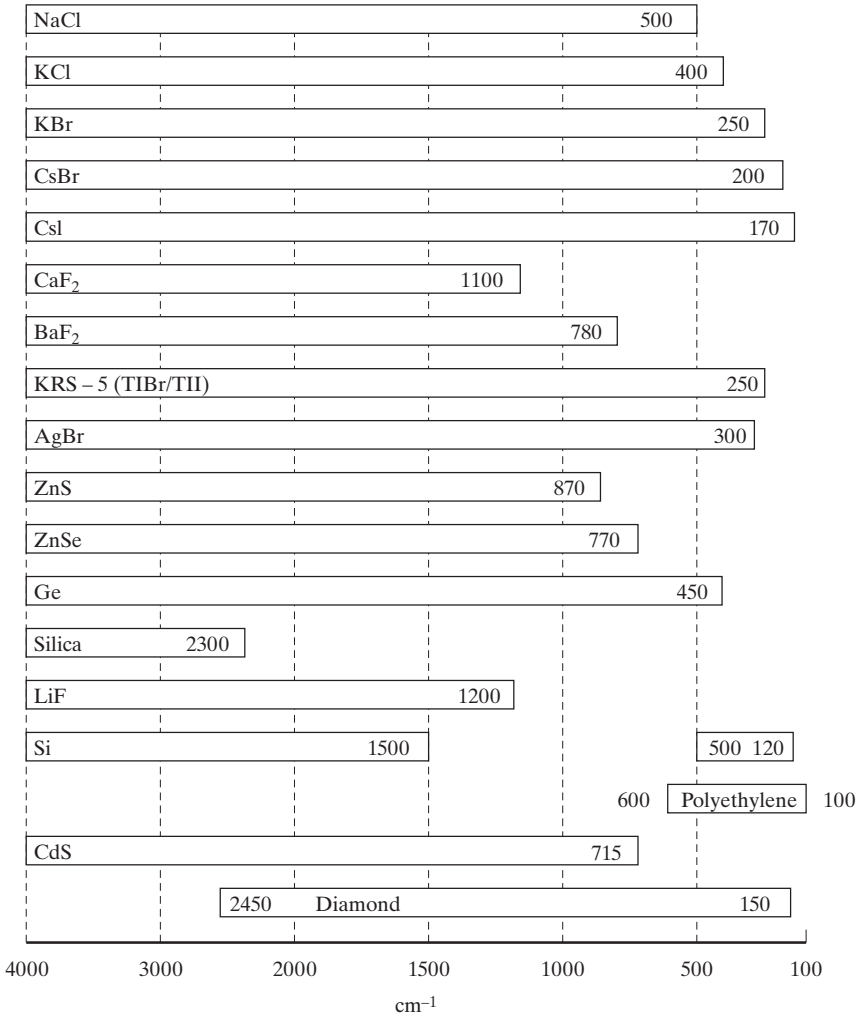


FIGURE 34 Optical characteristics of materials in the IR range. ATR crystals are usually made of diamond, germanium, ZnSe, or ZnS.

2.6 Raman Spectroscopy

Another vibrational technique, Raman spectroscopy is garnering attention for the forensic analysis of drugs[6,7], dyes[8–10], explosives[7,11], fibers[12–14], paints[15–18], and even body fluids[19–21]. Raman techniques differ from traditional vibration IR in that scattered radiation, rather than absorbed radiation, is studied. Furthermore, Raman interactions are dependent, not on the existence of polar bonds, but instead on the existence of **polarizable bonds** as shown in Figure 35.

A Raman spectrum is obtained by directing an intense laser beam onto the sample and examining the patterns of light scattered at higher and lower wavelengths relative to the wavelength of the incident laser beam. When scattering occurs, most of the photons are scattered at the same wavelength as the incident radiation, and this elastic scattering is called **Rayleigh scattering.** Interestingly, Rayleigh scattering contains no analytical information, but scattering on either side (inelastic scattering, called **Stokes scattering** and **anti-Stokes scattering**) of this band does. Excitation is with a laser in the visible or near-infrared region, and the scattering signal is measured at 90° or 180° to the light path of the source. In general, the larger the atom, the more polarizable the element's electron cloud is. As a result, water can be used as a solvent, because oxygen

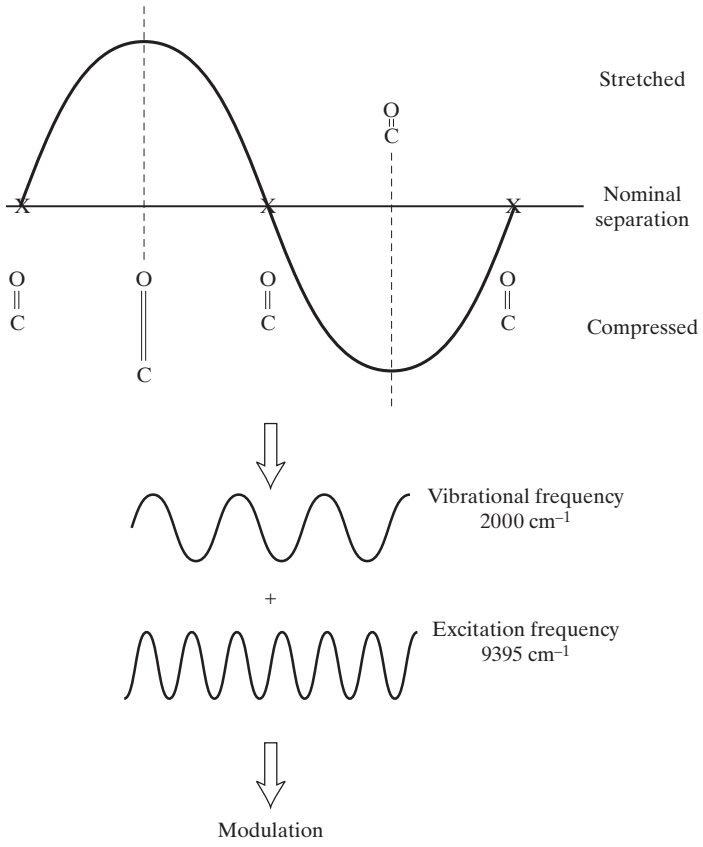


FIGURE 35 Frequency of a hypothetical chemical bond vibration. This bond expands and contracts at a frequency of 2000 cm^{-1}. How polarizable the bond is will determine how it scatters incoming light. At the maximum stretch, the polarizability of the bond will not be the same as when the bond is at maximum compression, so polarizability has the same frequency as the vibration.

and hydrogen are relatively small, and the bonds in water are not easily deformed by interaction with visible light. Because visible light is used for excitation, samples can be mounted on or contained in glass.

Inelastic scattering can result from interactions with the polarizable bond that produce signals (Stokes and anti-Stokes) at the excitation wavelength plus or minus the vibrational mode of the bond, as shown in Figure 36. The Stokes signal is the stronger of the two, but it is still several orders of magnitude weaker than the Rayleigh line. Further problems can arise when higher energy excitation sources, such as those in the visible and UV range, are used. These sources can cause fluorescence (Figure 37), which may produce a signal that will swamp the weak inelastic scattering signal. Despite these issues, Raman spectroscopy is finding a wider niche in forensic applications, given that it provides information that is complementary to absorption IR and is generally nondestructive.

3 MASS SPECTROMETRY

3.1 Overview

The name **mass spectrometer/mass spectrometry (MS)** is somewhat of a descriptive misnomer. Mass spectrometry works on the basis of separation, but not separation of electromagnetic energy into component wavelengths. Rather, a mass spectrometer disperses mass fragments so in this sense, "spectrometer" is a reasonable analogy, since the output is a spectrum of masses across a range of masses; an MS "scans" masses in the same sense that spectrometers scan electromagnetic energy. A good way to visualize

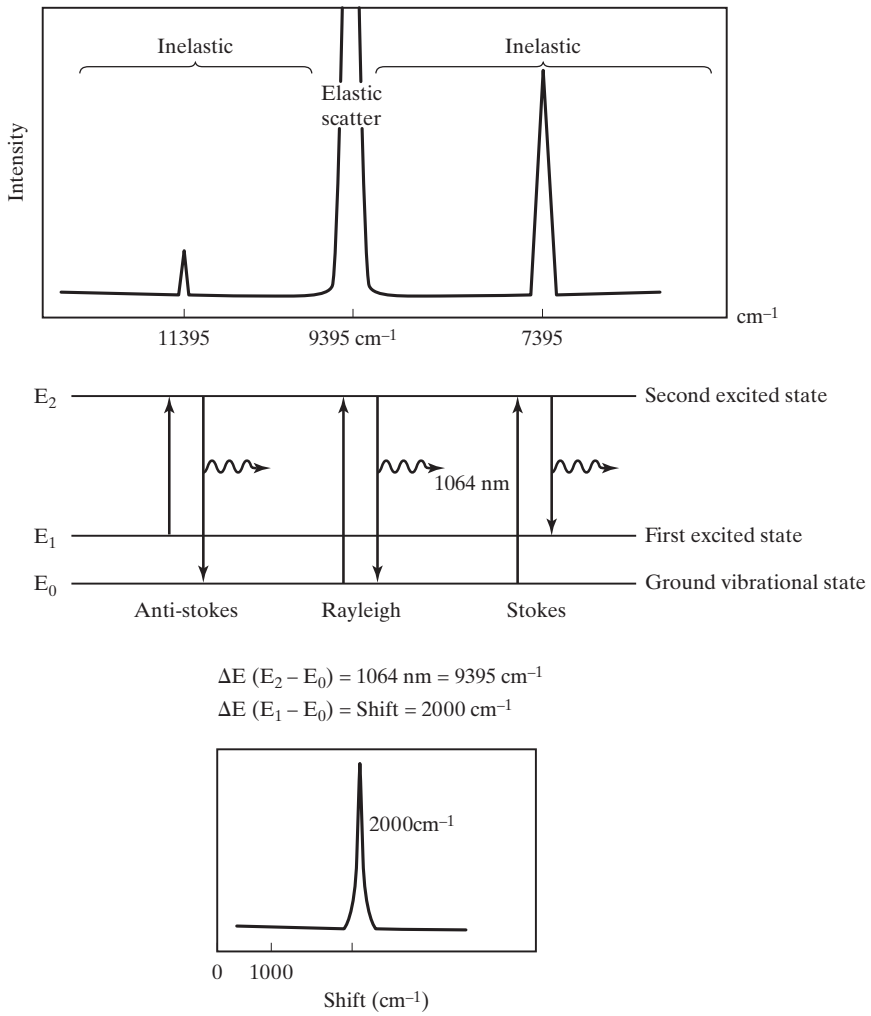


FIGURE 36 A Raman spectrum of a sample bond in a system using a near-infrared laser as the excitation source. Elastic scattering or Rayleigh scattering dominates the spectrum, with inelastic scattering at wave numbers plus or minus the equivalent of the vibrational frequency. The Raman spectrum can also be normalized and plotted as the shift in wavelength of the signal.

a mass spectrometer is as a mass filtering device, as shown in Figure 38. When this device is coupled to the output of some type of sample introduction system such as a gas chromatograph (discussed below), the flow is directed into a vacuum region, where the sample is ionized and fragmented to variable degrees. The ions are then introduced into a filtering device that separates them on the basis of their mass-to-charge ratio. Masses are often specified in units of Daltons (Da) and the ratio of mass to charge is represented by m/z. Ions arrive at the transducer and are converted to electrons. The signal is amplified by an electron multiplier and recorded.

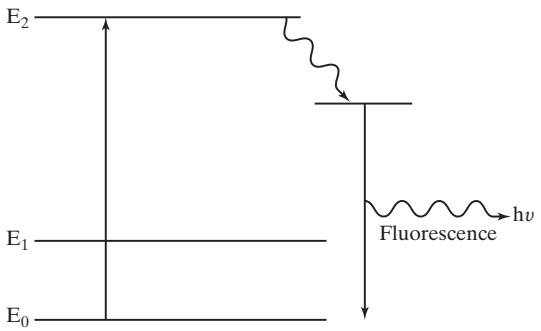


FIGURE 37 The fluorescence problem. If the laser is at a wavelength with sufficient energy to promote the molecule to the next higher state, the molecule can decay to a metastable state and then fluoresce, producing a signal that will swamp the weak scattering signal.

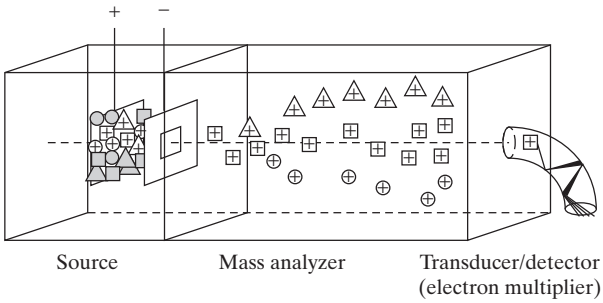


FIGURE 38 A generic mass spectrometer, which does with mass fragments what a grating does to light: filters it and separates it into individual components.

3.2 Basic Designs

There are many types of ionization modes and mass separation filters, the discussion of which is beyond the scope of this book. In forensic science applications, one of the most common MS designs is the **electron impact ionization/quadrupole mass filter** (Figure 39). Ionization and fragmentation are achieved by the collisions between molecules from the sample and electrons generated by a filament, as shown in Figure 40. This ionization technique is referred to as electron impact (EI), and it is considered to be a hard ionization technique. This means the fragmentation is fairly complete as opposed to a soft ionization technique. We will discuss an example of soft ionization shortly. In EI mode, few collisions result in ionization, but enough to generate both positive and negative ions, with positive ions usually being the polarity of interest. The positive ions are pushed into the focusing lenses by a repeller plate kept at a positive potential. The degree of fragmentation depends on the electron energy; standard values are 70 eV. The vacuum is necessary to prevent secondary collisions and combinations. Ions are focused into a tight stream by a series of electronic lenses and introduced into the quadrupoles, where alternating DC and radio-frequency currents determine the field and thus the ion pathways. At a given setting, only ions with a particular mass transit the quadrupoles safely, whereas all others collide with the quadrupoles or are ejected.

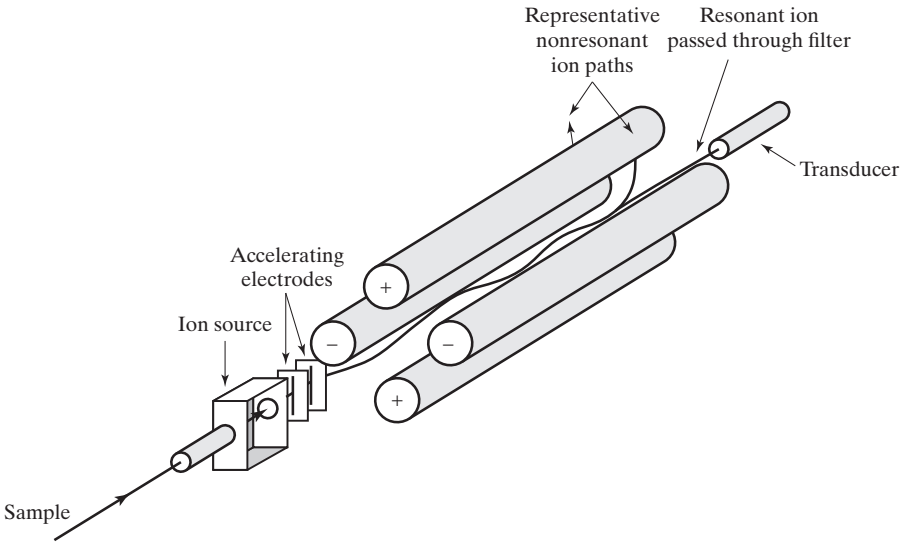


FIGURE 39 A quadrupole mass spectrometer. Alternating DC and radio frequencies applied to the quadrupoles dictate ion paths. At any given setting, only one mass will get through and this is called the *resonant mass.*

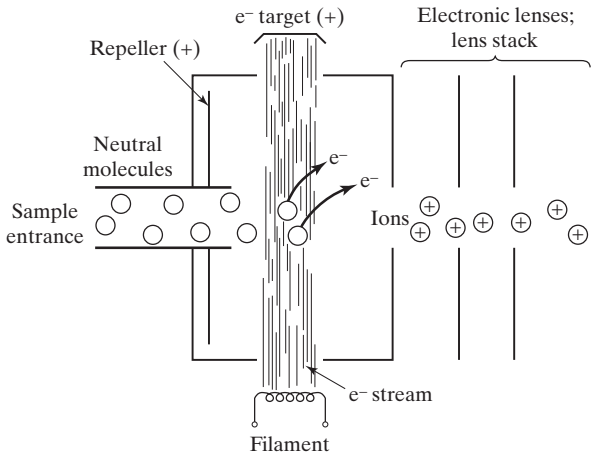


FIGURE 40 The inlet, ionization region, and lens stack of a typical mass spectrometer. The mechanism of ionization shown here is electron impact (EI).

Under standard EI conditions (70 eV), compounds are identified through the mass fragmentation pattern, library matches, and traceable standards. Key to identification is reproducible and controlled instrumental conditions. Electronics must be set such that patterns are reproducible in and across instruments. This is done by an internal calibration or tuning procedure in which a standard compound, usually perfluorotributylamine **(PFTBA)**, is introduced into the mass spectrometer and settings are adjusted or tuned until the resulting mass spectrum meets the required criteria of masses detected and relative abundances. The standard forensic library is the NIST/EPA/NIH Mass Spectral Library, which was acquired under instrumental conditions for PFTBA. Because the electronic settings alter mass intensities, if an instrument is out of tune (i.e., if it cannot produce a PFTBA spectrum with the required mass peaks and intensities), any calibration curves obtained under those tuned conditions have to be redone.

3.3 ICP-MS

For elemental analysis, particularly of the metals and semimetals, a mass spectrometer has obvious appeal; the challenge is in ionizing the sample prior to introducing it into the quadrupole (or other mass filter). The solution was the development of **inductively coupled plasma (ICP) torches**, which were described in the 1970s and became available as ionization sources for mass spectrometers in the 1980s. A schematic of an ICP torch is shown in Figure 41. Within the torch, there are three concentric quartz tubes. Argon flows through the tubes as shown, consuming several liters a minute of the gas. At the top of the torch are water-cooled induction coils that are powered by a radio frequency generator. The torch is ignited by a spark from a Tesla coil, which generates ions that then flow through the rapidly oscillating magnetic field generated from the coil. Frictional and collisional interactions heat the plasma (consisting of Ar^+ and electrons) to temperatures in excess of 6000 K. Sample that flows with argon through the center of the coil experiences temperatures of ~6500 K, resulting in rapid de-solvation, dissociation, atomization, and ionization.

The plasma stream cannot be directed into the mass spectrometer owing to the high temperature and flow rates. Thus, the design of the interface region is a primary technical challenge in ICP-MS. As shown in Figure 42, a differentially pumped design is used. The plasma is directed onto an orifice into a region that is maintained at a vacuum of about 1 torr. The sample cone allows a small stream of the plasma to enter, where it then passes through a second orifice called the skimmer cone and on into the high vacuum region. The beam is focused by a series of electronic lenses, some of which may direct the ion beam **off-axis**. This reduces the number of neutral species in the beam.

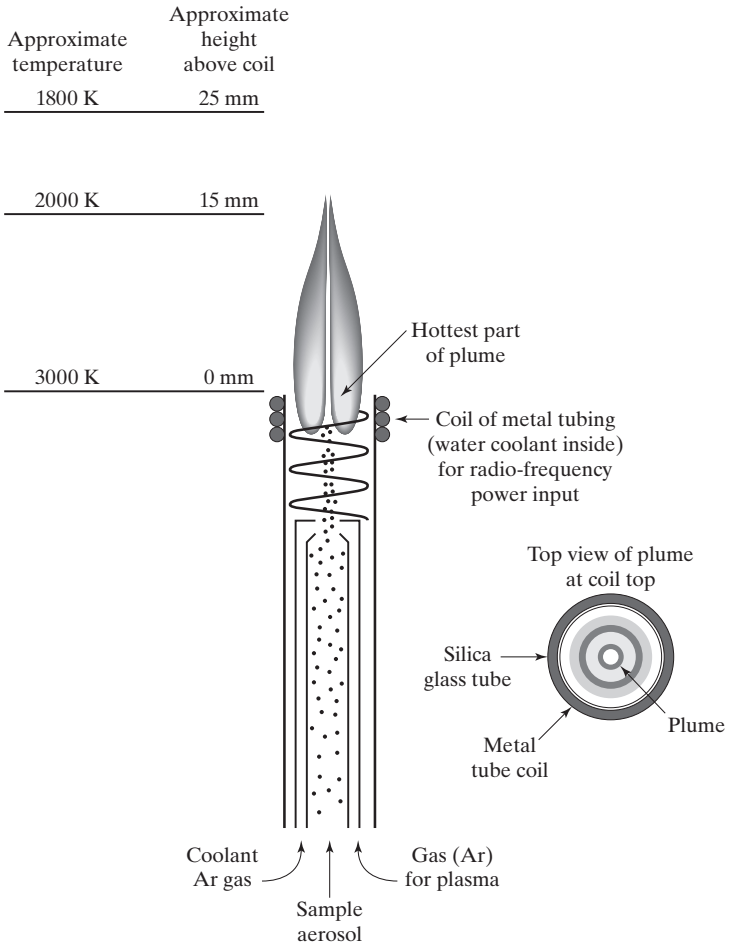


FIGURE 41 A plasma torch for ICP. A radio-frequency generator ionizes argon in the tube and accelerates the ions, maintaining heat by sustained collisions. Once the generator is off, the plasma stops forming.

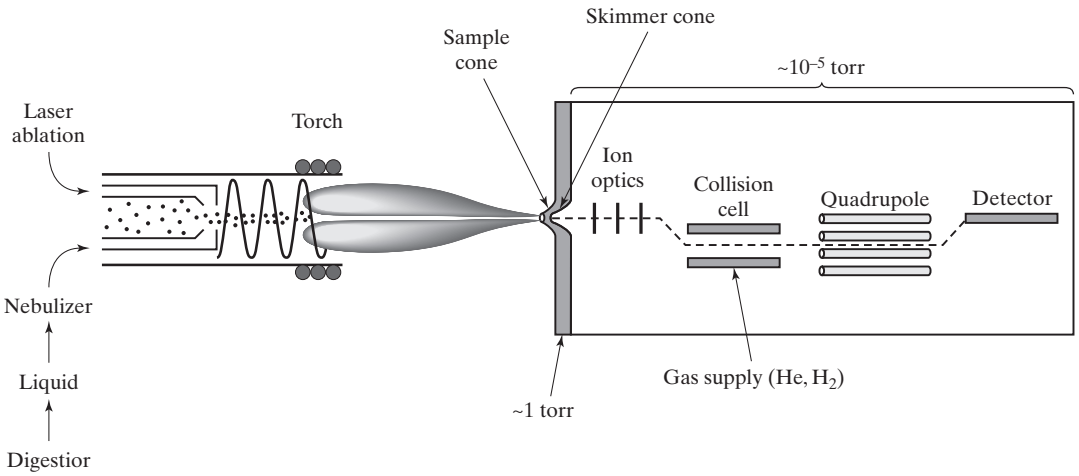


FIGURE 42 Schematic of a generic ICP-MS instrument. Sample can be introduced into the torch via pumping of liquid samples or by laser ablation. Note the collision cell is off-axis.

Another common feature is a reaction or **collision cell** that the focused ion beam enters prior to entering the quadrupole. The **reaction cell** is usually an octopole or a hexapole that operates in radio-frequency mode only (no DC), which has the effect of focusing the ion beam into the center of the cell. The function of the reaction cell is to allow for collisional dissociation of oxides or other polyatomic interferences species that have the same mass (isobaric) as an element of interest. For example, ^{35}Cl can combine with ^{16}O to form an oxide with a mass of 51 daltons, which is isobaric with $^{51}V^{+}$. Other examples include $^{40}Ar^{16}O^{+}/^{56}Fe^{+}$ and $^{40}Ar^{35}Cl^{+}/^{75}As^{+}$. Several designs are used for reaction cells; one example is based on **kinetic energy discrimination** (KED). Pressurizing the collision cell with helium causes the larger polyatomic ions to collide with helium more frequently than do the atomic ions of interest. More collisions means a greater loss of kinetic energy by the polyatomic compared to the atomic ions. A biasing voltage can then be placed on the entrance to the quadrupole that allows ions at or above that kinetic energy to enter the quadrupole. Although this approach cannot completely eliminate **isobaric interferences**, it does significantly reduce them.

APPLYING THE SCIENCE 2 You Are What You Eat

Once a person dies, the body can become the food source for a new ecosystem on the microscopic and macroscopic levels. Within minutes of death, blowflies can arrive and begin to consume tissues and eventually to lay eggs. These eggs hatch into larvae (maggots) that also feed on the corpse. Because the flies and larva feed on the body, they ingest the tissues as well as any compounds present at the time of death. For example, if a person dies of an overdose of heroin, which metabolizes rapidly to morphine, insects that feed on the body can have detectable levels of morphine found within their tissues. This process is part of a field of study called entomotoxicology. A recent report from the *Journal of Forensic Sciences* reported on an interesting application of ICP-MS to the study of insects and bodies. Suppose a person dies of a gunshot wound from a weapon fired at close range. As we will see in a later chapter, this can result in the deposition of metallic oxide primer residues (gunshot residue, or GSR) on and in the wound. Any arriving insects that feast on the wound site will ingest gunshot residue along with tissue, and this GSR should be detectable using the proper instruments and sample preparation. The current accepted standard for GSR is based on identification of GSR particles based on specific morphological features as seen in SEM. The particles are then analyzed using X-ray spectroscopy to confirm the presence of the metals characteristic of GSR. The authors hypothesized that such methods would be of limited value for decomposing flesh.

To investigate, they obtained pig carcasses and created knife and gunshot wounds on the bodies, which were left outdoors. Before significant decomposition set in, the two types of wounds were easily distinguished from each other based on physical appearance. After decomposition began, appearance alone was insufficient to distinguish knife wounds from gunshot wounds, a scenario that could easily occur in a forensic setting. Blowfly larvae were collected from the wound sites over time, along with tissue samples from the carcasses. Samples were frozen prior to analysis. Sample preparation involved the use of hydrogen peroxide, nitric acid, and microwave digestion followed by ICP-MS analysis. Results showed that the elements characteristic of gunshot residue were detectable in the larvae and tissues even in advanced decomposition stages, and the results were not dependent on environmental conditions. In this case, the ICP-MS approach yielded usable data under conditions where traditional GSR methods were severely limited.

Source: LaGoo, L., L. S. Schaeffer, D. W. Szymanski, and R. W. Smith. "Detection of Gunshot Residue in Blowfly Larvae and Decomposing Porcine Tissue Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). *Journal of Forensic Sciences* 55, no. 3 (2010): 624–32.

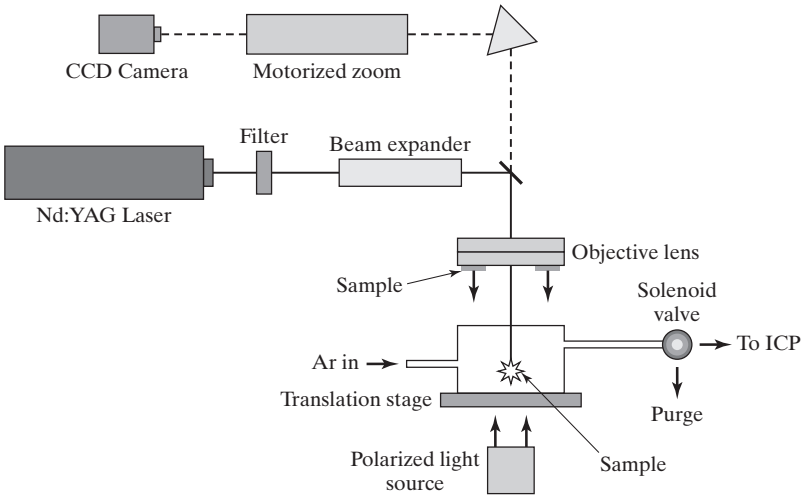


FIGURE 43 Laser ablation accessory for ICP-MS. A camera is used to visualize the sample and direct the laser onto the surface.

One of the strengths of ICP-MS is the ability to obtain isotope ratios for most elements. For example, lead, the primary metal in bullets, has four naturally occurring stable isotopes: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb with relative abundances of approximately 1.5%, 23.6%, 22.6%, and 52.3% respectively. Because these isotopes have different masses, they are separable and in a typical lead sample analyzed by ICP-MS four mass peaks will appear. The same situation occurs in organic mass spectrometry, where the chlorine isotopes ^{35}Cl and ^{37}Cl are easily distinguished. Because most metals have detectable levels of one or more naturally occurring isotopes, ICP-MS provides additional analytical information that can be used to classify, characterize, and distinguish materials such as bullets.

Until recently, sample preparation for ICP-MS was similar to that used for atomic spectroscopy techniques such as AA and AES. The sample (solid, semisolid, or liquid) is digested using an acid or acids and heat, filtration, and dilution in dilute acid such as 1% nitric. Microwave digestions are also used as part of the sample digestion. The resulting solution is drawn into a **nebulizer** and aerosolized, then into a spray chamber, and finally into the argon stream that enters the torch. This methodology is effective but is destructive, requires a fairly large sample size, and is time consuming and reagent intensive. An alternative sample introduction method is laser ablation (LA-ICP-MS) (Figure 43). To generate a sample plume, a laser such as a Nd:YAG is directed onto the surface of interest, resulting in ablation of fine particulates that are then drawn into the torch. The advantages of this method are obvious; the drawbacks are that only the surface is characterized, and quantitation can be difficult. However, the ease and convenience is contributing to increasing forensic interest in LA-ICP-MS with applications reported for bones and teeth[22], glass[23–28], fibers[29], and paper[30].

3.4 Tandem Mass Spectrometry

One of the most exciting developments in forensic applications of mass spectrometry is the adaptation of **tandem mass spectrometry**. Indeed, within the last 5 years, forensic toxicology laboratories have embraced tandem mass spectrometry at an extraordinary pace, and in some instances, these devices are supplanting GC-MS as the core instrumentation for identification and quantitation of drugs and metabolites. As was the case for ICP-MS, the key technological hurdle was development of an interface and ionization source that allowed for the linkage of liquid chromatography, with relatively high flows of solvents and buffers to be directed into the high-vacuum region of mass

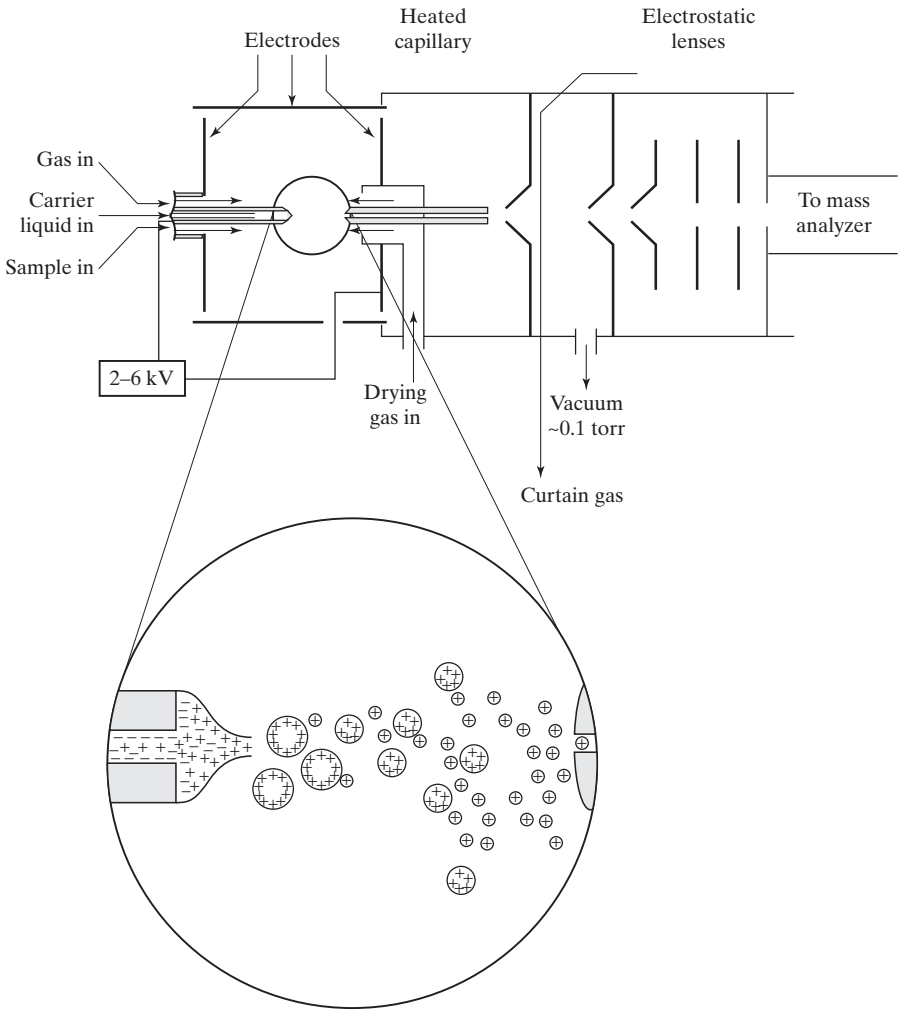


FIGURE 44 An electrospray interface from an LC column to an MS detector. A charge is imparted to the surface of the solvent drop, which is then heated. As solvent evaporates, the charges are drawn closer to each other until repulsion causes the drop to disperse via a "Coulombic explosion". The process is repeated until most of the solvent is removed.

spectrometers. Not surprisingly, the solution was similar—a differentially pumped region with a sample and skimmer cone arrangement. In addition, HPLC systems can be interfaced with a number of ionization sources. The most common in forensic applications are **electrospray ionization (ESI)** and **atmospheric pressure chemical ionization (APCI)**, with ESI currently dominating the commercial market. As with many topics in this chapter, tandem MS is the subject of several excellent books, and you are encouraged to explore these; a few are noted in the Recommended Reading section.

Electrospray ionization is ideally suited to liquid samples such as the effluent of a liquid chromatographic system. As the effluent of the HPLC enters the ion source, a surface charge is imparted to the sprayed droplet. Heated gases flow into the source, de-solvating the drops and bringing the like charges into close proximity. Once the coulombic repulsion becomes too great, a coulombic explosion occurs, further dispersing the droplets until essentially only gaseous ions and ion/molecule clusters enter the sample cone. The polarity of components in the source determines whether positive or negative ions are allowed to pass into the mass analyzer. Although ESI is not a universal ionization source, it has a wide range of applicability and is capable of ionizing large nonvolatile compounds, is including most drugs and metabolites.

Alone, ESI coupled to a quadrupole mass spectrometer did not offer significant analytical advantages over techniques such as GC-MS. ESI is the sample inlet and ionization source for the mass spectrometer in the same sense that a torch is for ICP-MS. However, ESI is a soft ionization method, the purpose of which is to produce molecular ions (in the positive mode) such as MH^+, and is significantly different from hard ionization sources such as EI used in GC-MS. EI sources, under controlled and verified conditions, reproducibly fragment molecules into patterns that are amenable to library searching. This is not the case with LC-ESI-MS. While it is possible to further fragment the parent ion MH^+ using collision cells, the fragmentation is typically less aggressive than EI and results in fewer fragments. Given the range of ionization and instrumental variables to be set, ESI conditions are difficult to standardize, and the resulting spectra are not amenable to library searching in the same sense that EI spectra are. In forensic applications, this is a significant limitation that was not addressed until tandem systems became available.

Tandem mass spectrometers are those that combine multiple mass filters in sequential chain (sequential in space or in time). Many designs are available that incorporate various combinations of quadrupoles, ion traps, and time-of-flight mass filters. Collectively and generically, this family of instrumentation is referred to as MS^n, where refers to the number of mass-selection steps that are possible with a given instrumental design. We will focus our discussion on triple quadrupole systems, since these have been the most widely adopted in forensic chemistry to date.

The triple quadrupole design is shown in Figure 45. Ionized sample is introduced into a differentially pumped region much as is used in ICP-MS. Ions are focused using lenses and, in some designs, by quadrupoles into a tight beam entering the first quadrupole, Q1. In a typical quantitative experiment, Q1 is set to allow passage of a single ion per compound, usually the parent or *precursor* ion MH^+ that forms as a result of the ESI process in the positive mode. These ions enter Q2, which acts as a collision cell. The cell is pressurized with a gas such as argon or nitrogen such that collisional dissociation of the admitted ions occurs. These ions are directed to Q3, where they are selectively filtered and sent to the detector. Different terms are used for this type of experiment such as MRM (multiple reaction monitoring) and SRM (selected reaction monitoring). An example is shown in Figure 46, with amphetamine and MDMA (Ecstasy) as example analytes.

Soft ionization in the positive mode produces *parent molecular ions* for each compound, as shown. Along with matrix materials, the ionized stream enters Q1, which selectively passes masses, 136 and 194. These two ions enter the collision cell and are fragmented, and the resulting ion stream is directed to Q3. As was the case for Q1, Q3 acts as a mass filter, passing on the ions shown, which are typical for these compounds under tandem MS conditions. The electronics are designed such that ions are scanned into the quadrupoles, which means that a resulting product ion (also called *daughter* ion) such as 119 is directly and unambiguously related to its parent MH^+, here the 136 of amphetamine. The transitions monitored by this experiment would be summarized as follows:

- Amphetamine: 136/91 and 136/119
- MDMA: 194/163 and 194/105

Such transitions allow for specific identification of most drugs and metabolites when tandem MS is combined with a chromatographic inlet.

The advantages offered by $LCMS^n$ are significant. LC methods have a much broader range of applicability than GC, which is limited to relatively volatile and thermally stable target analytes. The MRM methodology, through selective ion isolation and filtration, affords low detection limits (parts per billion or less) and simplified

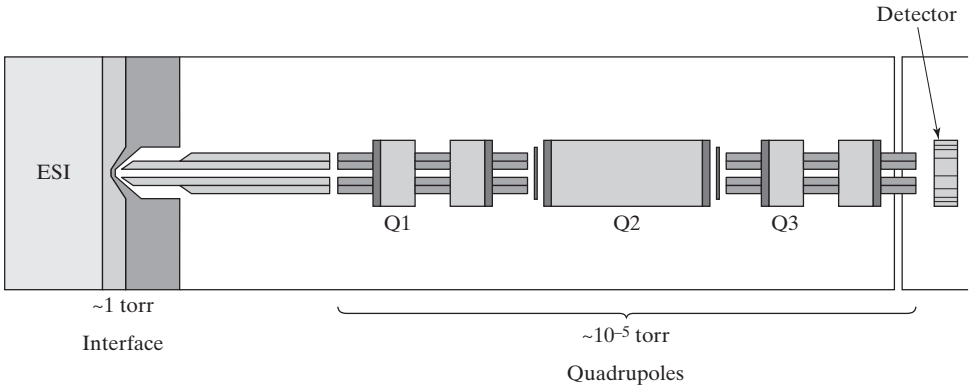
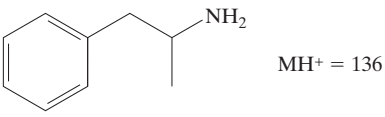
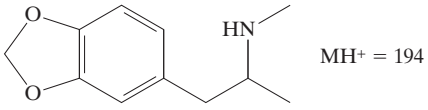


FIGURE 45 Generic triple quadrupole mass spectrometer with an electrospray ionization source.



Amphetamine (base)

Molecular weight = 135.21



MDMA (base)

Molecular weight = 193.24

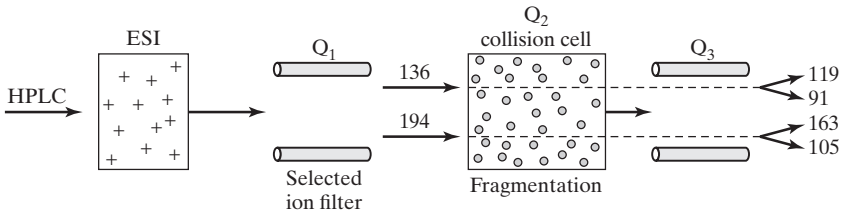


FIGURE 46 MRM/SRM mode of operation in triple quadrupole mass spectrometry.

sample preparation. The latter is due to the mass filtering capability of Q1, which eliminates all interferences except those that are isobaric with the target analytes being allowed through. Most of these can be eliminated through optimized chromatography and ionization, leaving few problematic isobaric interferences.

The caveats with LC-MS^n are few but important. Buffers and mobile phases must be carefully selected for compatibility with the ionizations source. Ion suppression can occur in the source and must be accounted for and minimized. Finally, and perhaps most important, it is worth emphasizing that the MRM/SRM protocol is a powerful quantitative tool, but it is not amenable to identification of general unknowns. To design assays, it is necessary to know what compounds are present or likely to be present in the sample and are being targeted so that an MRM transition can be implemented. A few stray peaks may appear in the chromatogram, but only for specified transitions. This information alone is insufficient for compound identification. This contrasts with GC-MS, where essentially anything that elutes and fragments can be detected and the mass spectrum searched.

A trend that bears watching is the rapid development in tandem systems that combine a quadrupole or quadrupoles with a time-of-flight (TOF) mass detection system. The advantage of this system is the mass accuracy and precision capability of a TOF detector, typically in the ppm range or better. This allows for a reliable mass determination to four or more decimal places, which greatly reduces the number of candidate structures. This capability addresses the limitations of triple quadrupole systems. Forensic toxicology labs are already adapting LC-Q-TOF systems as accurate screening instruments to complement the quantitative capabilities of tandem systems. As the cost of these tandem instruments decreases, it is likely they will become commonplace in forensic laboratories, MS and it would not be a surprise to see LC-MS^n systems become the GC-MS of the twenty-first century.

4 ELEMENTAL ANALYSIS

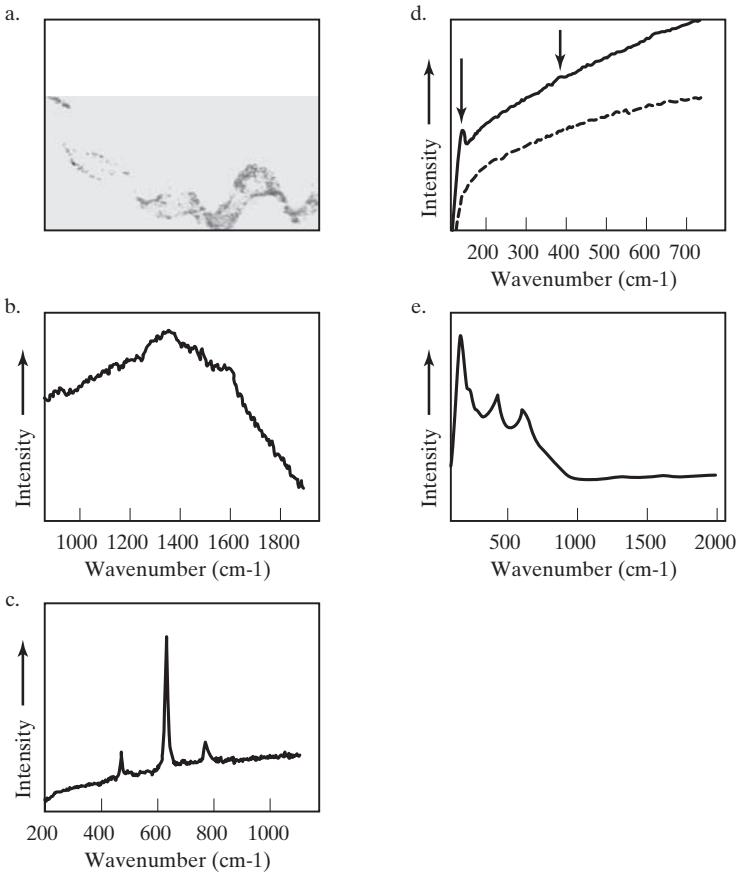
Elemental analysis in forensic chemistry has advanced significantly in the past decade and now includes mass spectrometry in addition to spectroscopic methods. Atomic spectroscopy methods such as atomic absorption (AA) and atomic emission (AES) spectroscopy are still used in forensic analysis, but generally not as frequently as inductively coupled plasma mass spectrometry (ICP-MS) and X-ray spectroscopy. Accordingly, we will focus on these two methods of elemental analysis.

APPLYING THE SCIENCE 3 Raman Microscopy and the Vinland Map

Beinecke Rare Book and Manuscript Library, Yale University

The Vinland Map surfaced in 1957. It appeared to be a map showing the coastline of North America and was purported to have predated Columbus's journey by 50 years. As with many objects of art and archaeology, the question of authenticity was immediately raised, but any chemical analyses undertaken had to be nondestructive. Raman analysis using a fiber-optic probe was one technique applied to the map, with results shown in the accompanying figures. Genuine medieval documents created with black inks often contain iron gallotannate, which slowly leaches from the ink into the parchment underneath. Over time, the migrating iron

causes the parchment to yellow and become brittle, leaving a faint yellow border around black inks (Figure 2). In this case, however, the black ink was found to be carbon based, and the parchment showed no evidence of the damage expected from an iron gallotannate ink. Rather, it appears that a forger placed a yellow line on the parchment before overwriting with the black ink in an attempt to simulate the appearance of a nearly 500-year-old document.



▲ Image courtesy of the Beinecke Rare Book and Manuscript Library, Yale University.

(a) Black ink with yellow underlayer, from the Vinland Map. (b) Black ink from the Vinland Map. (c) Chromite ($FeCr_2O_4$) from a specimen sample. (d) Anatase and plain parchment from the Vinland Map; solid line, anatase in yellow line; dotted line, plain parchment. (e) Ilmenite ($FeTiO_3$) from a specimen sample. Figures and captions reproduced with permission from the reference cited below, Copyright 2002, American Chemical Society.

Source: Brown, K. L., and R. J. H. Clark. "Analysis of Pigmentary Materials on the Vinland Map and Tartar Relation by Raman Microprobe Spectroscopy." *Analytical Chemistry* 74 (2002), 3658–3661. Copyright American Chemical Society 2002.

4.1 X-Ray Fluorescence (XRF)

The advantages of X-ray methods include the ease of sample preparation, the ability to analyze small samples, and the nondestructive nature of the analysis. X-ray detectors can also be integrated into scanning electron microscopes, a topic to be discussed in a later section of this chapter.

X-ray energy is far more energetic than that in the ultraviolet range. If an X-ray photon has an energy that exceeds the ionization energy of an electron, absorption of that photon can eject the electron outright. Furthermore, the ejected electrons are from core levels—those closest to the nucleus. In terms of quantum numbers, these levels correspond to $n = 1, 2$, and 3, which are usually referred to with the older shell notation K, L, and M. When an inner-shell electron is ejected, an outer-shell electron "falls" to fill the vacancy and releases X-ray photons (fluorescence) in the process illustrated in Figure 47.

Because electrons may fall in a cascading fashion, the spectrum can contain several lines that are related to a transition, as shown in the figure. The letter designation refers to the quantum level to which the electron falls, whereas the α, β, γ notation refers to how many levels the electron falls. Because more than one electron per level may fall, the transitions are numbered. An L_α transition refers to an electron that falls into the L level from one level above (α). Most transitions are to an inner shell (K or L).

X-ray spectrometry is a family of related techniques for elemental and crystal structure analysis that derive information from the emitted radiation and from the ejected electrons. X-ray fluorescence (XRF) can be based on the energy of the emitted photons

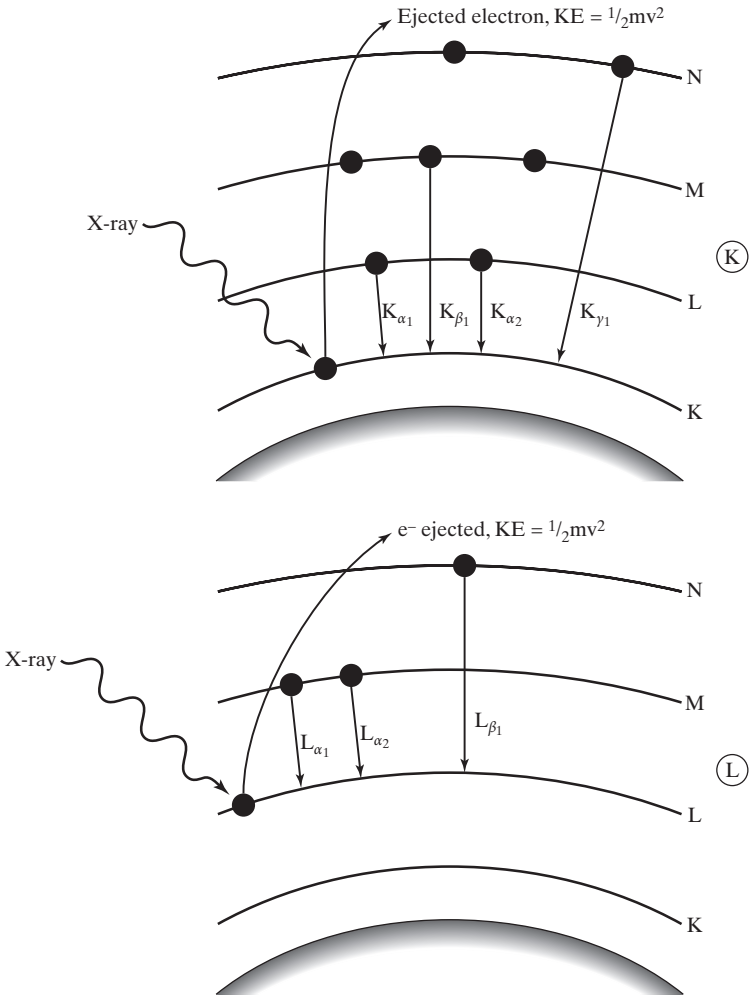


FIGURE 47 Process that generates an XRF spectrum.

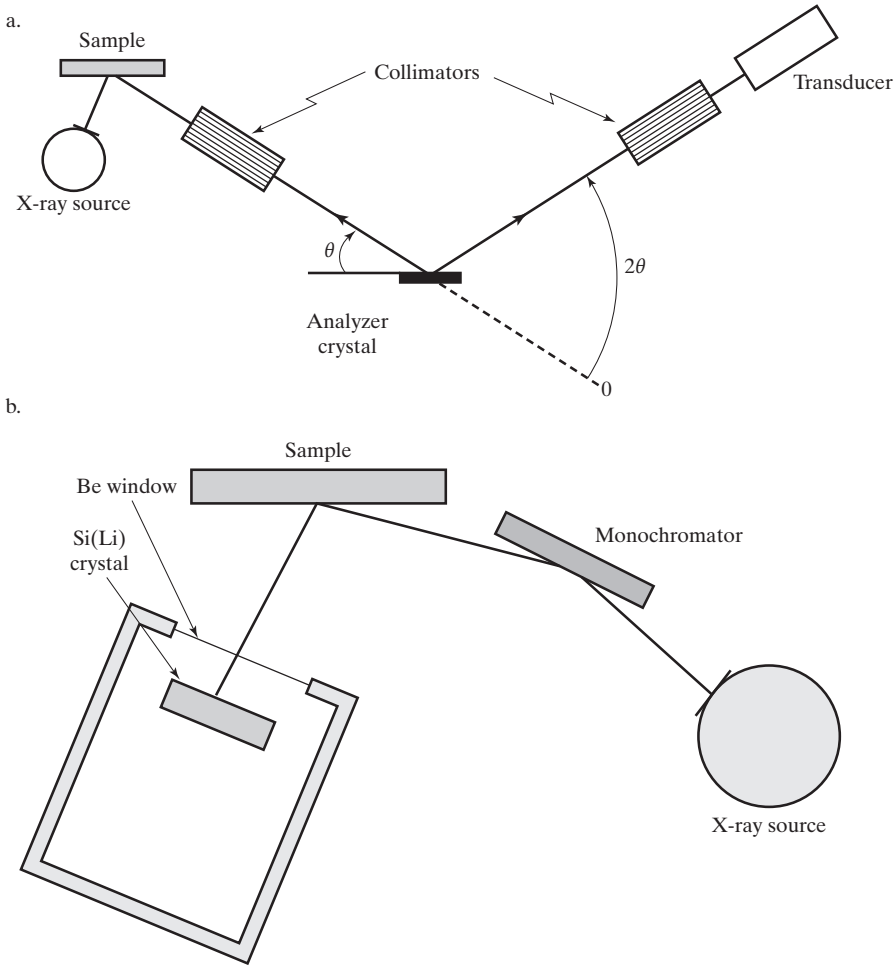


FIGURE 48 (a) A box diagram of a WDS X-ray spectrometer. (b) A box diagram of an EDS spectrometer.

(energy-dispersive spectrometry, or EDS) or on the wavelengths emitted (wavelength-dispersive spectrometry, or WDS). Both techniques are illustrated in Figure 48.

Wavelength-dispersive XRF operates much as does any other emission method in which emitted light is physically dispersed by a grating to isolate individual wavelengths. Because the wavelengths of X-ray energy are so small, it is not feasible to construct a grating. Instead, crystals with predictable geometries are used to generate constructive and destructive reinforcement patterns analogous to those generated by a grating. EDS does not use a monochromator but rather calculates the energy of the X-rays that strike the detector and converts those energies to equivalent wavelengths.

Other X-ray techniques include X-ray diffraction (XRD), which reveals information about crystal structures. It is used less frequently than XRF in forensic science. Other related techniques, such as Auger spectroscopy, have yet to find significant applications in forensic chemistry.

5 MICROSPECTROPHOTOMETRY

Microscopes and spectrometers both use electromagnetic energy to probe matter, so why not use a microscope as the source of energy so that very small samples such as single fibers can be easily studied? Until recently, the roadblock was technological.

Because the amount of energy directed through a microscope is so small, the intensity of that energy is limited. Accordingly, the development of suitable detectors was one of the keys to implementing practical microspectrophotometry (MSP), particularly for the infrared range. In the last 15 years, MSP has come of age and is beginning to displace traditional macrospectroscopy in the forensic laboratory. Any mode of spectroscopy can theoretically be adapted to microspectroscopy. Molecular and atomic methods are now available, and the instruments that perform such analyses are an increasingly common sight on the forensic bench. Most MSP tests are nondestructive or minimally destructive, and the instruments are now more affordable for forensic labs. In some cases, such as UV/VIS spectroscopy, microspectrophotometry is reviving interest in techniques that had been marginalized or nearly abandoned in forensic chemistry.

The marriage of a microscope, designed to work with visible light, and colorimetry is the simplest proposition, because the optics of both operate in the same spectral range. Thus, glass lenses, objectives, and other optical components can be made of the same material. The situation is more complex when a visible microscope is combined with a spectrophotometer that operates outside the visible region, such as the IR or UV. In these cases, optics must be capable of focusing and directing energy in all applicable ranges of wavelength. The realization that mirrors could be used in lieu of or in combination with lenses was a pivotal advance for MSP. A **Cassegrain system** uses highly polished stainless-steel mirrors to focus rays of UV, VIS, and IR energy and thus serves as an objective "lens" across many spectral regions. Reflection, transmission, and ATR modes can be accommodated by this design, as shown in Figures 49. The design also provides a large numerical aperture and can have large working distances, topics previously discussed in Section 1.

Another factor constraining microspectrophotometry was the intensity of the source. The trade-offs among intensity, slit width, and energy are amplified when energy must be focused on a very small portion of the sample. As a result, intense light sources such as lasers or extremely bright lamps are required. Lasers have the added advantage of being tightly focused. The final consideration is the detector, which, as all transducers do, converts photons to electrons, but with extraordinary efficiency. Because analytical signals are so small, background noise must be minimized. Many MSP detectors are based on semiconductors, and a number of them are cooled either by electrothermal processes or with liquid nitrogen.

Early microspectrophotometers were designed as adjuncts to an existing spectrometer. Energy from the source was directed into the microscope, where it interacted with the sample and then traveled back to the bench and the existing detector. This approach had the advantage of dual use (bench and scope) but typically optimized the

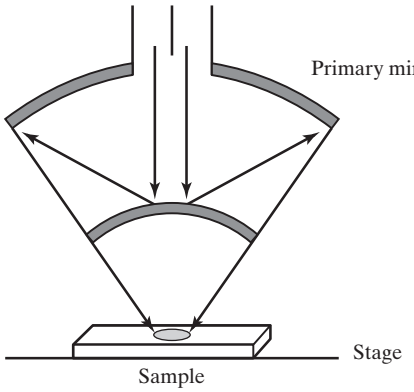


FIGURE 49 Simplified depiction of a Cassegrain focusing system using polished mirrors in lieu of lenses to focus electromagnetic energy on a sample.

Image courtesy of Criac Technologies

FIGURE 50 A commercial microscope base with a UV/VIS/NIR spectrophotometer detector and imaging system. Photo courtesy of Dr. Paul Martin, Criac Technologies.

macrospectroscopy rather than the micro. Newer instruments are stand-alone devices with microscope bases and spectrometers supplied as integrated components. One common accessory is a precision motorized stage that allows for computer control of sample positioning, which is crucial when the size of the image is as small as a few microns. Computerized stage controls also facilitate automated surface mapping of samples.

5.1 UV/VIS Microspectrophotometry

The availability of UV/VIS spectrophotometers has revitalized the examination of evidence for characteristics associated with color. Fibers, inks, and paints in particular can be examined nondestructively and characterized by a spectrum as well as the analyst's judgment of their color. Prior to the availability of UV/VIS MSP, obtaining a visible spectrum of a fiber involved destructive extraction followed by colorimetry. Large numbers of fibers were needed, and potentially useful inter- and intrasample variations in color were lost. Particular applications of MSP to color determinations are addressed in the application chapters that follow. Applications include colorimetric analysis of inks, paint, dyes, and even hair color.

5.2 IR and Raman Microspectrophotometry

As with macroscopic work, microvibrational spectroscopy has found its broadest use in forensic chemistry. FTIR techniques are particularly amenable to micro applications, given the ability to simultaneously collect energy across the wavelength range and average a large number of scans to reduce noise and enhance sensitivity. All modes of IR, including transmittance, reflectance, and ATR, have been adapted to FTIR microscopy. Detectors used include the mercury–cadmium–tellurium (MCT) design, which requires cooling, and the deuterium–triglycerine sulfate (DTGS) design, which does not. Some designs have adapted a novel diamond ATR objective through which the user can sight and obtain an IR spectrum. In many forensic laboratories, micro-FTIR, and particularly micro-ATR, are replacing bench FTIR for applications such as drug and paint characterization, given the ease of ATR, the small sample size needed, and the nondestructive nature of the test. Raman microspectrophotometry has also proven valuable, particularly for ink and pigment analysis, although it is still not considered a routine forensic procedure.

5.3 Microelemental Analysis

XRF is readily adaptable to microscopic platforms and is used for surface mapping applications. Forensically, the most important micro-XRF method is that associated with scanning electron microscopy (SEM), which uses electron beams as opposed to photon beams to visualize a sample. Consequently, X-ray emission is a natural and exploitable byproduct of this interaction. The prevalent adaptation for forensic work is the SEM-EDS configuration illustrated in Figure 51. Electrons are emitted from a filament or other source and are focused onto the sample, whereupon the beam scans the surface. There are instruments that target transmitted electrons (transmission electron microscopy, or TEM), but they are not widely used forensically.

Electrons are scattered analogously to photons, with heavier elements scattering more electrons relative to lighter ones. In the output display, scattering correlates with increased brightness. Because electrons are exploited for imaging, but photons are not, there is no color information in the signal, although false coloring can be added to the image. The sample must be conducting and is usually coated with a thin layer of gold. Secondary electrons are those emitted when the incident electrons eject an inner-shell electron, resulting in X-ray fluorescence. These electrons are ejected at angles and

energies correlated with surface shapes and features. The resulting image can be magnified by a factor of a million or more compared with a few hundred times in conventional microscopy. The added benefit of identifying topography and elemental composition is critical in the forensic identification of gunshot residue, a topic to be addressed in detail in a later chapter. SEM-EDS is also used in analysis of trace evidence, such as paint chips, bullets, and glass.

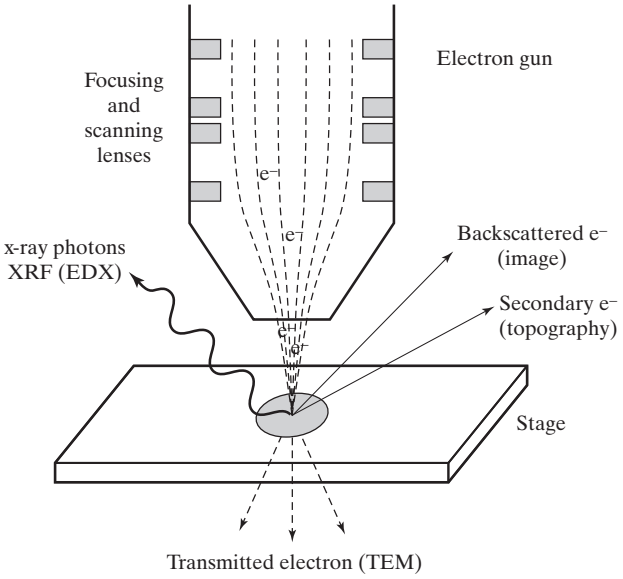


FIGURE 51 Simplified SEM-EDS. Scattered electrons create the image, ejected electrons relate to topography, and the wavelength of the photons correlates with an element.

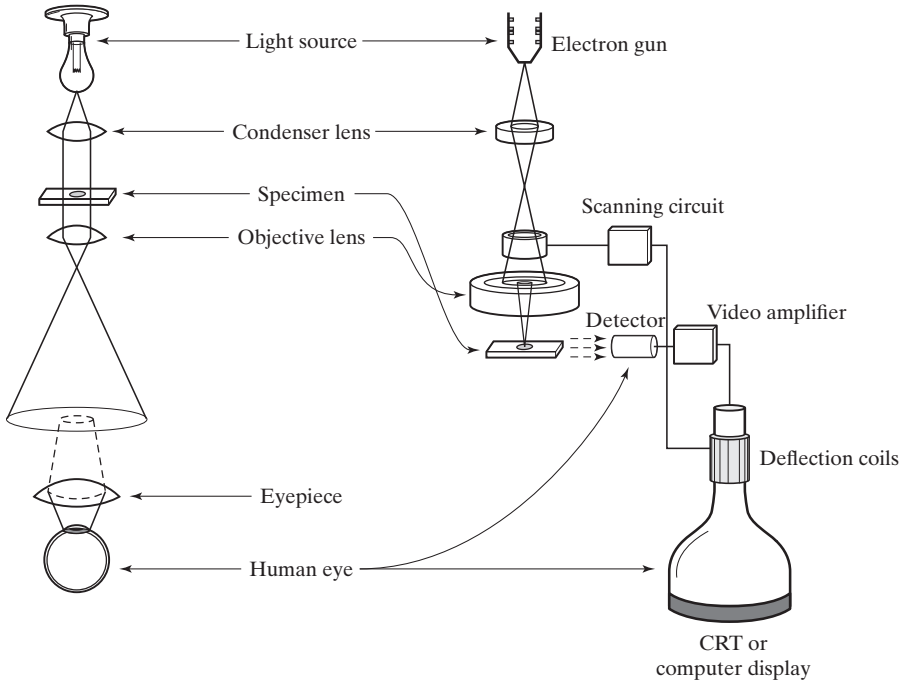


FIGURE 52 SEM and optical microscopy share the same generic features, including a source, focusing lenses, and a detector. The optical path of the microscope is inverted for comparison.

6 ELECTROPHORETIC INSTRUMENTS

Once the exclusive purview of biologists, biochemists, serologists, and DNA analysts, electrophoresis is entering the realm of small-molecule separations and consequently now plays a role in forensic chemistry. Electrophoresis can be conducted on a bed of gel, in a tube of gel, or in a tube of liquid buffer to name a few methods. **Ion mobility spectrometry** is a gas-phase form of **electrophoresis** that has become a primary field-deployable forensic instrument for detection of drugs and explosives.

Electrophoresis separates ions and molecules on the basis of differences in their size-to-charge ratios (compared to the mass/charge ratio exploited in mass spectrometry), which in turn dictate how fast they move through an electric field. The velocity of a charged particle or an ion in an electric field is given by

$$V = \mu_e E \tag{9}$$

where μ_e is the mobility of the ion, and E is the electric field strength, determined by the voltage applied across the region through which the ions move. As charged species, ions move under the influence of the field, based on the charges they carry, tempered by the friction created by the ions as they move. Electrophoresis was originally used to separate proteins on the basis of the charges that can be acquired by protonation and deprotonation of the amino, carboxyl, and other groups. The pH of the solution determines the charge on a protein. This should sound familiar based on our discussion of protonation and deprotonation of drugs as a function of pH in the last chapter. The gel provides resistance to flow proportional to the size of the ion, and separation is based

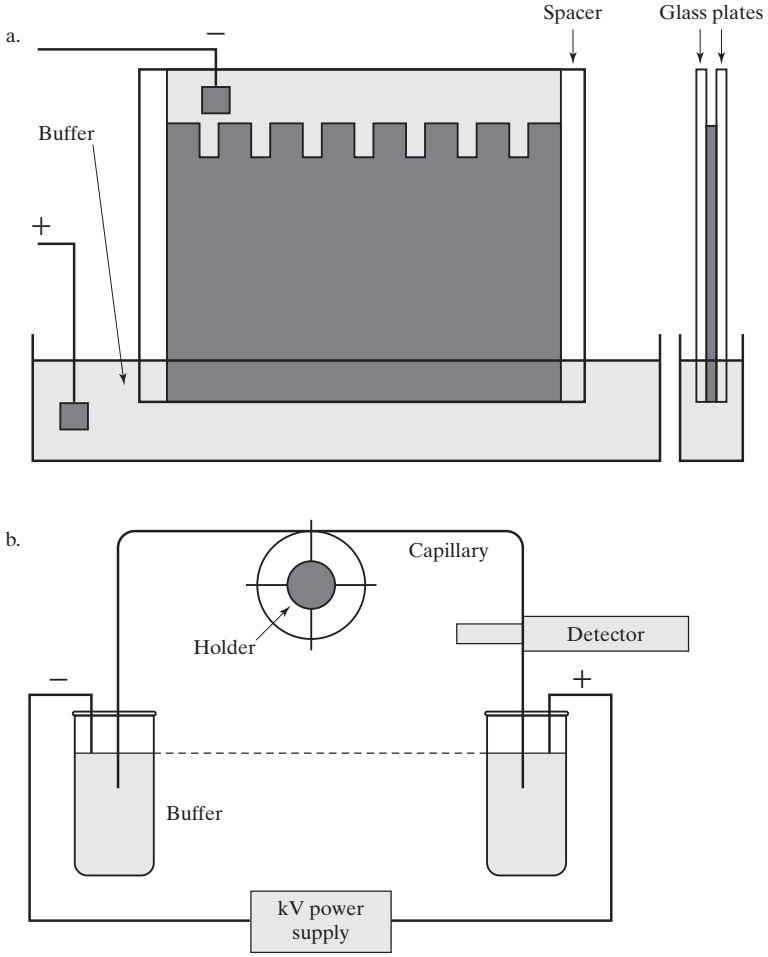


FIGURE 53 (a) Slab-gel electrophoresis. A power supply generates the electric field, and the buffer ensures that the pH remains constant. (b) Electrophoretic separation in a capillary tube.

on the balance between friction and induced movement in the electric field. The process is illustrated in Figure 53.

Capillary Electrophoresis: Slab-gel techniques were used in forensic serology until the late 1980s to separate and detect polymorphic serum proteins found in blood and body fluids. Around the time that DNA began to supplant traditional blood group typing in forensic serology, capillary electrophoresis (CE) was introduced to separate small molecules. Ironically, by the turn of the century, CE-based instruments dominated DNA typing.

One of the advantages of CE systems is their simplicity. A capillary tube (composed of SiO_2) is placed with both ends in a buffer system so that separation will take place within the capillary rather than in a slab. The mode of separation is altered as well, to exploit an **electroosmotic flow** generated within the tube. As shown in Figure 54, polar sites attract a layer of hydrated cations at the tube's inner surface. These cations are attracted to the cathode and flow regardless of what is introduced into the capillary. As a result, neutral species as well as positive can be detected. Even negatively charged small ions are eventually dragged to the detector by the electroosmotic flow. Modifications to the surface of the tube, to the pH, to the electric field, and to other experimental parameters impart unmatched flexibility to CE.

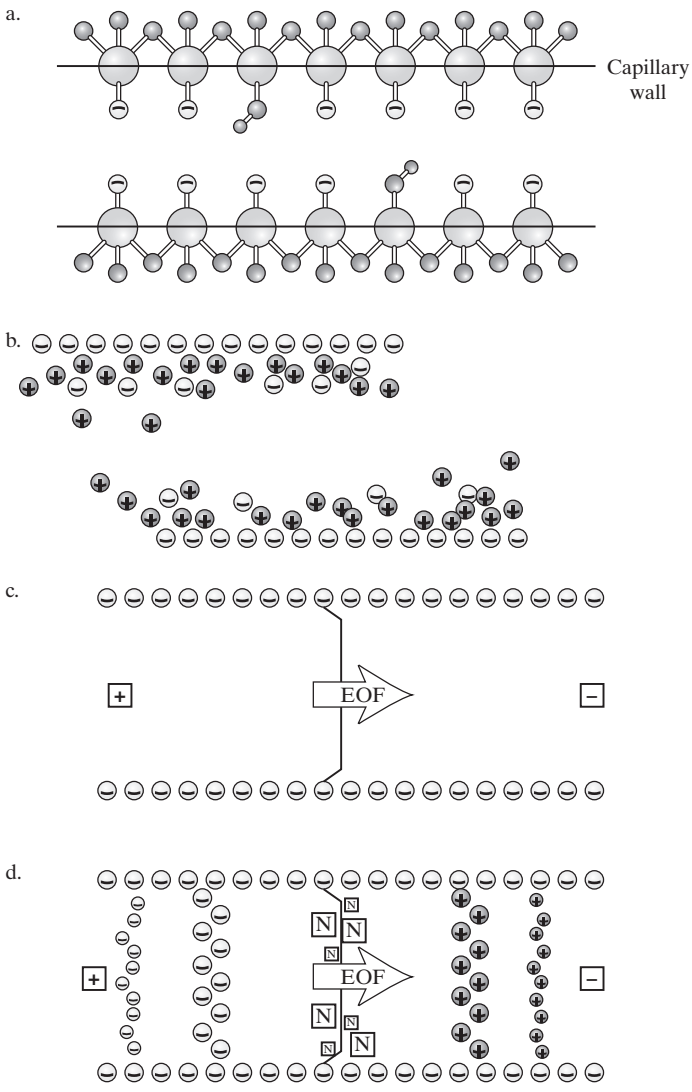


FIGURE 54 Generation of electroosmotic flow. (a) Exposed Si—O— groups on the capillary wall attract hydrated cations (b) and form a layer along the capillary wall. When the potential is applied, the positive layer migrates (c) toward the cathode (–). This flow is strong enough to move neutrals and anions at different rates while accelerating the movement of any analytical cations in the system.

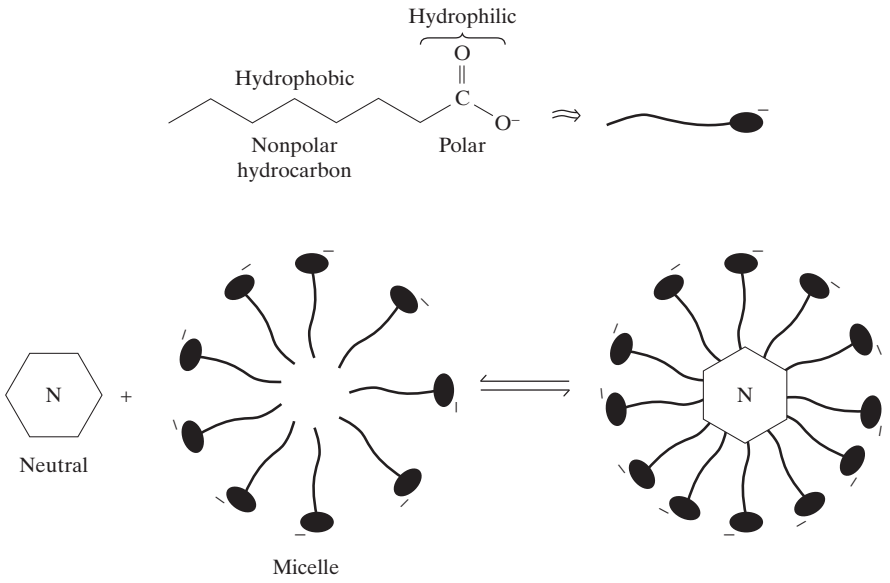


FIGURE 55 Soap is surfactant molecule that can form micelles in water; neutrals can move in and out of a micelle, with the degree of association dependent on factors such as the size and polarity of the neutral.

CE can be operated in several modes. The approach described in the previous paragraph is referred to as **capillary zone electrophoresis**, or CZE. A variant called *capillary gel electrophoresis* uses a capillary tube filled with gel, essentially a miniaturized version of slab-gel techniques. The third variant, and the one of most forensic significance, is **micellar electrokinetic chromatography** (MEK or MECK). MEK is a modification designed to improve the separation of neutral species, which are easily separated from cations and anions, but not well separated among themselves.

MEK achieves improved separation by adding another discriminating interaction. **Micelles** (Figure 55) form when surfactant molecules such as soaps and detergents are added to water. Soap is a molecule with a hydrophobic hydrocarbon portion and a hydrophilic ionized end. When soap is added to water at a sufficient concentration, micelles form. Neutrals can interact and associate with (i.e., selectively partition) the micelles, which themselves are charged and move with the electroosmotic flow according to that charge. If a micelle is negatively charged, it moves slowly toward the detector—more slowly than the neutrals do. However, because the neutrals interact with the micelles, the progress of the neutrals is impeded in proportion to their degree of association with the micelles. This is another form of chromatography in which separation is based on differences in partitioning between two phases, and MEK is often described as a hybrid of CE and chromatography. The micelle is like a "column" on which the solute is retained; the longer the retention, the slower the migration. This process is illustrated in Figure 56.

The detector module of a CE system is usually spectroscopic. Hence, its sensitivity is a function of path length, which introduces one of the fundamental problems of linking a flowing separation system to a detector designed for static measurements. The volume used in CE is on the order of nanoliters, and that already minuscule volume is constantly moving. The simplest detectors are those based on UV absorption and are implemented by "burning" a clear window into the capillary at the point where detection is to occur. By creating a Z-shaped bend in the tube (Figure 57), the path length can be increased, as long as the path is not so long as to allow more than one separated component into the detection zone. Laser excitation and fluorescence is another more sensitive option and is used for DNA typing. Electrochemical detectors have been used, as has mass spectrometry, but UV modes dominate forensic implementations.

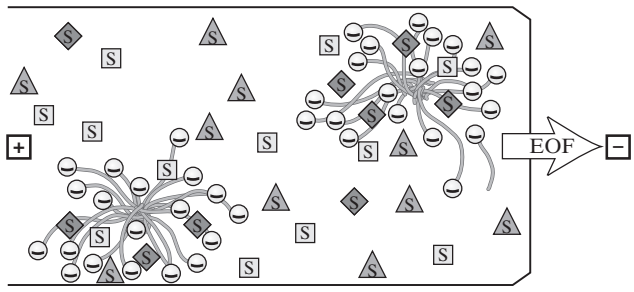


FIGURE 56 Separation in MEK, using micelles to carry neutrals along. *S* = sample.

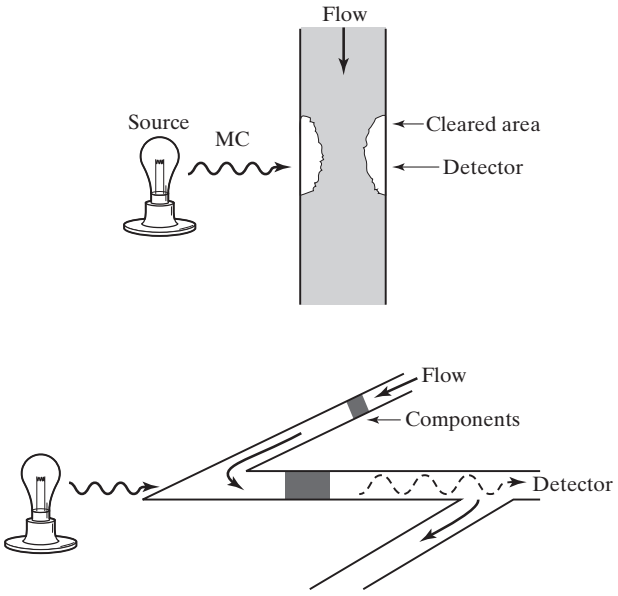


FIGURE 57 Use of a Z-shaped flow cell to increase the path length for spectrophotometric detection in CE. The same principle has been applied to detectors in liquid chromatography.

Because of its versatility, CE is making inroads into forensic laboratories and in forensic research.[31–33] Instrumentation is also fairly inexpensive and easy to maintain, a prerequisite for wide adoption. Toxicology in particular has embraced CE for drugs and analytes, which can be acidic, basic, or neutral, and hydrophobic or hydrophilic.

Gas-Phase Electrophoresis: Ion Mobility Spectrometry (IMS): In airports, seaports, and border crossings, ion mobility spectrometry (IMS) has become a standard field screening method for explosives and drugs. Although the instrument operates in the gas phase at atmospheric pressure, the basis of separation is the size–charge ratio. In fact, one of the original names for the technique was *gas-phase electrophoresis*. IMS instruments (Figure 58) are best known to the public as the instruments used at airports to analyze swipes from luggage or and handheld detectors for chemical weapons. The instrument operates at atmospheric pressure and requires small amounts of power, important criteria for any portable system.

As sample enters the inlet, it is ionized through a complex series of reactions. The first step is ionization of N_2 by ^{63}Ni, a β emitter. Because the instrument runs at ambient pressures, there exists a reservoir of reactant ions of the form $H(H_2O)_n^+$ that surrenders protons to M^+ ions, depending on relative proton affinities. What enters the drift region is a complex mixture of M^+ and other cluster ions that move toward the detector under the influence of an applied electric field, analogous to the situation in gel electrophoresis. In IMS, an opposing drift gas flow collides with the clusters, impeding the

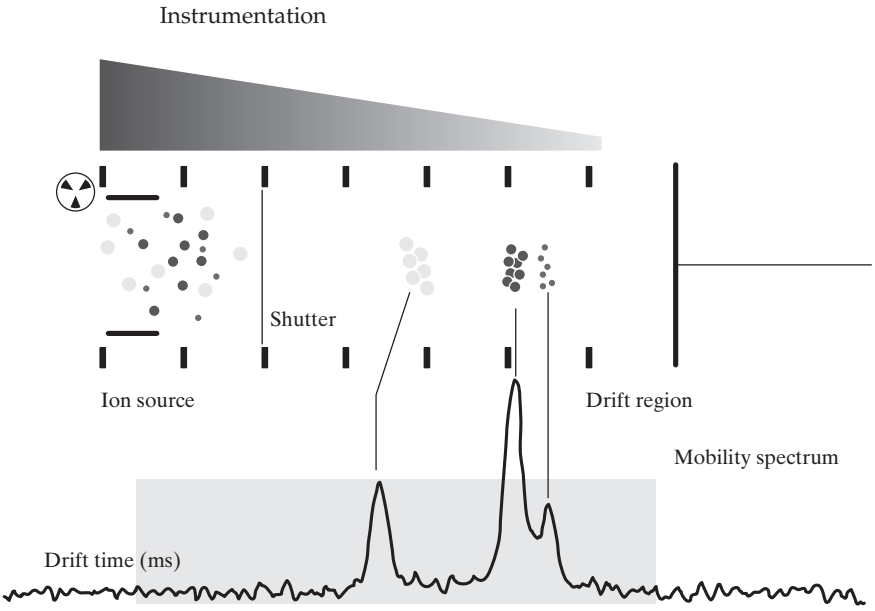


FIGURE 58 An ion mobility spectrometer and mobility spectrum. Ion molecule clusters move toward the detector and separate on the basis of size and charge. Ion drift time is recorded in milliseconds (ms). Image courtesy of Dr. G. A. Eiceman, New Mexico State University.

progress of the larger ones more than that of the smaller ones. The drift gas acts as the gel does, resisting migration in proportion to size. The smaller charged species arrive at the detector before the larger ones. The same process occurs in the negative mode, but with a different group of reactant ions, such as O_2^- and Cl^-. Fundamentally, IMS measures the collisional cross-sectional area of the ions and ion/molecule clusters.

The output of the instrument is a plot of signal intensity versus drift time. **Mobility spectra** are information poor in much the same sense that UV/VIS spectra are. A mobility spectrum cannot definitively identify compounds alone, but linkage to libraries and databases allow for the range of potential analytes to be reduced to a small population. In screening applications, this is an acceptable trade-off, as long as false negatives are minimized. At airports, IMS is used to screen baggage for explosives and is found at security checkpoints. Attendants may wipe a pad over luggage or computers and place the pad in the instrument to obtain a fast negative-mode scan for potential explosives. Recently, IMS has been combined with gas chromatographs and with time-of-flight mass spectrometers to create hyphenated instruments. The newest generation of IMS instruments has been significantly miniaturized such that the portion of the instrument shown in Figure 58 is slightly bigger than a quarter.

7 HYPHENATED INSTRUMENTATION AND INLETS

Hyphenated instruments are those that couple a sample introduction system to a detector system. We have already mentioned several including GC-MS and LC-MS^n. In these examples, the chromatographic system separates complex mixtures such that (ideally) only one analyte enters the detector at a time. Because gas chromatography and liquid chromatography are so important in forensic chemistry, it is worth discussing and reiterating the basics of these techniques with an eye for how they are coupled with detectors used in forensic chemistry. Although mass spectrometry would seem to be the ideal detection system, there are still many applications in which other detectors are the logical choice. We will discuss the **hyphenated systems** that are most common in forensic chemistry, but we begin with what should be a quick review of chromatography.

Chromatographic Inlets: Gas chromatography remains a workhorse inlet of forensic chemistry, be it for drug analysis, toxicology, or identification of accelerants.

High-performance liquid chromatography[†] is used extensively in toxicology, particularly when the technique is linked to a mass spectrometer. As inlets, both methods effect separation by selective partitioning, the theory of which was covered in the previous chapter. In gas chromatography, analytes are volatilized and introduced into an inert carrier gas stream that is directed through a capillary tube[‡] (Figure 59). The tube is coated with a material in which the analytes partition (usually) on the basis of their relative polarities and boiling points. Just as more interactions with micelles slow the progress of neutrals in MEK, more interaction with the stationary phase means a longer retention time in the column. Capillary columns provide large surface areas for interaction, facilitating excellent separation and resolution. The principal limitation is in the capacity of the column. With thin films, it is easy to saturate the solid phase.

In a separations module employing a flowing system, the goal is to separate or resolve each component of a complex mixture into a discrete and pure packet separated in space and time from all other components. Doing this requires a tightly packed grouping with maximal separation between groups, expressed by the resolution or efficiency of the column. Figure 60 shows a mixed sample introduced into the flowing system. Partitioning and separation take place in the column. The ideal situation (Figure 60b) occurs when the separated groups of molecules are tightly packed together and the separation between groups is large. The resulting chromatogram will have narrow peaks with baseline resolution (Figure 60c). Tight groups but little separation (Figure 60d), or broad groups both lead to overlapping peaks and mixtures entering the detector.

The most frequently cited measure of column efficiency is the number of **theoretical plates,** given by

$$N = 16\left(\frac{t_{r_i}}{w_i}\right) \qquad \textbf{(10)}$$

[†]HPLC generically, meaning high-*performance* or high-*pressure* liquid chromatography.

[‡]Forensic GC is capillary based; packed-column applications are rare.

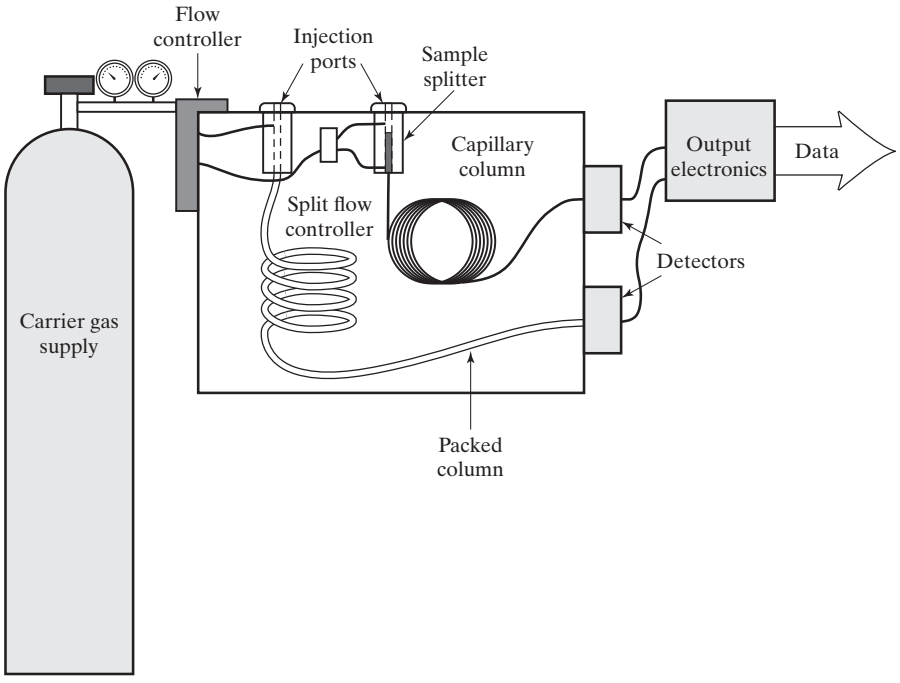


FIGURE 59 A gas chromatograph with packed and capillary columns shown for purposes of illustration. Forensic applications are capillary-column based.

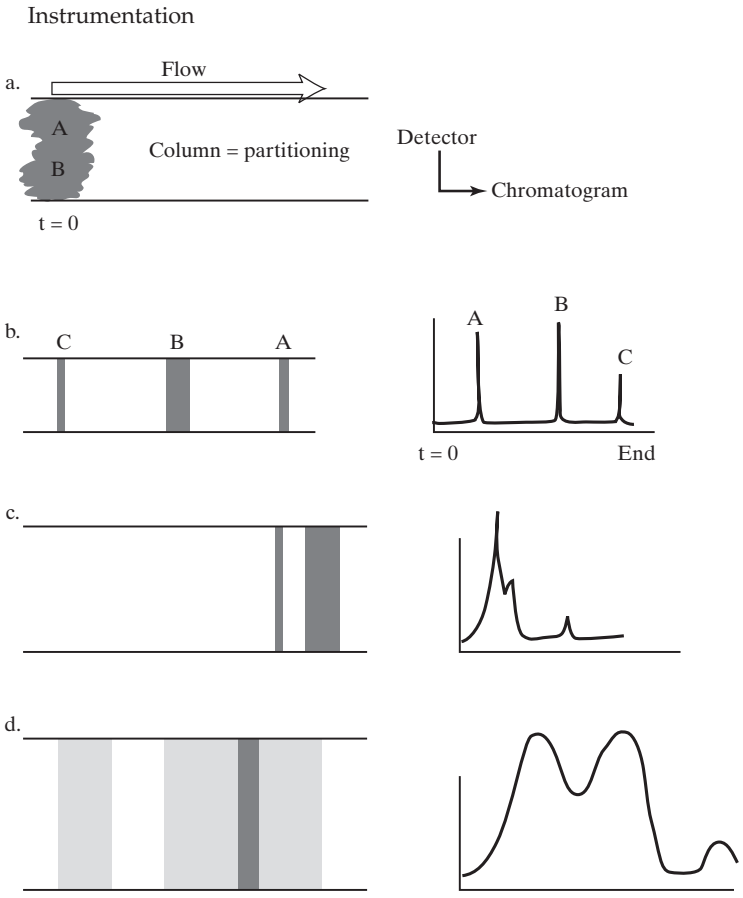


FIGURE 60 Separation of a complex mixture.

where t_r is the retention time of the peak being used and w is the width of that peak at the baseline; larger is better, and capillary columns can have N values of 10,000 or greater. Also used is the height equivalent of a theoretical plate (HETP),

$$H = \frac{L}{N} \tag{11}$$

where L is the length of the column.

Although a detailed review of GC and HPLC is beyond the scope of the present treatment, a brief review of the key relationships is appropriate. The **van Deemter curve**, shown in Figure 61, is a plot of efficiency (HETP, equation 11) as a function of mobile phase velocity (the flow rate). Three terms contribute to determining the shape of the curve. The minimum point is the flow rate that gives the best separation efficiency for a given mobile phase. Note that curves for different gases have different minima but a similar shape. The first factor that causes peaks to spread is diffusion. When analyte travels through the column, molecules follow different paths and a Gaussian distribution of spreading results. In a capillary GC column, where stationary phase is coated on the walls of the column rather than on beads packed into it, this term (A) drops to negligible values and has the effect of dropping the curve toward the x-axis. The second term is longitudinal **diffusion**, in which any concentrated mass of material naturally spreads out over time. As the flow rate increases, time in the column decreases, as does the contribution of this factor to band broadening (B/μ).

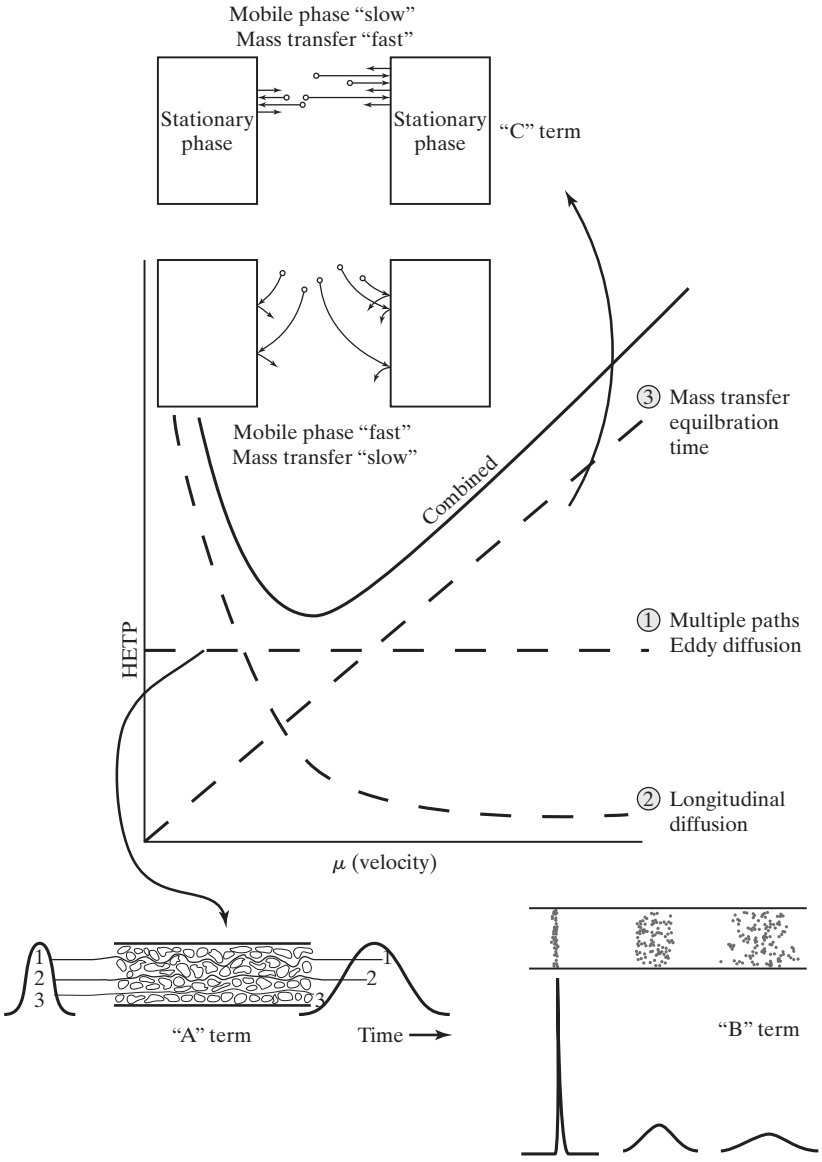


FIGURE 61 The three factors that contribute to band broadening and efficiency of a chromatographic separation, plotted as a function of velocity (μ) of the mobile phase, be it liquid or gas.

The third term that adds to band broadening is the time allowed for equilibration between the mobile and stationary phase $(C\mu)$. Partitioning requires an analyte to associate with the stationary phase:

$$\text{Analyte (mobile)} \longleftrightarrow \text{Analyte (stationary)} \qquad \textbf{(12)}$$

The kinetics of this interaction is important. If the transfer of the analyte to the stationary phase and back (**mass transfer**) occurs quickly relative to the flow rate, the analyte has many opportunities to interact without being left behind in the stationary phase. If the flow is rapid (the mobile phase is "fast" in Figure 61), some of the analyte associated with the stationary phase is left behind the solvent front, leading to band broadening. Accordingly, this type of broadening is worsened by increasing the flow rate, since that would slow equilibration. The combined expression—the van Deemter equation—is

$$\text{HETP} = A + \frac{B}{\mu} + C\mu \qquad \textbf{(13)}$$

and it can be applied in analogous forms to any chromatographic or electrophoretic separation.

The liquid phase analog of GC is high-pressure liquid chromatography (HPLC), in which small volumes of solvent are directed through a column tightly packed with beads coated with the stationary phase. The system operates under elevated pressure, which facilitates the tight packing and high surface area for interaction. HPLC is ideal for analytes that do not volatilize easily, such as large polymers and sugars. Thermally unstable compounds, including the drug LSD, formaldehyde, and many explosives, are amenable to HPLC without derivatization. The ability to combine solvents, which can include aqueous solutions and buffers in the mobile phase, adds to the versatility of HPLC. Unlike what happens in capillary GC, band broadening due to different paths is significant, but the expression for efficiency is still based on theoretical plates and HETP.

HPLC techniques cover a larger range of analytes than GC does, as shown in Table 1. The interactions should be familiar; they are drawn from the same group of interactions as in solid-phase extraction with some additions. Recently, instrument and column manufacturers have created systems that significantly improve the separation power of traditional HPLC. In these systems, called UPLC® (standing for ultra-performance liquid chromatography), particulates are used to pack the column that are much smaller than in traditional HPLC(~ 2 um μ or less). The result is much improved separation power using smaller columns; the caveat is that the pumping system must be capable of higher pressures and consistent delivery of small solvent volumes at pressures higher than in traditional HPLC. The term often used to characterize UPLC columns and assays is *peak capacity*, or the number of peaks that can be resolved within

TABLE 1 HPLC Separation Modes

Separation Mode	Separation Mechanism	Application
Normal Phase (NP)	Partitioning between a polar stationary phase and a less polar mobile phase	Organic-soluble polar analytes and water-soluble nonionic analytes; usually of a molecular weight <2000
Reversed Phase (RP)	Partitioning and/or adsorption between a nonpolar stationary phase and a more polar mobile phase	Organic-soluble nonpolar analytes and water-soluble polar nonionic analytes; usually of a molecular weight <2000
Hydrophobic Interaction (HI)	Adsorption between a nonpolar stationary phase and an aqueous mobile phase	Aqueous-soluble, denatured proteins and peptides
Ion Pairing (IP)	Interactions between a nonpolar stationary phase and a more polar, usually aqueous, mobile phase containing an ion-pairing reagent	Aqueous-soluble ionic analytes
Ion Exchange	Interactions between cationic and/or anionic stationary phase and aqueous mobile phase	Aqueous-soluble ionic analytes
Gel Permeation Chromatography/ Gel Filtration Chromatography/ Size Exclusion Chromatography (GPC/GFC/SEC)	Size sieving of analytes through or around pores in either a polymeric or a bonded silica stationary phase	Organic-soluble analytes, typically MW >2000, but not exclusively
Chiral	Physical interaction with an optically active stationary phase	Optically active analytes

a specified unit of time. Using these ultrafast techniques, runtimes of a minute or less have been reported. It remains to be seen how rapidly these instruments and methodologies will make their way into widespread forensic application.

Detector Systems: GC: In any hyphenated instrument, a critical design concern is the interface between the separations module and the detector. This was made clear in our previous discussion regarding mass spectrometry. The issue was also hinted at in the previous section with the discussion of the "Z" **flow cell** for capillary electrophoretic systems. The task is simplified for GC relative to HPLC, because there is no solvent to contend with, yet the overall issue of flow out of the column, either too much or too little, must be addressed. All detectors have a characteristic selectivity and sensitivity; inescapable trade-offs are involved. In forensic chemistry, two detectors are widely used in addition to mass spectrometry: a **flame ionization detector (FID)** for arson cases and screening applications, and a **nitrogen–phosphorus detector (NPD)** for drugs and metabolites.

The flame ionization detector (FID, Figure 62) is sensitive to compounds that contain C — H bonds, and the more of these bonds, the stronger is the response. In forensic science, an FID is used for the analysis of blood alcohol and for fire debris to detect the presence of accelerants such as gasoline. GC-FID is also employed for other screening applications and for developing methods. The flow of carrier gas and analytes exiting the column is supplemented with a supply of hydrogen and air (O_2) that is ignited, resulting in the production of free radical cations such as $CHO^{\cdot}$. Some free radicals are positively charged and so are attracted to the cathode (collector). The detector output is a plot of the current generated by the detector as a function of the run time. With capillary GC systems, a makeup gas such as nitrogen is often needed to maintain the optimum fuel–oxidant ratio for efficient combustion. The nitrogen–phosphorus detector (NPD) is a variant of the FID with comparable sensitivity, but greater selectivity. A small heated bead of an alkali salt such as rubidium sulfate is used to generate ions, a process that is at maximum efficiency in the presence of nitrogen- and phosphorus-containing compounds. Forensic toxicologists employ NPD in screening tests because most drugs and metabolites contain nitrogen.

The FID and NPD are used in screening applications because they are not specific detectors. A peak in a chromatogram indicates a detector response, and the population of molecules that could have produced that peak is limited. However, even the NPD detector responds to thousands of compounds. Analytical conditions used in sample preparation and instrument operation further constrain the possibilities, as does the retention time and the use of traceable standards for comparison. Peak areas combined with retention times are used for quantitative purposes, but definitive identification, particularly of unknown peaks, is not possible using a single column and single detector.

EXAMPLE PROBLEM 4

Of the solvents pentane, hexane, and carbon disulfide, which is the best choice for analyzing arson debris with GC-FID?

Answer:

All three solvents are acceptable for the extraction of nonpolar gasoline components, since all three are themselves nonpolar. If the extract is to be analyzed by GC-FID, carbon disulfide is the best choice because the FID detector is not very sensitive to this solvent. The other two contain C — H bonds, and as a result, the solvent peak could mask the response to materials found in the fire debris. Finally, CS_2 is unlikely to be found in the sample, whereas pentane and hexane might be if the fire debris does contain an accelerant.

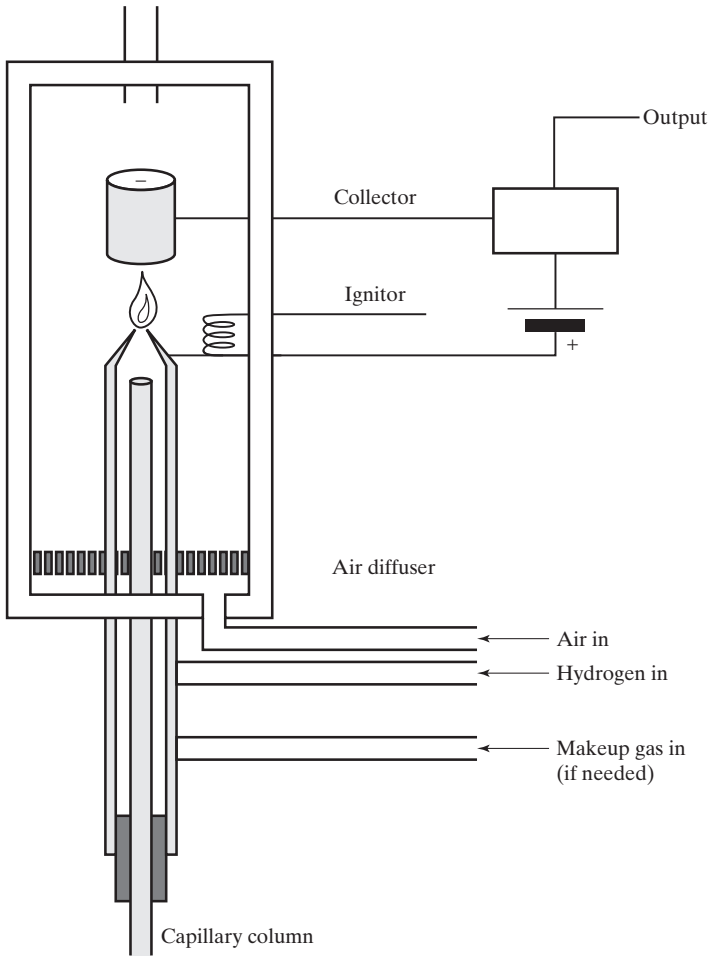


FIGURE 62 Schematic of an FID.

Detector Systems: HPLC: Similar detectors are used for capillary electrophoresis and HPLC, with many of the same design considerations and limitations. Three detectors are common: UV single wavelength, UV/VIS **photodiode array (PDA)**, and mass spectrometry.

The fixed-wavelength UV detector essentially is a marriage of a flow cell and a UV/VIS spectrophotometer. Absorbance at one wavelength at a time can be detected, but most such detectors can operate at more than one wavelength. A photodiode array detector uses a grating to disperse the light that is transmitted through the sample onto a grid of photodiodes placed such that the location of each photodiode correlates with the physical location where a given wavelength of light will impinge. In practice, two gratings are used to provide not just linear, but two-dimensional, dispersion. As with a mass spectrometer, the diodes must be scanned, requiring a finite amount of time that is always at odds with a flowing system design. The advantage is being able to obtain a full UV/VIS scan for each component, rather than just absorption at one wavelength, analogous to the comparison of an FID and MS.

Summary

This chapter presented a whirlwind tour of the instruments most commonly employed in forensic chemistry. These instruments are usually not the newest, nor do they necessarily reflect the cutting edge of analytical instrumentation—and there is good reason for this. First, analytical techniques must prove their worth and exhibit a good cost–benefit ratio as applied to forensic casework, a process that takes years of testing and research. Second, instruments and methods must be rigorously tested and validated to meet legal requirements. Third, cost and intercomparability of those results are essential. Instruments must be rugged and suitable for heavy use and automation. Finally, the cost of obtaining and maintaining the instruments must fit into constrained budgets. In the forensic context, "elegant and exotic" does not always translate to practical or feasible. The emphasis remains on the tried and true.

Hyphenated instruments have come to play a central role in forensic analytical chemistry, taking their place alongside microscopy and spectroscopy as the primary instruments of the modern laboratory. As mentioned at the beginning of the chapter, the microscope and the spectrometer were the pillars of the first forensic laboratory; add to that a GC-MS, and a modern forensic chemistry laboratory takes shape. Ten years from now, LC-MS^n may well be standard equipment for forensic laboratories, along with LA-ICP-MS. Many factors dictate what instrumentation is used in forensic chemistry, including purchase and operating costs, availability, and of course, acceptance by the scientific and legal communities. The most important advancement in recent years has been the development of reliable and affordable IR microspectrophotometers, hyphenated instruments, and capillary electrophoresis.

Key Terms and Concepts

Angle aperture (AA)
Anisotropic
Anti-Stokes scattering
Aperture
Atmospheric pressure chemical ionization (APCI)
Bandwidth
Baseline resolution
Bertrand lens
Birefringence
Capillary zone electrophoresis/CZE
Cassegrain system
Collision cell
Condenser/condenser lens
Depth of focus
Dichroic
Diffuse reflectance
Diffusion
Dispersion
Electron impact ionization/quadrupole mass filter
Electronic transition
Electroosmotic flow
Electrophoresis
Electrospray ionization (ESI)
Evanescent wave
Extinction
Eyepiece
Field of view
Flame ionization detector (FID)
Flow cell
Focal length
Hyphenated system
Inductively coupled plasma (ICP) torch
Interferogram
Interferometry
Ion mobility spectrometry
Isobaric interference
Isotropic
Kinetic energy discrimination (KED)
Köhler illumination
Mass spectrometer (MS)
Mass transfer
Micellar electrokinetic chromatography (MEK)
Micelles
Microspectrophotometry
Mobility spectrum
Monochromator
Nebulizer
Nitrogen–phosphorus detector (NPD)
Numerical aperture (NA)
Objective lens
Ocular lens
Off-axis
Optic axis
PFTBA
Photodiode array (PDA)
Pleochromism
Polarizable bonds
Polarized light
Polarized light microscopy (PLM)
Principal focus
Pseudocrystalline
Radiationless transition
Raman spectroscopy
Rayleigh scattering
Reaction cell
Real image
Resolving power
Retardation
Retardation distance

Scanning electron microscopy (SEM)
Snell's law
Specular reflection
Stokes scattering
Surface absorption–reflection (SAR)
Tandem mass spectrometry
Theoretical plates
Transducer
van Deemter curve
Virtual image
Working distance
X-ray diffraction (XRD)

Problems

FROM THE CHAPTER

1. What is the best wavelength to use to examine the smallest visible samples? Why?
2. On the basis of the information presented in Exhibit A, explain why blue light bends more (is refracted to a larger degree) than red light.
3. Draw a box diagram of a simple microscope next to that of a simple spectrophotometer. Trace the light paths and show common components and features. On the basis of your drawing, how would you expect a polarizing IR microscope to function? Draw a schematic.
4. Why are fluorescent and phosphorescent emissions always redder than the source used to stimulate the emission?
5. How would a gas-phase UV spectrum of acetone compare with one obtained in the liquid phase? From a forensic point of view, would this provide any additional information or value?
6. If the resolution of an FTIR depends on the distance the mirror travels, why are relatively long moving distances such as 2 or 4 cm not used? What is the trade-off?
7. Why would the electron clouds of bonds made with larger atoms be more polarizable than those from smaller atoms?
8. What is the difference between a KED reaction cell used in ICP-MS instruments and the collision cell used in triple quadrupole mass spectrometers?
9. A large N value is not the sole criterion for selecting a GC column for a given separation. What other factors must be taken into account?
10. Which of the three contributing factors to band broadening in chromatography is independent of the flow rate? Explain why.

INTEGRATIVE

1. Suppose a sample of urine contains quinine, a molecule that can be excited by visible light and that fluoresces strongly. How would the design of a simple colorimeter–spectrometer have to be modified to detect the fluorescence and not the transmitted light coming from the excitation source?
2. The development of the technology for micro-Raman methods has been much more difficult than for traditiona IR methods. What factors have contributed to this difficulty?
3. In the case of the assassination of President John F. Kennedy, a key piece of forensic evidence was provided by the analysis of bullets and fragments recovered. Research this case, describe how the elemental analysis was done, and discuss some of the limitations of the instrumental technique used. How would such an analysis be accomplished today?
4. Use the concepts of scattering and polarizability to draw links between atomic and molecular scattering, the refractive index, and Raman spectroscopy.
5. The calibration of spectroscopic methods is based on Beer's law and a linear relationship of the form $y = mx + b$. Theoretically, b should be zero, but usually it is not. Why?
6. Most manufacturers send test runs of 5–10 peaks with new GC columns and calculate the number of theoretical plates for each peak rather than just for the first one. Why?
7. One way to dramatically increase the sensitivity of a quadrupole mass spectrometer is to use a technique called selected ion monitoring (SIM). Explain how this technique works and why it improves sensitivity. Is there a compromise between a wide range of masses scanned and SIM? Explain.
8. Discuss and explain why the intensity of the source in any type of spectroscopy fundamentally controls the instrument's LOD and LOQ. Why does interferometry change this control?
9. Spectroscopy in the UV/VIS range produces broad bands for reasons discussed in the text. In addition, rotational and vibrational modes contribute to band broadening even though these quantum states and transitions are not targeted. Explain. Would performing the analysis in the gas phase alter the contribution of these modes? Would any additional forensic information be gained?

FOOD FOR THOUGHT

1. Although the Michel-Levy chart is easy to use, there are difficulties associated with it and its applications. What might those difficulties be? In what other areas could this be an issue in forensic analysis?
2. a. From the perspective of "fitness for purpose," which of the following methods would best be suited to characterizing a single bullet fragment recovered

from a body: SEM imaging coupled with X-ray spectroscopy, bulk analysis using acid digestion and ICP-MS, or LA-ICP-MS? Create a table listing the pros and cons of each in the context of this example. If your lab had all three methods available, which would you select based on fitness for purpose?

b. Research how the bullets involved in the assassination of John F. Kennedy were analyzed, and critique the procedures and results.

c. A recent report commissioned by the FBI and published by the National Research Council discusses the use of elemental analysis and "chaining" as a method of analysis and interpretation of the composition of bullets. Find this report, read it, and describe what chaining is and how it is used. Critique the method and offer alternatives, keeping in mind the typical forensic laboratory capabilities and time pressures.

3. Does an HPLC-PDA instrument provide the same level of qualitative and quantitative information as a GC–MS?

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Drugs as Physical Evidence: Seized Drugs and Their Analysis

OVERVIEW AND ORIENTATION

This chapter is our first to delve into the chemical analysis of physical evidence. We will look at drugs and seized drugs specifically, focusing on chemical concepts. In both seized-drug analysis and toxicology, there are two primary goals—identify substances that are present and that are of forensic interest; and second, if applicable, quantify the amount present. Because classification is a prelude to identification, we'll start with classification. Forensic scientists are classifiers. Those who work with body fluids classify a red material first as a biological fluid, next as blood, then as human, and finally by DNA type. Fingerprint analysts begin their work by classifying the major features of a fingerprint as a loop, an arch, or a whorl. From there, fine features are used to locate a fingerprint within an increasingly smaller group. Thus, **classification** is the process of placing an exhibit of evidence into successively smaller categories. Ideally, classification results in a category that contains only one member.

Forensic chemists are also classifiers. Is the evidence biological or physical? Answering that question allows analysts to assign the evidence to a smaller category. Drugs can be classified as acidic, basic, or neutral, but that is just one of many ways drugs are categorized. The first part of the current chapter discusses the many ways in which drugs are categorized and how these categorizations overlap and interact. An important tool in categorization is presumptive testing. We will look at the tests used in some detail and from a decidedly chemical perspective to provide a deeper understanding of what color change is and what it means, and

what it does not mean. We will discuss in general terms how seized drugs are analyzed. Finally, we will take a brief look at some of the more common types of seized drugs, divided by acid and base characteristics. We will explore a current forensic and law enforcement problem, the clandestine synthesis of methamphetamine, again focusing on the chemistry of the process.

1 WHAT IS A DRUG?

A **drug** is a substance that when ingested, is capable of inducing a physiological change. There are many modes of ingestion, including swallowing, injection, inhalation, and absorption through the skin. All drugs are toxic; it is the dose that differentiates a therapeutic drug from a poison. Drugs are used to treat or prevent disease, to alleviate pain, to promote sleep, or to induce other physiological responses. Medicines are combinations of drugs and inert ingredients, but the terms "drug" and "**medicine**" are often used interchangeably. Aspirin is a drug, but if it is part of a preparation used to treat a cold or the flu, that preparation is called a medicine. An example is shown in Figure 1.

Drugs can also be abused, but the definition of what constitutes drug abuse changes over time and differs among societies. Cocaine was an ingredient in Coca Cola®, LSD and a compound related to methamphetamine (Ecstasy or MDMA) were used in psychotherapy, and methamphetamine was used by American soldiers from World War II through the first Gulf War in 1991.

Marijuana and related preparations were used medicinally in ancient times, and the active ingredient in the drug has been found to be useful in treating glaucoma, anorexia, and the nausea associated with chemotherapy. The active ingredient in marijuana is now prepared synthetically and marketed as the drug Marinol®. Although these social and historical considerations may not affect how the forensic chemist performs an analysis, they do dictate what the target analytes are.

Pearson Education

Bruce Rolff/Shutterstock

FIGURE 1 Aspirin by itself is a drug, but when aspirin is combined with other active ingredients, such as in a cold preparation, that preparation is a medicine.

EXHIBIT A

The Dose Makes the Poison

"All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy." Paracelsus (1493–1541).

M. B. Orfila is considered the father of forensic toxicology, but the title "father of modern toxicology" could belong to Paracelsus. Among his many contributions was the reintegration of the emerging science of chemistry with medicine, something not seen since the age of the Greeks. Paracelsus heralded the coming Renaissance, during which science and, eventually, forensic science would arise. A physician and alchemist, Paracelsus appreciated the value of the scientific method and experimentation. He also realized that what is a beneficial medicine at a low dose can become a toxin at a higher dose. The use of mercury as a treatment for syphilis was his suggestion—and not such a bad one, based on the limited knowledge of the time. Like all poisons, mercury, by definition, has antimicrobial properties.

Source: Gallo, M. A. "History and Scope of Toxicology," in *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 6th ed., ed. C. D. Klaassen. New York: McGraw-Hill Medical Publishing Division, 2001, 3–10.

2 CLASSIFICATION AND CATEGORIES

2.1 By Origin and Function

Drugs can be classified on the basis of their acid–base character. Although this approach is useful and meaningful to chemists, it is one system of many and not a common one in the legal context. A drug can also be classified by origin (i.e., by how it was obtained). This classification system includes the categories **natural product, semi-synthetic**, and **synthetic**. **Alkaloids**, for example, are extracted from seed plants and are natural products. Because these compounds are basic, they have an alkaline character, leading to the name. A large number of drugs are alkaloids, including the opiate alkaloids (derived from the opium poppy) and compounds such as caffeine. Other plant-derived drugs include cocaine, aspirin, opiates, and tetrahydrocannibinols (active ingredients of marijuana). Heroin, like aspirin, is a semi-synthetic compound that is made by the acetylation of morphine. Hormones and steroids are obtained from or synthesized by animals, humans, or genetically engineered bacteria and are also semi-synthetic. Compounds like diazepam (Valium) are synthetic. Compounds that once were obtained only from plant matter, such as Δ^9–tetrahydrocannabinol (THC), can now be synthesized. **Dronabinol** is synthetic THC and the active ingredient in Marinol. As a result of the new capabilities in drug synthesis, classifying drugs by origin has become problematic.

2.2 By General Effect

In addition to classifying drugs by their acid–base character, forensic chemists often categorize drugs on the basis of the physiological consequences of ingestion. This scheme leads to five groups: **analgesics**, **depressants**, **hallucinogens**, **narcotics**, and **stimulants**. Note that drugs may fall into more than one category; for example, narcotic drugs are also CNS depressants.

Analgesics: These are drugs that relieve pain. Among the common analgesics are aspirin, ibuprofen, naproxen sodium, and morphine. Aspirin and related drugs are nonsteroidal anti-inflammatory drugs (**NSAIDs**), which stop pain by reducing fever and inflammation. They do so by blocking the function of prostaglandins, fatty acid derivatives found

EXHIBIT B

What a Difference a Century Makes

The original formula for Coca-Cola was invented by chemist John S. Pemberton, who wanted to create the perfect medicinal drink. He had heard about extracts of the coca plant and knew of its purported stimulant and aphrodisiac effects. His first concoction was "Pemberton's French Wine Cola," launched in 1885. It sold particularly well in Atlanta, the city that became the corporate headquarters of the Coca-Cola company. He continued working on the beverage, striving to make a "temperance drink" based on coca extracts and the kola nut, but without the bitter taste typical of alkaloids. The addition of sugar and corn syrup helped, along with some citric acid to counter any oversweetening. The name Coca Cola came from the two key ingredients: the kola nut and cocaine. By the turn of the century, public sentiment was turning against cocaine, given its potential for addiction, and cocaine had been removed from the formula by 1929.

Library of Congress

▲ *An early advertisement for Coca-Cola.*

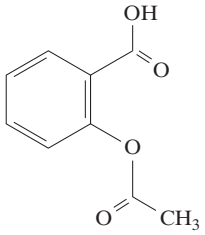
associated with cell membranes. Prostaglandins affect many processes, including inflammation. The NSAID drugs prevent the synthesis of prostaglandins, thereby reducing inflammation and the pain associated with it. Aspirin also inhibits pyrogens, which play a role in the fever response. Specifically, pyrogens ("fire starters") are released by white blood cells in response to injury or infection. Pyrogens act on the hypothalamus and stimulate both the release of prostaglandins and heat-producing processes in the body. This physiological cycle describes the mechanism of action of aspirin.

Morphine and other opiates reduce pain by a different mechanism. Although the complete mechanism is not fully understood, morphine appears to reduce pain by attaching to sites called opiod receptors scattered throughout the **central nervous system (CNS)** and also in the gastrointestinal tract. By binding to sites in the CNS, opiates such as morphine block the transmission of nerve impulses that relay the sensation of pain to the brain. Because morphine can bind to multiple sites, the side effects of pain relief include sleepiness and a sense of well-being. Morphine also interacts with the sites in

EXHIBIT C

Aspirin

The history of aspirin (acetylsalicylic acid) is typical of that of many other drugs and starts with folk knowledge. As early as 400 B.C., the Greek physician Hippocrates recommended that his patients chew on willow bark when they had a fever or pain. It is likely that the use of aspirin-based folk remedies predates even this ancient practice, perhaps by centuries. It took nearly two thousand years for chemists to enter into the story and synthesize the active ingredient of aspirin. By the 1800s, salicylic acid, closely related to acetylsalicylic acid and also found in willow bark, was available. The drug often produced stomach pains, a side effect also associated with aspirin. A German chemist, Felix Hoffman, is generally credited with the first synthesis of acetylsalicylic acid. Hoffman performed his work at a company with a familiar name: Bayer. This company, the name of which has become synonymous with aspirin, was not active in pharmaceuticals until the 1890s. Many of the firm's early drugs were actually by-products of dye manufacture a complex chemical process and not far removed from drug synthesis. Aspirin hit the market in 1899, and by 1915 it was available over the counter. Still, it wasn't until the 1970s that scientists unraveled the mechanism of the drug. Although aspirin was originally sold as an analgesic, many people taking aspirin do so to lower their likelihood of suffering a heart attack or for related reasons, not to relieve pain.



Aspirin
Acetylsalicylic acid

▲ *Structure of aspirin, acetylsalicylic acid*

the brain associated with pleasurable sensations—sites that are activated by endorphins found in the brain. (Interestingly, the term "endorphin" means "endogenous morphine.") As a result of its mimicry of endorphins, morphine can produce pleasurable sensations and euphoria.

To summarize and greatly simplify, aspirin reduces pain by inhibiting the pain-inducing event, whereas morphine intercepts the pain signal after it is produced (Figure 2). The different mechanisms help explain why morphine is addictive and aspirin is not. Aspirin stops inflammation and pain, but it does not produce euphoria along with the pain reduction. Conversely, morphine can cause feelings of deep relaxation and euphoria as a side effect of pain relief. The side effects of morphine create more potential for abuse and lead it to be classified also as a narcotic.

Depressants: These are a class of drugs that depress functions of the CNS generally, resulting in a slowed heartbeat, a reduction in anxiety, and, in some cases, the promotion of sleep. **Barbiturates**, tranquilizers, sleep aids, and ethanol are depressants. The benzodiazepine family of synthetic drugs, introduced in the 1960s, has become one of the largest classes of prescribed drugs and includes familiar brand names such as

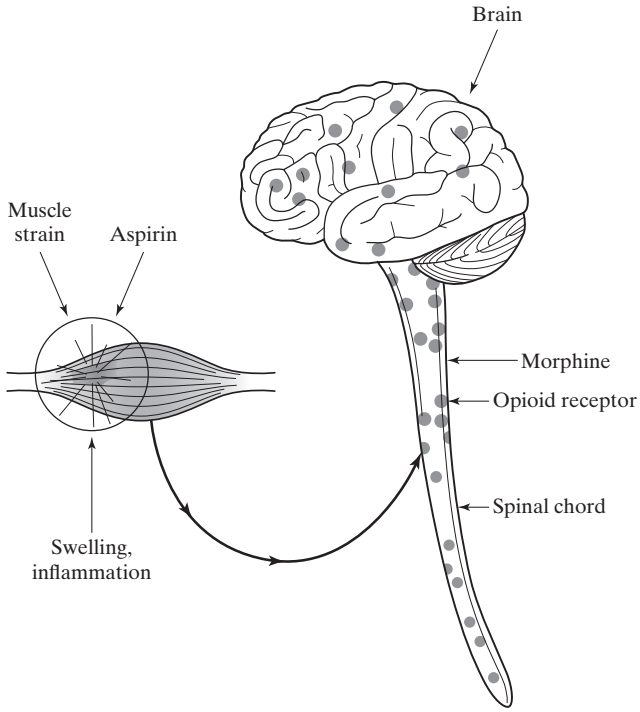


FIGURE 2 Both aspirin and morphine treat pain, but they stop it by two different mechanisms. Aspirin attacks the cause of the pain, while morphine blocks the nerve impulse that signals pain.

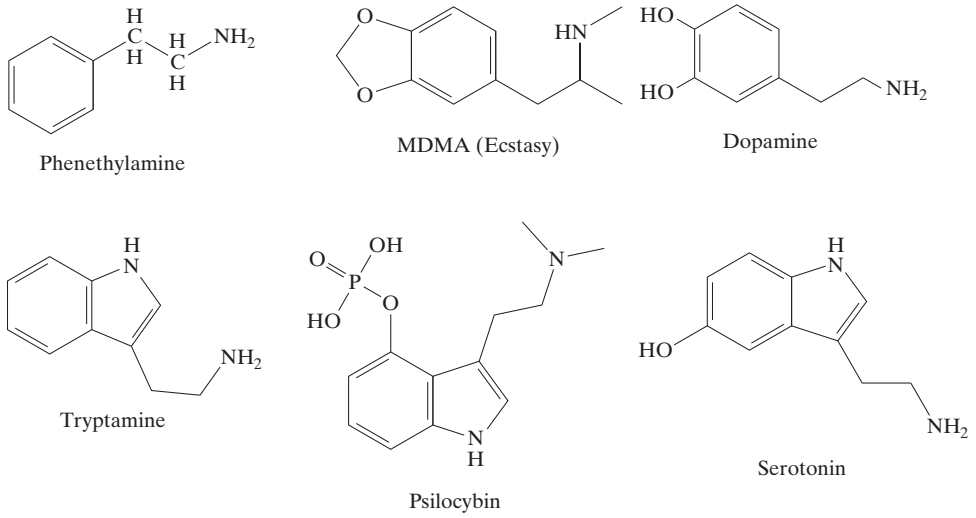
Valium. As a mechanistic example, this group of drugs works by interacting with gamma-amino-butyric acid (GABA) receptors in the brain.[1] The GABA receptors are the most common inhibitory neurotransmitter in the brain and central nervous system; the benzodiazepines are able to bind with GABA receptor sites and generate the inhibitory response, resulting in depression of the CNS.

Hallucinogens: These are drugs that alter the perception of time and reality, a difficult effect to quantify. Movement, thought, perceptions, vision, and hearing are also affected. LSD, mescaline, and marijuana are examples of hallucinogenic drugs. A number of stimulants, such as methamphetamine, are hallucinogens at high doses. The pharmacology of this class is complex and cannot easily be summarized,[2] but some generalizations can be made. There are two classes of hallucinogens: those based on the phenylethylamine (also called phenethylamine) molecular skeleton and those based on tryptamines. Hallucinogens create their effect by interacting with receptors in these two neurotransmitter systems, but the delineation is not as clean as the structures suggest.[2] Methamphetamine and Ecstasy (MDMA, related to amphetamine) are phenethylamines, whereas psilocybin, derived from psilocybin mushrooms, is a tryptamine.

Narcotics: Narcotic drugs have analgesic effects and tend to depress the CNS and promote sleep. Opiate alkaloids (drugs derived from the opium plant) are the best-known narcotics and include morphine, codeine, heroin, hydromorphone, oxycodone, and hydrocodone.

Stimulants: In contrast to narcotics and depressants, this group of drugs stimulates functions of the CNS, induces alertness, and interferes with sleep. Common stimulants include cocaine, amphetamine, and methamphetamine. At high doses, many stimulants are hallucinogenic. The mechanisms of action within the class vary widely. Cocaine, for example, can block the absorption (called reuptake) of dopamine, serotonin, and norepinephrine, leading to generalized stimulation and potential hallucinogenic responses.[3]

TABLE 1 Hallucinogens



2.3 By Use

Some drugs are grouped together on the basis of how they are used and how they are abused. Within a group, the chemical structures are typically similar, as are the physiological effects. Most of these substances will be discussed individually later. Four examples of classes based on use and abuse are predator drugs, club drugs, human-performance drugs, and inhalants.

Predator Drugs: Also known as date-rape drugs and drug-facilitated sexual assault (**DFSA**) agents, these substances are used to incapacitate a victime for sexual purposes. Current date-rape drugs, aside from alcohol, are ketamine, Rohypnol (flunitrazepam), and gamma-hydroxybutyrate (GHB) and related compounds. When the drug is mixed in a drink, the effects can range from disorientation to unconsciousness and loss of short-term memory. Victims may awaken several hours after an assault with no memory of the event or the few hours leading up to it. Consequently, they may delay seeking treatment until the drug and metabolites are no longer detectable by traditional toxicological methods.

Club Drugs: These are drugs used at parties and clubs frequented by young people; many are also predator drugs. In addition to the compounds listed as predator drugs, Ecstasy (MDMA) is a club drug. Other hallucinogens, such as LSD and psilocin mushrooms, are sometimes included, as are the stimulant–hallucinogens phencyclidine (PCP) and methamphetamine.

Human-Performance Drugs: These drugs consist of substances that improve or impair one's performance, most notably anabolic steroids and alcohol. **Anabolic steroids** include dozens of drugs, mostly acquired by prescription, based on testosterone. These drugs are abused by athletes in attempts to increase their muscle mass and decrease the recovery time after strenuous training and competition. Abuse has been found at the high school level.

Inhalants: Unlike the other groups of drugs listed in this section, **inhalants** are substances that are inhaled to produce their desired effects. Most inhalants are not intended to be

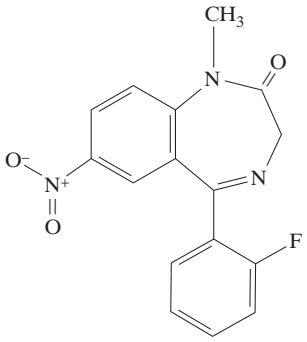


FIGURE 3 The structure of Rohypnol.

used as drugs. Examples of inhalants are paint thinners, nitrous oxide (laughing gas), gasoline, cleaners, and nail polish. Any substance that has a volatile component can be used as an inhalant, and in general, these substances have depressant effects similar to those of alcohol.

Note that drugs can be classified under multiple systems. Rohypnol (flunitrazepam, Figure 3), for example, is a basic drug, a benzodiazepine, synthetic, a depressant, and a predator drug. It is also a federally controlled Schedule IV drug, a categorization based on legislative, rather than chemical or physiological characteristics.

2.4 Classification by Schedule: The Controlled Substances Act and Listed Chemicals

For the forensic chemist, the legal categorization of a drug is nearly as important as the chemical and physiological one. The term *drugs of abuse* applies to drugs and related compounds that are subject to regulations and laws because of their potential to be abused and to cause harm. Abused drugs are usually addictive, causing physiological dependence, psychological dependence, or both. Physical addiction is traceable to a biochemical or physiological change caused by repeated use of the substance. In the case of morphine, for instance, repeated regular use can lead to a decrease in the number of active opioid receptors in the brain and CNS. Since fewer receptors are active, the user cannot simply stop taking the drug without feeling symptoms of withdrawal. Ever-increasing doses are needed to elicit the desired effect, a phenomenon referred to as *tolerance*, which accompanies physical addiction. Psychological dependence does not have a direct physiological cause but, rather, is rooted in emotional needs and responses.

In the United States, drugs are federally regulated under the **Controlled Substances Act**, passed in 1970. The act divides drugs into categories called *Schedules* on the basis of their medical uses and potential for abuse. For violations, the act specifies criminal penalties and fines. The federal **Anti-Drug Abuse Act** of 1986 expanded the list to include "designer drugs," synthetically produced analogs of controlled substances. The current version of the CSA and schedules can be accessed at the Drug Enforcement Administration website, www.dea.gov.

Although substances such as cocaine and heroin require extensive extraction and chemical processing before they are ingested, drugs such as PCP, GHB, and methamphetamine are relatively simple to make, requiring little more than basic chemistry skills and access to the Internet. An effective tool for minimizing illicit production is to limit access to the precursors. Accordingly, the Controlled Substances Act has been modified to include many of the key precursor chemicals needed for making methamphetamine and other clandestinely produced drugs, such as the hallucinogen PCP. Rather than list all precursors as controlled substances, the **Chemical Diversion and Trafficking Act (CDTA)** was passed in 1988 and amended in 1993. This legislation created two lists of regulated chemicals that are controlled to deter diversions of these compounds for clandestine synthesis. (The lists are also available at the DEA website.) Notable among the chemicals on List I are "ephedrine, its salts, optical isomers, and salts of optical isomers," as well as "phenylpropanolamine, its salts, optical isomers, and salts of optical isomers." All these precursor substances are used in the synthesis of amphetamine and methamphetamine. Note the inclusion of "all salts and isomers," typical wording in the regulations. Compounds such as iodine, sulfuric acid, and diethyl ether are on List II. These are necessary ingredients for many clandestine drug syntheses but have legitimate uses. The federal **Methamphetamine Anti-Proliferation Act** (MAPA) of 2000 placed limitations on the availability of pseudoephedrine and phenylpropanolamine (PPA) in over-the-counter medication; PPA was withdrawn from the market by an unrelated FDA action in 2000.

TABLE 2 Controlled Substances and the Controlled Substances Act

Schedule	Medical Use	Controls on Prescriptions	Required Security	Potential for Abuse	Addiction Potential	Examples
I	None accepted	None; used only for research	Vault or safe	Highest	Severe	LSD, heroin, MDMA (Ecstasy), GHB
II	Some accepted uses with restrictions	Written prescription with no refills	Vault or safe		Severe	Morphine and many related opiates, cocaine, amphetamine and methamphetamine, most barbiturates, oxycodone
III	Accepted uses	Written or oral (phone in), limits on refills and time	Secured area		Moderate to low	Ketamine, anabolic steroids, some codeine preparations
IV	Accepted uses	Written or oral (phone in), limits on refills and time	Secured area		Limited	Benzodiazepines such as Valium, mild sleep aids
V	Accepted uses	Over the counter, written, or oral (phone in), limits on refills and time	Secured area	Lowest	Limited	Selected preparations of codeine

Source: United States Drug Enforcement Administration, http://www.dea.gov/pubs/csa.html and http://www.dea.gov/pubs/scheduling.html. Accessed August 30, 2011.

EXHIBIT D

The Government Steps In

In the United States, the first "recreational" drugs were the opiates, such as morphine and opium, introduced by Chinese immigrants in the mid-1800s. San Francisco was the first city to pass a law regulating drugs, in 1875. The first federal law regarding drugs was the Pure Food and Drug Act of 1906, which required labeling on patent medicines. The first federal agency with a responsibility for drug control, the Bureau of Revenue, was formed in 1915. This organization was a precursor to the Drug Enforcement Administration (DEA).

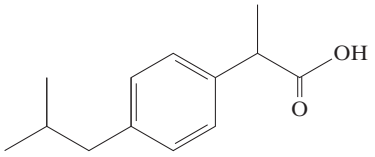
Library of Congress

EXAMPLE PROBLEM 1

Question: Classify the drug ibuprofen based on acid/base/neutral characteristics, effect, and Schedule on the CSA.

Answer:

This type of question requires the use of reference materials, including online sources. To identify the chemical class, the first information needed is the structure. This can be obtained from *Clarke's Handbook*, the *Merck Index*, or the National Library of Medicine's free search utility, CHEMID Lite which (http://chem.sis.nlm.nih.gov/chemidplus/chemidlite.jsp), is illustrated here.



Ibuprofen
(±)-2-(*p*-Isobutylphenyl) propionic acid

FIGURE 1. Structure of Ibuprofen and name as obtained from the NLM portal.

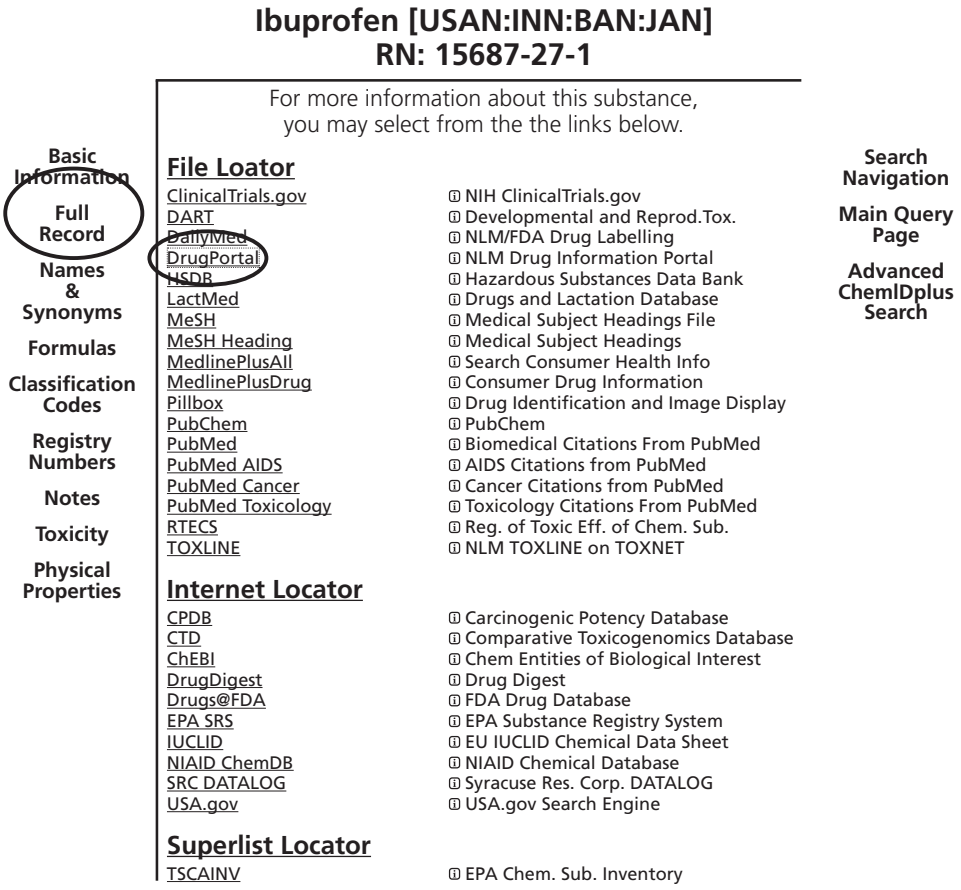


FIGURE 2. Screenshot of the NLM CHEMID Lite search results. The "Full Record" link yields structures, names, registry numbers, chemical and physical properties, and other data. The "Drug Portal" and "HSDB" link provide more detailed information and toxicological data.

The NLM portal is especially useful because it provides other links, such as to the Drug Information Portal and to the Hazardous Substance Database (HSDB), both of which provide a wealth of additional information about the compound. At HSDB, for example, once you locate the structure of ibuprofen, you can click on the full record to find the structure, names, and available chemical and physical data. You can quickly identify it as a monoprotic acid compound based on the structure and the chemical names. The link to the Drug Information Portal reveals that ibuprofen is "a nonsteroidal anti-inflammatory agent with analgesic properties used in the therapy of rheumatism and arthritis." The only remaining question is in regard to the CSA. You may already know that ibuprofen is not a controlled or listed compound, but if not, venture to the DEA website (http://www.deadiversion.usdoj.gov/schedules/index.html), where a complete list of controlled substances can be found, searchable in many different ways. A quick alphabetical search reveals that ibuprofen is not controlled.

3 DRUGS AS PHYSICAL EVIDENCE

3.1 Physical Evidence: The Five P's

The analysis of materials suspected to be or to contain controlled substances represents the largest portion of the workload in most forensic laboratories. When suspected controlled substances are submitted as physical evidence (**exhibits**), the forensic chemist must identify and, in some cases, quantify the controlled substances present. The most common forms of drug evidence seen can be summarized as the "**five P's**": powders, plant matter, pills, precursors, and paraphernalia. **Powders** include colored powders from crystalline white to resinous brown, and many, such as heroin and cocaine, are derived directly or indirectly from plants. Many solids that are submitted as evidence are not really powders but are oily and odiferous, while some can be described (unofficially and informally) as "goo." Hashish, a concentrated form of marijuana, lies between plant and powder. Typical **plant matter** exhibits are marijuana, mushrooms, and cactus buttons. As biological evidence, plant matter must be stored properly to prevent rotting and degradation prior to analysis; failure to do so can generate the aforementioned goo.

Pills, such as prescription medications or clandestinely synthesized tablets, are common forms of physical evidence. In cases where the evidence is or appears to be commercially manufactured (over the counter (OTC) or prescription) drugs, tentative identifications can be made visually, using references such as the ***Physicians' Desk Reference*** (**PDR**).[4,5] In other cases, the pills may have different markings, such as crosses or other imprints. The markings and dimensions of commercial products are referred to collectively as *pharmaceutical identifiers*, and these can be useful in the analysis of such evidence. Amphetamines, methamphetamine, and LSD are often sold in pill form, although typically the pills are cruder than those produced commercially.

Precursors are compounds or materials used in the clandestine synthesis of drugs such as methamphetamine. Some precursors are controlled and listed on Schedules, whereas others are not. For example, 1-phenylcyclohexylamine (PC) and 1-piperidinocyclohexanecarbonitrile (PCC) are listed on Schedule II, as are precursors used in the synthesis of PCP. (PCP is listed on Schedule II as well.) Illicit methamphetamine was once predominantly made starting from phenyl acetone (phenyl-2-propanone, or P2P), now listed on Schedule II. Methamphetamine is now usually made starting from pseudoephedrine, an ingredient in over-the-counter cold and allergy remedies. Lysergic acid and lysergic amide, precursors of LSD, are listed on Schedule III. Other precursors are not necessarily controlled but must still be identified as part of investigations of clandestine synthesis. *Immediate precursors* are those requiring only one or two simple steps to convert to the controlled substance; *distant precursors* require additional steps.

Drug **paraphernalia** are the implements and equipment used in the preparation and ingestion of drugs. Typical items include syringes (a significant biohazard to the

FIGURE 4 A roach clip, used to hold a marijuana cigarette. Image courtesy of the Oklahoma State Bureau of Investigation.

analyst) and cookers used to prepare heroin and other drugs; pipes and bongs (water-filled vessels used in smoking marijuana); and razor blades, mirrors, and straws, used for snorting cocaine. Such items present both a sampling challenge and an analytical challenge, since only traces of material may remain. Typically, the items are rinsed with a solvent to extract the residues. Although effective, this is a destructive step that significantly alters the evidence. If this step is unavoidable, the analyst should adhere to any laboratory or legal requirements regarding the preservation of extracts.

Of the five P's, the cases most frequently submitted are those in which the exhibits are plant matter suspected of being or containing marijuana. Methamphetamine, cocaine, and heroin round out the "top four," although the mix and numbers vary across regions and states.[6] Over the past ten years, there has been increasing concern over predator drugs, methamphetamine, and MDMA (Ecstasy or XTC), while cocaine abuse appears to have leveled off. Heroin abuse is generally increasing.

Forensic chemists also are called upon to analyze other types of evidence that is not easily categorized. For example, phencyclidine is a controlled substance that is often seized in liquid form, usually a greenish-colored solution with an overwhelming smell. Other unusual exhibits might include spray cans, bags, or rags soaked with inhalants. Apples, candy bars, and fruit are often submitted soon after Halloween. In these cases, the challenge comes down to sample preparation and the matrix.

3.2 Cutting Agents

In addition to identifying and quantitating a target drug, drug chemists often must identify **cutting agents** (also called **diluents**) added to many drug exhibits. A similar term, **excipients**, is used in a similar vein, referring to inactive ingredients in commercial preparations. Cutting agents are used to stretch the supply of a controlled substance. Cutting agents are chosen on the basis of their physical and chemical similarity to the controlled substance. Cocoa powder can be used to cut powders that have a tan or brown appearance, while cornstarch (Fig. 7) has a dry, powdery appearance that is ideal for cutting drugs with a similar morphology.

The taste of a cutting agent is a crude measure of its chemical similarity to a target drug. For example, heroin, which is an alkaloid, has a bitter taste that is

FIGURE 5 A lightbulb modified as a crank bulb, used to smoke methamphetamine. Image courtesy of the Oklahoma State Bureau of Investigation.

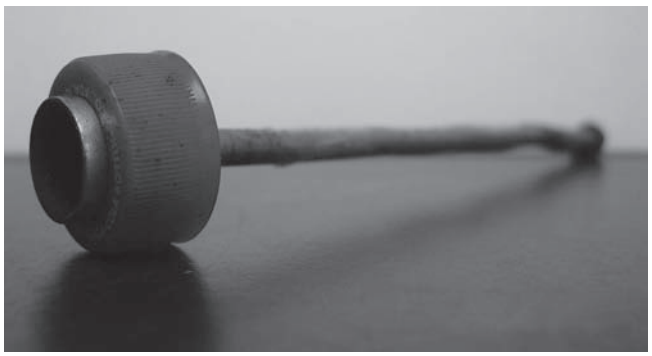
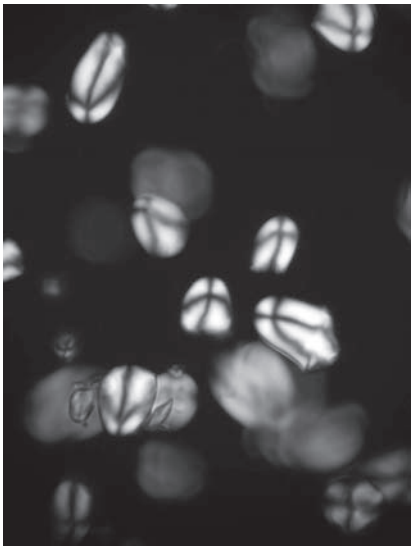


FIGURE 6 A pipe made from a bottle cap. Image courtesy of the Oklahoma State Bureau of Investigation.

mimicked by the cutting agent quinine. Similarly, cocaine is a topical anesthetic that is used in eye surgery, so it should be no surprise that cocaine is often cut with local anesthetics such as procaine, lidocaine, or tetracaine. Other common cutting agents include sugars, such as mannitol, inositol, and sucrose (table sugar); baking soda; and caffeine. The identification of diluents is an important part of drug profiling, a topic to be introduced shortly.

The convention we will use to categorize cutting agents is one seen in the literature.[7,8] Diluents (**thinners**) are substances that are not drugs and that have no pharmacological properties. Baking soda and sugars fall into this category. **Adulterants** are active and typically (but not always) have effects that are grossly similar to the target drug's effects. Caffeine added to cocaine is an example in which both the drug and the adulterant are stimulants.

Impurities are materials found with the drug (if it is a natural product) or added to it inadvertently during processing. Cocaine and heroin are drugs that originate from plant matter and thus are usually accompanied by a number of impurities. Codeine, for example, is found in the opium poppy and is frequently an impurity in heroin. *Contaminants*, a subcategory of impurities, are substances that find their way into the sample by accident. If heroin is extracted with the use of lime ($Ca(OH)_2$) contaminated with barium, the barium that ends up in the heroin is a contaminant that originated as a contaminant in the lime.



Suzanne Bell

FIGURE 7 Grains of cornstarch viewed under polarized light, showing the distinctive cross pattern. Starches such as cornstarch are common *diluents*—pharmacologically inactive fillers.

3.3 Profiling

Informally referred to as "chemical fingerprinting," **profiling** a drug sample involves analyzing the sample's composition beyond simple identification and quantitation of the controlled substance(s) present. Profiling data are used to categorize drug samples into similar groups to provide investigative information, such as learning that the samples have a common origin.[†] Additional goals of profiling can include any or all of the following:

- Elucidation of the synthetic pathway or extraction method used
- Identification of diluents, adulterants, and impurities
- Identification of the drug's geographic origin for plant-derived exhibits

Overview: Profiling a drug sample (Figure 9) includes physical examination and organic and inorganic analysis. Continuing with the heroin example, the first step is often an examination of the color and appearance of the sample (sparkling powder vs. oily appearance), and a microscopic characterization of diluents such as starches or sugars. Analysis of particle sizes has been employed as part of physical characterization as well.[9] Organic analysis of major and minor constituents reveals information about the drug and also the chemical methods used to process and prepare it. With heroin and other plant-based drugs, *isotopic ratios* (ratios of stable natural isotopes in plant tissues—also called **stable isotope ratios**) can be related to the environmental conditions prevailing at the time and location when and where the poppies were cultivated. Plants draw nutrients such as nitrogen from the soil, along with calcium, magnesium, and other elements, so relative amounts of these elements can be informative. Although we

[†]In drug analysis, profiling a drug and identifying its source does not individualize evidence or identify a common source in the same sense as DNA and fingerprints may. Rather, finding a common source might indicate that several samples came from the same processing laboratory.

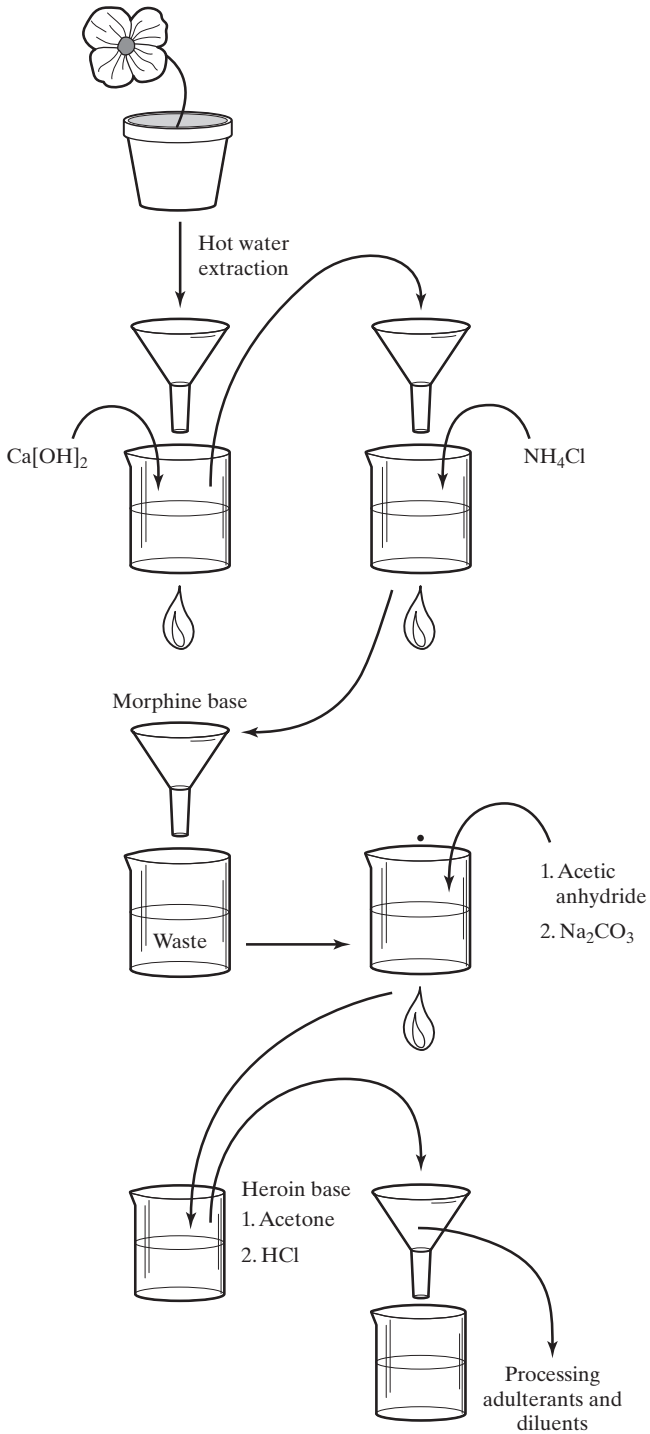


FIGURE 8 Simplified depiction of one common method of preparing heroin from opium. The morphine base filtered out in the second step will contain many related alkaloids, which can be partially or fully acetylated along with the morphine. Each step in the process contributes constituents that can be useful in profiling and linking a sample to a batch.

will not discuss stable isotope analysis in detail here, there are several sources in the Further Reading section if you are interested in pursuing this topic further.

Coextracted Components: With plant-derived drugs, alkaloids are extracted along with the drug or its precursor. In the case of opium, morphine is the target compound and precursor to heroin, but other alkaloids are inevitably carried throughout processing. Codeine,

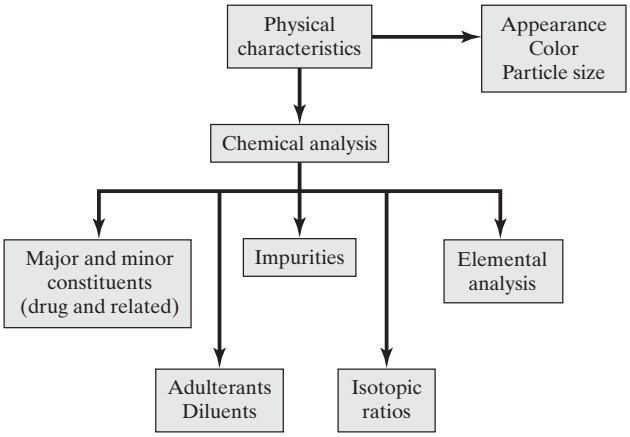


FIGURE 9 Aspects of profiling a drug sample. Not all profiling involves all the steps shown; for example, elemental analysis is infrequently used, whereas analysis of adulterants and diluents may be incorporated into a laboratory's standard protocol. Statistical analysis can be used to identify groups and interpret the data.

thebaine, papaverine, noscapine, and other trace alkaloids will be extracted along with the morphine and will interact and react along with morphine as the process continues. These ratios of opium alkaloids and chemical derivatives are similar within a batch, but variable outside of that batch. In addition, because of the chemical similarity of the aforementioned compounds, analytical methods such as TLC, GCMS, or HPLC optimized for heroin or cocaine usually will also separate and identify those impurities. As a result, impurities can be analyzed simultaneously with the necessary evidentiary analysis.

Impurities: Each stage of processing introduces impurities into a batch, much as any laboratory analysis can be contaminated by impure reagents, dirty glassware, etc. Acids and bases can be contaminated with trace metals and ions, as can water. Solvents can carry organic contaminants or can themselves be contaminants. Residual solvents and any characteristic impurities contained within them can be occluded within the crystal matrix of the final salt product. The use of different solvents at different processing stages contributes to residuals.

Adulterants and Diluents: Adulterants and diluents added to a batch can provide useful information regarding batches and groups. Common adulterants in heroin are acetaminophen (referred to as **paracetamol** outside the United States), caffeine, and lidocaine, all of which chromatograph well and can be detected simultaneously with the heroin. Diluents tend to be highly variable. Many are hard to identify during routine analysis, since some are removed in the sample preparation steps. Even if a diluent can be isolated, its identification often requires more time than can be spared in routine cases. Sometimes a quick microscopic examination of residues is sufficient to identify starches or sugars. However, materials such as mannitol or baking soda are not so easily tackled. The limiting factors in most forensic laboratories are not technology or equipment, but caseload and time.

4 ANALYSIS OF SEIZED DRUGS

This section will examine the nuts and bolts of forensic drug analysis, starting with **presumptive** color and precipitate (**microcrystal**) **tests**. These tests trace back to early qualitative organic chemistry. To investigate their mechanisms is to take a trip through the history of analytical chemistry, **functional group** chemistry, condensation reactions, and the nature of color. Effective ways to categorize drugs are classification by functional group or by chemical properties. The former, although useful in discussions of presumptive tests, becomes cumbersome, given that many drugs have multiple functional groups. Consequently, the approach we will use here is one introduced previously; classification by acid/base character. Secondary classifications, such as by the general effect of the drug (is it a narcotic, a stimulant, and so on) and the drug's schedule are also noted.

The focus of the discussions on drugs in this chapter and the next is not the mechanics of the testing and analyses but, rather, fundamental chemistry, given that the latter defines and drives the former. Laboratory methods and protocols applied to drug analysis fill several volumes, and discretion being the better part of valor, no attempt

APPLYING THE SCIENCE 1 Occluded Solvents and Impurities in Cocaine and Heroin Samples

A 1997 report in *Science and Justice: Journal of the Forensic Science Society* illustrates the value of organic characterization using GCMS. In this study, the authors employed a static headspace method to evaluate impurities and occluded solvents found in cocaine and heroin exhibits.

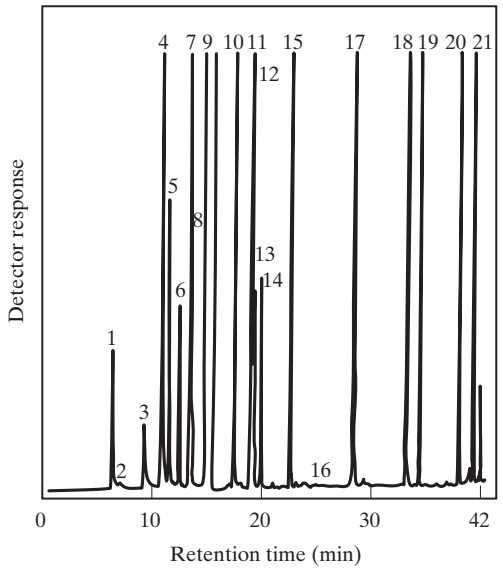


FIGURE 1 Chromatogram of solvent standards identified in cocaine and heroin samples in this study. Elution order: 1: acetaldehyde; 2: methanol; 3: ethanol; 4: acetone; 5: isopropanol; 6: diethylether; 7: dichloromethane 8: methylacetate; 9: carbon disulfide; 10: methylethylketone; 11: ethylacetate; 12: hexane; 13: chloroform; 14: butanol; 15: benzene (from carbon disulfide); 16: methylisobutylketone; 17: toluene; 18: *m*- and *p*-xylene; 19: *o*-oxylene; 20: *m*-decane (internal standard); 21: 1, 2, 3-trimethylbenzene.

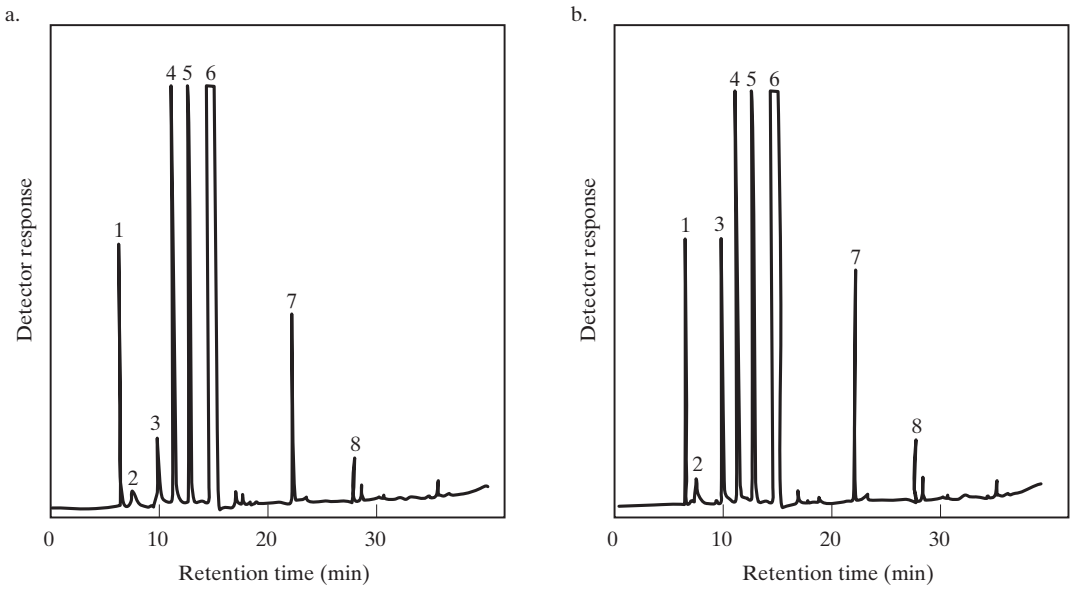


FIGURE 2 Occluded solvents identified in cocaine samples (a) 95/90.2; (b) 95/90.3. 1: chloroform; 2: methanol; 3: ethanol; 4: acetone; 5: diethylether; 6: carbon disulfide; 7: benzene (from carbon disulfide); 8: *m*- and *p*-xylene.

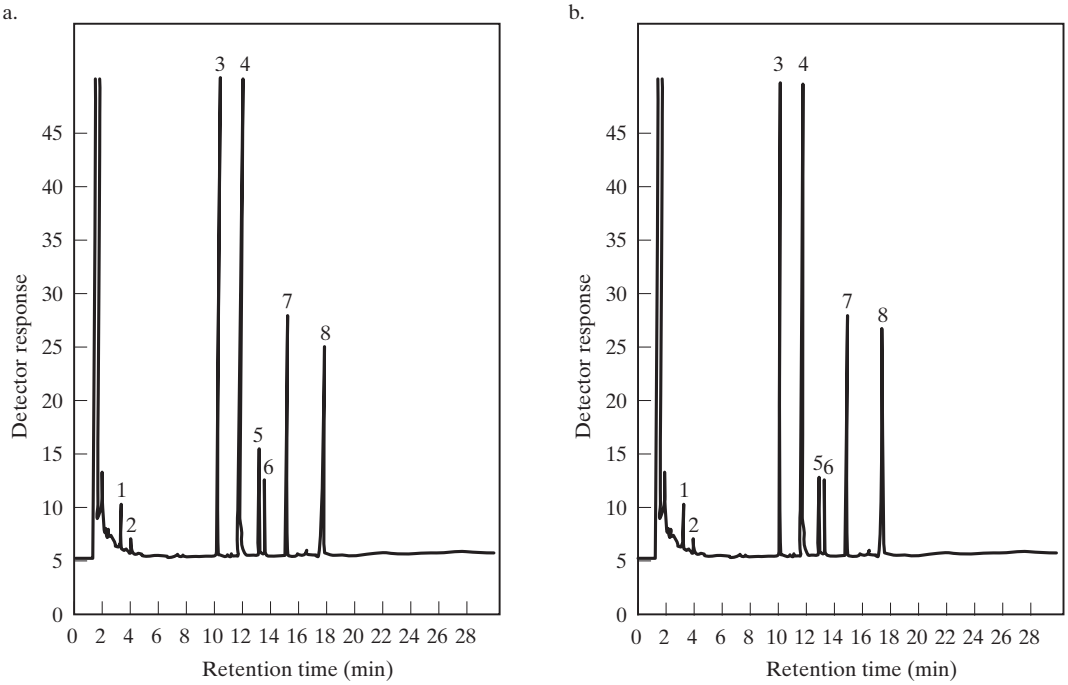


FIGURE 3 Impurities found in cocaine samples (a) 95/90.2; (b) 95/90.3. 1: ecgonine methyl ester-TMS; 2: ecgonine-TMS; 3: heneicosane (internal standard); 4: cocaine; 5: benzoylecgonine-TMS ; 6: norcocaine-TMS; 7: *cis*-cinnamoylecgonine methyl ester-TMS; 8: *trans*-cinnamoylecgonine methyl ester-TMS.

Source: Cartier, J., et al. "Headspace Analysis of Solvents in Cocaine and Heroin Samples." *Science and Justice: Journal of the Forensic Science Society*, 37 (1997): 175–81. Figures reproduced with permission, the Forensic Science Society, Copyright 1997.

will be made to reproduce that material here. For analytical references, *Clarke's Analysis of Drugs and Poisons* and the *Merck Index* are recommended as supplements to a laboratory's procedure manual.[10,11]

The analytical approach to drug analysis is the forensic approach: narrowing down possibilities until the drug is identified. With physical evidence, the initial screening is a visual examination, followed by presumptive and screening tests such as TLC. The final step is a definitive identification of the controlled substance or a determination that the sample does not contain one at detectable levels. This step usually necessitates instrumental analysis, such as GC-MS or infrared spectrometry. Other instruments that have been employed are HPLC-MS, NMR, Raman spectrophotometry, and CE, but the emphasis here is on techniques that are routinely used and are available to the crime laboratory chemist or toxicologist. The method of choice for extraction and sample preparation is determined from the results of initial presumptive tests based on simple color changes.

4.1 Presumptive Color Tests and TLC Developers

4.1.1 OVERVIEW Drug analysis and, to a lesser extent, toxicology utilize traditional color-based presumptive testing, targeting both drugs and diluents.[14] In a recent survey, 86% of responding laboratories reported using **spot testing** (another term used to describe presumptive testing) for drug analysis. The most frequently used **color tests** were the **Duquenois–Levine** (for marijuana), **cobalt thiocyanate** (for cocaine and related drugs), **Marquis** (for opium derivatives, amphetamines, and other alkaloids), and ***p*-DMAB** (*p*-dimethylaminobenzaldehyde, for LSD) tests.[13] Presumptive color tests arose from organic qualitative analysis dating back to the 1800s, and many

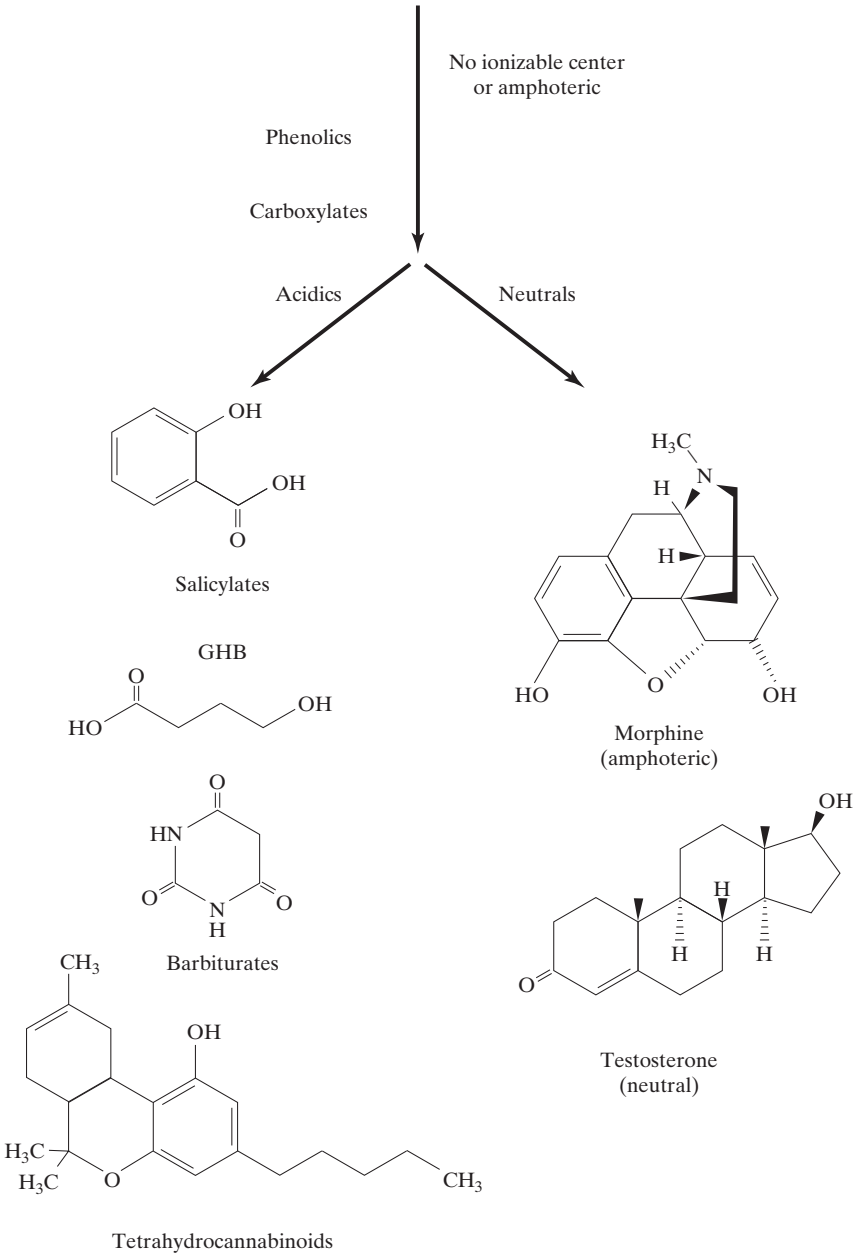


FIGURE 10 Acidic and neutral drugs are the drug classes addressed in this chapter. Neutral drugs consist of those with no ionizable center, such as testosterone and related steroids, and those that are amphoteric, such as morphine.

are still used in courses incorporating organic qualitative analysis. The appearance of color or a change in color is evidence that a chemical reaction has occurred.

Color tests target the type of compound and functional groups. Drugs of interest in forensic chemistry are characterized by a relatively small number of functional groups, the most important of which are phenols, aromatic rings, and basic nitrogens (primary, secondary, or tertiary amines). Because many drugs have more that one active moiety, color testing is more complicated than the simple identification of the drug's functional group. In general, presumptive tests have detection limits from 1 to 50 µg discounting complicating factors such as diluents.[13] The time of contact is an important consideration in color testing; leaving samples in the reagents, many of which contain

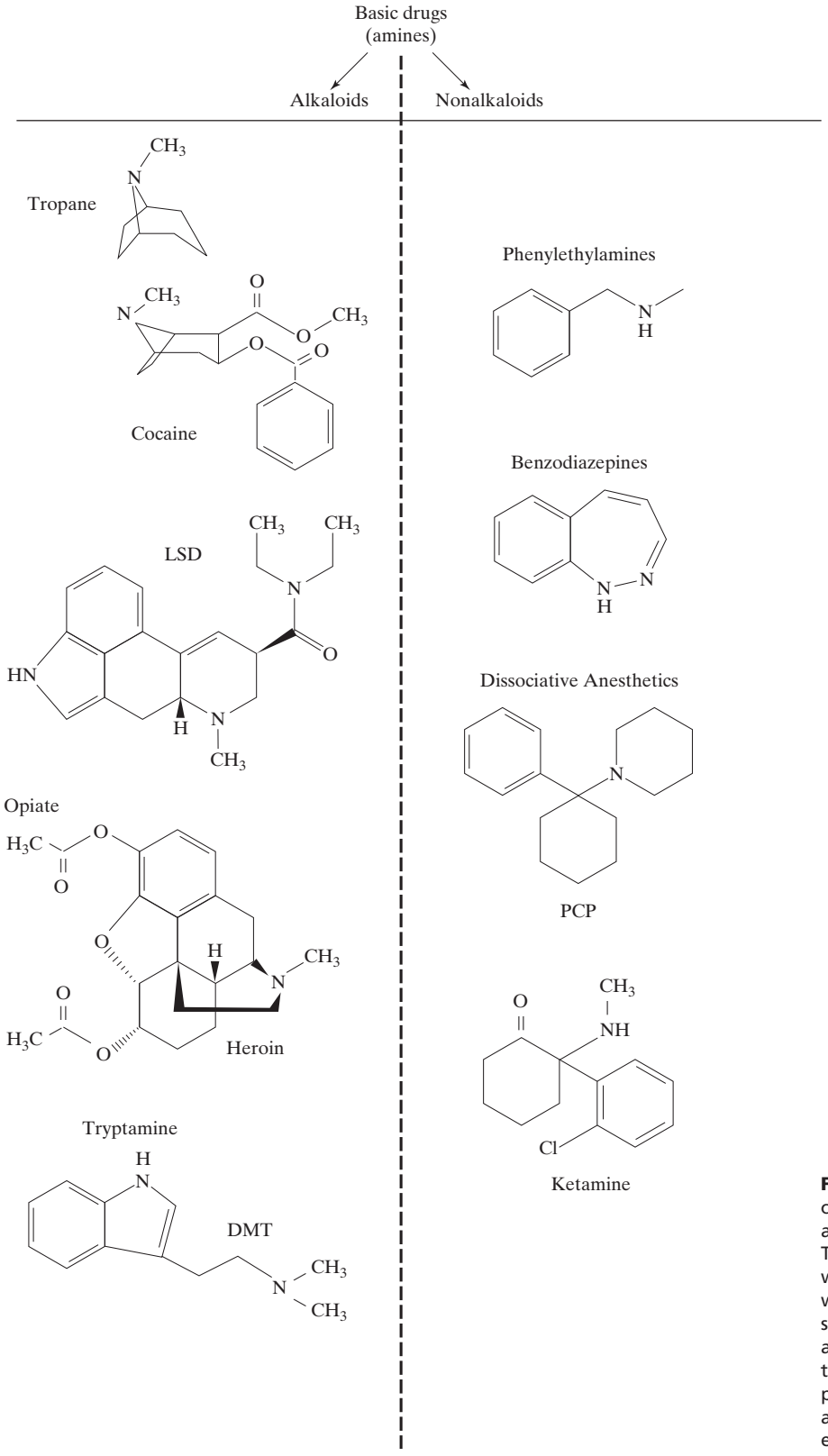


FIGURE 11 Basic drugs and classes, the largest subgroup in the acid–base classification scheme. The alkaloids are (or at least once were) derived from plant matter, whereas nonalkaloids are generally synthetic or semi-synthetic. Tropane alkaloids include cocaine, and tryptamines include mescaline and psilocyn. Caffeine and theophylline are xanthine alkaloids. Valium is an example of a benzodiazepine.

strong acids, can lead to colors unrelated to the presence of the target analyte. Finally, most color-test reagents can be used as developers for TLC and vice versa.

When an unknown powder, plant matter, or some other substance arrives at the laboratory as physical evidence, a series of color tests are performed, assuming that the amount of sample is sufficient. Most tests require a few milligrams, but the response depends on the concentration of the target analyte and may be influenced by the presence of other substances, such as the diluents. Analyst judgment rather than a set flowchart usually dictates which tests will be used and in what order. If green plantlike material is submitted, there would be little point in performing a test targeting cocaine or heroin, at least initially. Brownish tarry material suggests *hashish* or *heroin*, whereas tan powders often contain heroin, methamphetamine, and related controlled substances. Blotter papers, gel windows, or tiny tablets are common dosage forms of LSD. Evidence consisting of tan or whitish powders or pills is most suited to an extensive battery of color tests. As has been noted, when powders are encountered, the two tests used most frequently (initially) are usually the cobalt thiocyanate test for cocaine and the Marquis test for a variety of alkaloids. Some color tests are also precipitation tests in which the new solid accounts for the color change, but this is not universally true. The Duquenois (also Duquenois–Levine) test results in the formation of a colored solute that is selectively partitioned into a chloroform layer.

Spot tests are usually performed with a **spot plate** (glass or ceramic) or a disposable test tube. Results are easier to see with a white background, although a few spot tests work better on a black spot plate. To perform the test, the analyst places a small amount of the questioned powder in a well of the plate and then adds the reagent. For tests that require successive reagents or a liquid–liquid extraction, test tubes are used. As presumptive tests, all will react with more than one substance, but to an experienced chemist, subtle differences are usually apparent. Control samples that include a blank or negative control (a reagent only in the spot plate or test tube) and a known sample or samples should be analyzed in parallel with the questioned material.

4.1.2 WHAT IS COLOR? A color change is the outward evidence of a chemical reaction, just as the evolution of a gas indicates chemical decomposition. Appearance or change of color points to an alteration in chemical bonding that accompanies a reaction. This change is manifested by some surprisingly complex reaction chemistry. **Colorants** are substances or materials that can absorb or emit electromagnetic energy in the visible range; two types of colorants (**dyes** and **pigments**) are of particular interest in forensic chemistry. Many of the color changes observed with presumptive-testing reagents are the result of dye formation, a subject that will be addressed shortly. In general, presumptive color tests produce color changes based on two mechanisms. The first is dye formation, and color is detected because of changes in molecular structure that allow for visible light to interact with molecular orbitals. The second mechanism is alteration of d orbitals in transition-metal complexes, allowing for visible light interactions. We will discuss examples of both types in detail with the goal of understanding the fundamentals of the chemistry behind them. The unifying theme is that the reaction of a drug with a reagent produces an energy difference between orbitals (atomic or molecular) such that visible light has sufficient energy to promote an electron.

Molecular Orbital Transitions: A reasonable place to begin a discussion on color is to relate human vision to the spectrophotometric detection of visible light. Molecular compounds are covalently bonded, meaning that atomic orbitals combine to form **molecular orbitals,** denoted as σ, π and so on. If a sample powder appears white, it is reflecting all wavelengths of visible light in the 400–700-nm range. In other words, none of the energy gaps in the molecular orbital structure correlate with visible energy. No visible light is absorbed and no color is observed.

EXHIBIT E

A Light Review

Electromagnetic radiation can be described as a particle and as a wave; the key equations are as follows:

In the wave model, $c = \lambda\nu$ where λ is the wavelength, ν is the frequency in Hz (s^{-1}) and c is the speed of light, 3.0×10^8 m/s. In the particle model, $E_{photon} = h\nu$ where h is Planck's constant $= 6.63 \times 10^{-34}$ J·S. The bridge between the two models is $E = hc/\lambda$ derived by combining the foregoing expressions.

In UV-VIS spectroscopy, the energy of an absorbed photon drives the molecule into an excited state via the promotion of an electron in accordance with the relationship $M + h\nu \rightarrow M^*$ The energy is dissipated via collisions in solution. For a photon to be absorbed, its energy must match that of the energy gap to be traversed, such as the HOMO-LUMO gap discussed later in this chapter.

For example, the natural colorant β-carotene consists of a conjugated system of double bonds and a λ_{max} of 455 nm. As a consequence of absorbing this blue-green light, the compound appears orange (the complementary color). The energy gap is calculated by first converting the wavelength to meters (to match units of Planck's constant) and then substituting:

$$455\text{nm} \times \frac{1\text{ m}}{10^9\text{ nm}} = 4.55 \times 10^{-7}\text{ m}$$

$$\Delta E = \frac{6.63 \times 10^{-34}\text{ J·s} \times 3.0 \times 10^8\text{ m/s}}{4.55 \times 10^{-7}\text{ m}} = 4.37 \times 10^{-19}\text{ J per photon}$$

If upon the addition of a few drops of Marquis reagent, a red color appears, red light is being reflected and the complementary color of light is being absorbed by the sample. Because absorption in the UV/VIS range correlates to an electronic transition between molecular orbitals, it follows that the electronic structure and bonding arrangement were altered by the addition of reagent. The change may have occurred in the ingredients in the reagent, the sample, or both. The rearrangement of molecular orbitals means that blue light now matches some energy gap between the orbitals, a gap that did not exist before the Marquis reagent was added to the white powder. The color change indicates that a chemical reaction occurred.

Light from the sun or some other type of broad-spectrum light source emits a mix of the visible wavelengths and is referred to as white light. If a substance or surface reflects the light diffusely or at random angles, the light appears white. By contrast, if the material absorbs all wavelengths, it will appear black. Between these extremes

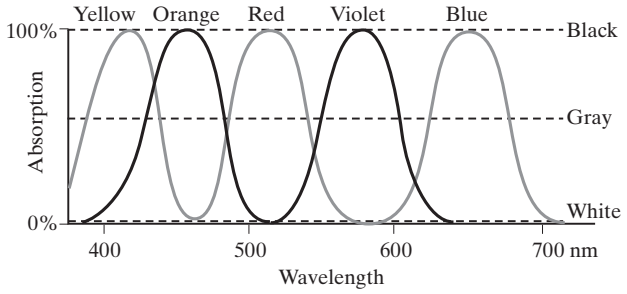


FIGURE 12 Superimposed spectra of the spectral colors by wavelength. If all are absorbed equally, a viewer will perceive black; if all are reflected equally, a viewer will perceive white. If all wavelengths are partially absorbed in equal proportion, a viewer will perceive gray.

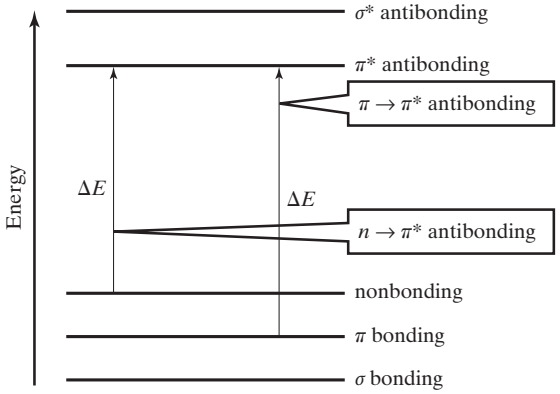


FIGURE 13 The two electronic transitions feasible with UV/VIS radiation, ranging from 200 nm to 700 nm.

is the color gray, which is what is seen if some constant percentage of intensity at all wavelengths is absorbed. White, gray, and black are referred to as **achromatic**—literally, lacking color. If a specific color is perceived, the light is **chromatic**.

Absorption of energy in the visible range is governed by Beer's law,

$$A = \epsilon_\lambda bc \qquad \textbf{(1)}$$

where A is the absorbance, ϵ is the molar extinction coefficient of the sample at wavelength λ, b is the path length, and c is the concentration of the sample. Many dyes have high extinction coefficients, in the range of 10^4-10^5 $cm^{-1}M^{-1}$ per mole and as a result are intensely colored.[14] This is important in color testing, since the more intense a color, the easier it is to perceive and the smaller the amount of the sample required.

UV/VIS light is of relatively low energy and capable of exciting only two kinds of electronic transitions. The $n \longrightarrow \pi^*$ transition is shorter (has a smaller energy gap) and involves a lower energy, or "redder," photon than the $\pi \longrightarrow \pi^*$ if, in a given molecule, an energy gap corresponds to red light of 650 nm, the sample will reflect all light but 650 nm. In other words, red light is subtracted from the white light, and what the eye then perceives is the complementary blue-green color. Note that an organic compound must have π electrons if there is to be any possibility of absorption of a UV/VIS photon. Finally, recall that in solution there are no single sharp transitions. Even for one discrete energy gap, a Gaussian distribution defines the possible transitions. Add to that more gaps and more distributions, and what results is an endless variety of colors as perceived by the eye.

The portion of the molecule capable of absorbing a photon is called the **chromophore**. This term is generic in the sense that even a UV absorber can be called a chromophore, although the human eye cannot detect any color resulting from a UV transition. To generate color, the transitions require lower energy photons corresponding to smaller energy gaps. One way to decrease the size of the gaps is through **conjugation** (Figure 14). Because the gaps are smaller, visible light has enough energy to promote electrons through absorption, imparting color. The more conjugation in the system, the longer is the wavelength of light absorbed and the darker is the perceived color.

EXHIBIT F

A Hundred Thousand Shades of Green

It is estimated that the average human visual system can discriminate about a million different colors. If we assume the seven basic colors of red, orange, yellow, green, blue, indigo, and violet (ROYGBIV), then the human eye perceives roughly 142,000 shades of green alone.

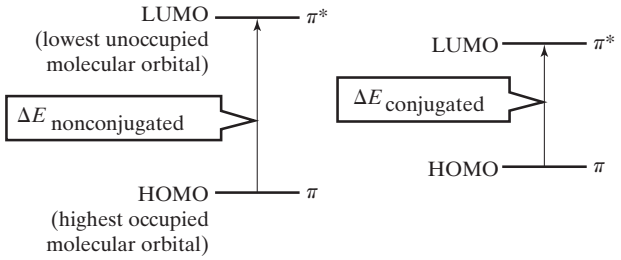
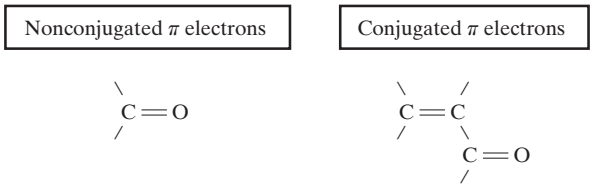


FIGURE 14 The effect of conjugation is to reduce energy gaps. As a result of conjugation, the photon energy required to promote an electron is less than it would be in the absence of conjugation. If there is sufficient conjugation, the electron can be promoted with visible light.



Another method of altering transitions and imparting color is via the addition of other functional groups. An **auxochrome** is a group that is not by itself a chromophore but a group that will alter the wavelength or intensity of the chromophore. Oxygen and nitrogen contain unshared electrons and possess π electrons that are available to interact with an aromatic system. This property decreases the energy gap and increases the wavelength of absorbed light. When these unshared pairs are removed, as in the case of the anilinium ion, λ_{max} drops back to the same value as for unsubstituted benzene. Changes to absorption maxima can be characterized as shown in Figure 15. When the wavelength of absorption increases, the energy of the light absorbed decreases, or becomes "redder," corresponding to a *blueshift* in the observed color. This kind of shift is referred to as a **bathochromic shift,** the opposite, a shift to absorbance of bluer light and a *redshift* in appearance, is called a **hypsochromic shift**. Increasing the intensity (increasing ϵ_λ) correlates with a **hyperchromic shift,** and decreasing it corresponds to a **hypochromic shift**. These shifts are summarize in Figure 16.

You may have noticed that the benzene ring chromophore is a common element in many colored structures, including many drug molecules, and substituents on the rings can have dramatic effects on absorbance.[15] When a substituent adds unshared electron pairs to an aromatic system, as in the case of —OH and amine groups, the effect can be significant and can depend on the pH, since protonation–deprotonation changes the electronic characteristics of the chromophore. Finally, absorption characteristics and color are affected by the presence of electron donors and acceptors. To simplify, whenever two electrons are added or donated to a molecule, a new energy level and potential transition results. Example donor groups are those with unshared electron pairs such

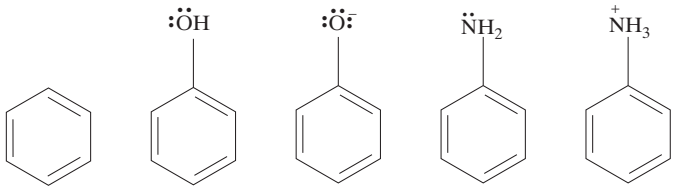


FIGURE 15 The effect of auxochromes on absorbance. Auxochromes change the electronic character of the molecule, including the availability. The combination of auxochromes and conjugation is important in generating the visible colors characteristic of many color tests.

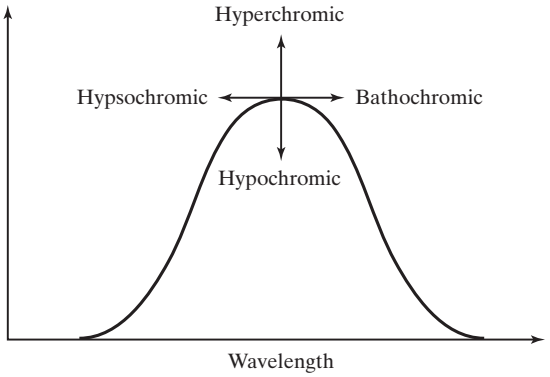


FIGURE 16 The addition of an auxochrome can affect the absorbance of a chromophore by altering the wavelength, intensity of absorptivity, or both.

as amines. Acceptor groups are those that stabilize the added electrons via resonance structures; examples are nitrate and carbonate. Keeping these fundamentals in mind, we can usually understand color changes caused by presumptive-test reagents and can interpret the results in a general sense. Given that the tests are presumptive, this level of understanding is sufficient.

Dyes and Dye Formation: A number of presumptive tests generate color through dye formation. Like pigments, dyes are colorants, but the two terms are often confused.[14] A *pigment* is a suspension of insoluble materials in a solvent, whereas a *dye* is a solution of the colorant. Colorants can be organic, inorganic, natural, or synthetic, but it is solubility that differentiates a dye from a pigment. For purposes of presumptive testing, the distinction is not crucial, but it becomes so in other areas of forensic interest, such as paint and ink analysis.

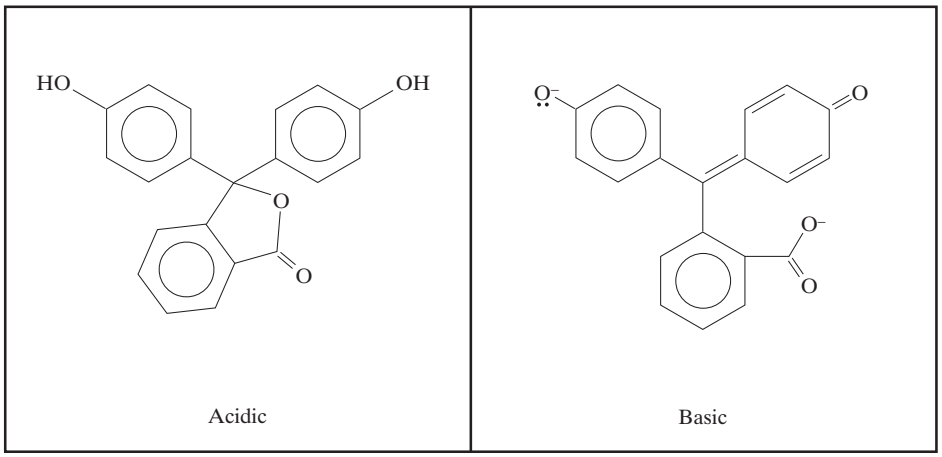
Of the many reaction pathways that can lead to dye formation, four are central in presumptive color testing:

- *Di- and triarylmethines*. These dyes are characterized by systems of two or three linked substituted phenyl rings (**aryl groups**). Common functionalities attached to the phenyl rings include dimethylamine, OH, and O^- groups. Some of these molecules can exist as cations. There are also multiple-linked ring dyes in this class, characterized by an **aza linkage** ($-N=$) between the substituted phenyl rings.
- *Azo dyes, diazonium salts, and azo coupling*. **Azo dye** chemistry is one of the most important subjects in colorant chemistry. The defining characteristic of azo dyes is the azo linkage, of the form $-N=N-$. The reactions in which groups are linked through the azo group are called *coupling reactions*. Like the arylmethines, these dyes can exist as cations and can form salts, called **diazonium salts**. An example is Fast Blue B, a diazonium cation that was once widely used as a spray reagent for TLC and as a color test for the active ingredients in marijuana. Owing to concerns about its carcinogenicity, it is rarely used now, but substitutes work in accordance with comparable principles.
- *Nitro and nitroso dyes*. These are dyes that contain NO_2 functionality.
- *Carbonyls*. These are dyes that contain the carbonyl group. A large number are based on the structure of anthraquinone.

Other Structural Features: The colored products of presumptive testing frequently contain carbonium ions. Alternatively called **carbocations,** these ions have a positively charged carbon atom in their structure. The carbon atom in a carbocation that is highly substituted or found within a conjugated system can be quite stable. A number of proposed structures for colored products include carbocations.

EXAMPLE PROBLEM 2

The indicator phenolphthalein is colorless under acidic conditions and an intense pink color under basic conditions. It is also used as a presumptive test for blood. What accounts for the color change?



Acidic Basic

Answer:

Under acidic conditions (pH at least 2 pH units below the pKa), the two phenolic groups are protonated. Under basic conditions, these deprotonate and become a donor/acceptor pair that increases conjugation, decreases the existing energy gaps, and allows for absorption in the blue range. This produces the perceived pinkish red color. Note also that under basic conditions the ring opens and another unshared pair is donated to the conjugated structure.

EXHIBIT G

Drugs and Dyes

These two families of compounds have much more in common than might first be expected, and both are, and continue to be, important in forensic chemistry. Many drugs and dyes have an acid–base character, and many are based on similar structures, such as aromatic and other amines. The drug and dye industries came of age in the mid-1800s, and the first synthetic dye, mauve (aniline purple), arose from an attempt to make quinine to treat malaria. In 1856, an 18-year-old-chemist named William Henry Perkin was studying at the British Royal College of Chemistry when he accidentally created the dye from a mixture of aniline and toluidines. He left school and founded what was to become one of the largest segments of the chemical industry: the production of dyes and pigments.

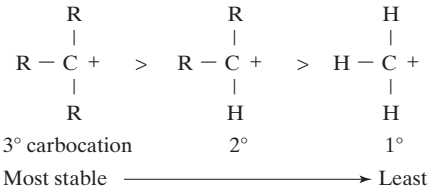
In 1863, the company that was to become Bayer (of aspirin and pharmaceutical fame) was founded in Germany by Friedrich Bayer (1825–1880) and Johann Friedrich Weskott (1821–1876), both of whom were dye makers. The pharmaceutical group was not formed until 1881. Aspirin, developed by Bayer chemist Felix Hoffman, was commercialized in 1899, a year that marked the initial emergence of the drug industry as a separate entity.

4.1.3 EXAMPLES OF PRESUMPTIVE COLOR TESTS *The Marquis Test*: This test is arguably the most versatile and widely used color test in drug analysis. Its chemistry is complex and not completely understood.[16] The color produced is apparently the result of a relatively stable carbocation[17,18] formed through the action of formaldehyde,[16] although a **free-radical mechanism** has also been suggested.[19] The first step is addition of the formaldehyde to an aromatic ring that has an amine group in a side chain. The intermediate product of this reaction is an alcohol that is available for later reactions. The carbocation can then be attacked by nucleophiles such as the alcohol or the electrons in the double bond of the original structure. The product is a dimer of the original molecule with more conjugation, which in some cases can be revealed by the appearance of a color. Creating a dimer often increases conjugation and facilitates interaction with visible light. Analysis using low-temperature NMR showed that the reagent will react with some aromatics to form carbonium ions,[20] and a comprehensive spectral analysis employing UV, IR, and NMR spectroscopy pointed to a polymerization step. However, these reports are dated, and structures and mechanisms presented here are best considered as proposed rather than confirmed.

The Marquis reagent reacts with amphetamine and methamphetamine to produce the orange-red product shown in Figure 17 and in the color insert.[16] The proposed

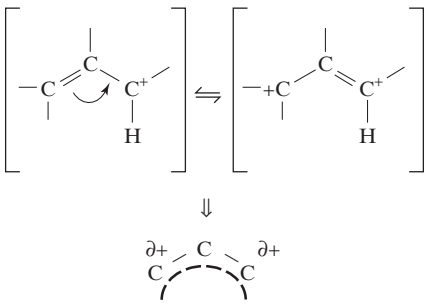
EXHIBIT H

Carbocations



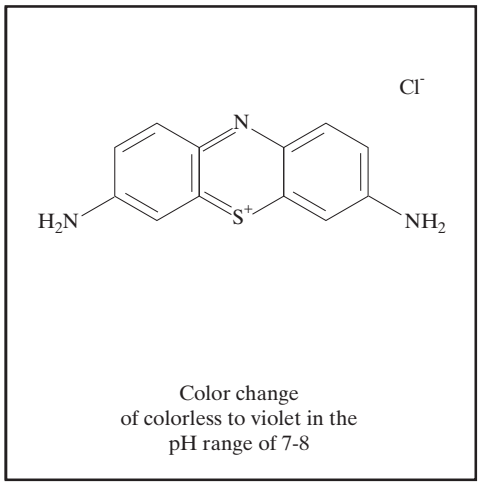
Recall from organic chemistry that carbocations are encountered as intermediates in many reactions. Because these compounds are electron-deficient species, they are strongly electrophilic and generally reactive. Carbocations are stabilized by substitution, and highly substituted carbocations may be stable enough to remain as carbocations and to be isolated as products of a reaction. Conjugation, the key to producing color, also stabilizes carbocations through resonance structures. If a carbocation is sufficiently stable, it can form salts, just as any other cation can.

Carbocations should not be confused with free radicals or carbanions. A free radical is similar to a carbocation in that it has three bonds, but rather than having a deficit of two electrons, a free radical has one unshared electron and, as a result, is highly reactive. A carbanion has an unshared pair of electrons in addition to three bonds and carries a negative charge. A carbene carbon has two bonds and one unshared pair of electrons. Carbenes are rarely isolated, because of their propensity to react with one another to form dimers.



EXAMPLE PROBLEM 3

The following dye is used as an indicator in acid–base titrations: Rationalize the color changes.



Answer:

The amine groups are protonated under acidic conditions and deprotonated in neutral–to–mildly basic color ranges. Deprotonation has the same effect here as it did with phenolphthalein, allowing unshared pairs of electrons to interact with the aromatic system. Under acidic conditions and protonation, there is less conjugation than under basic and unprotonated conditions. The indicator is cyanine.

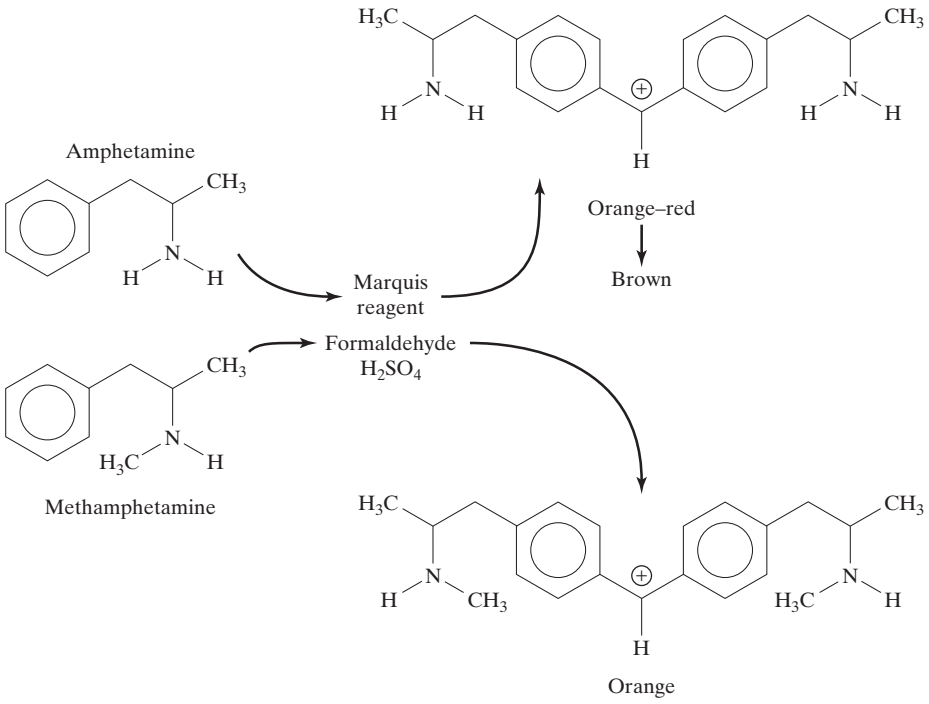


FIGURE 17 The reaction of the Marquis reagent with amphetamine and methamphetamine.

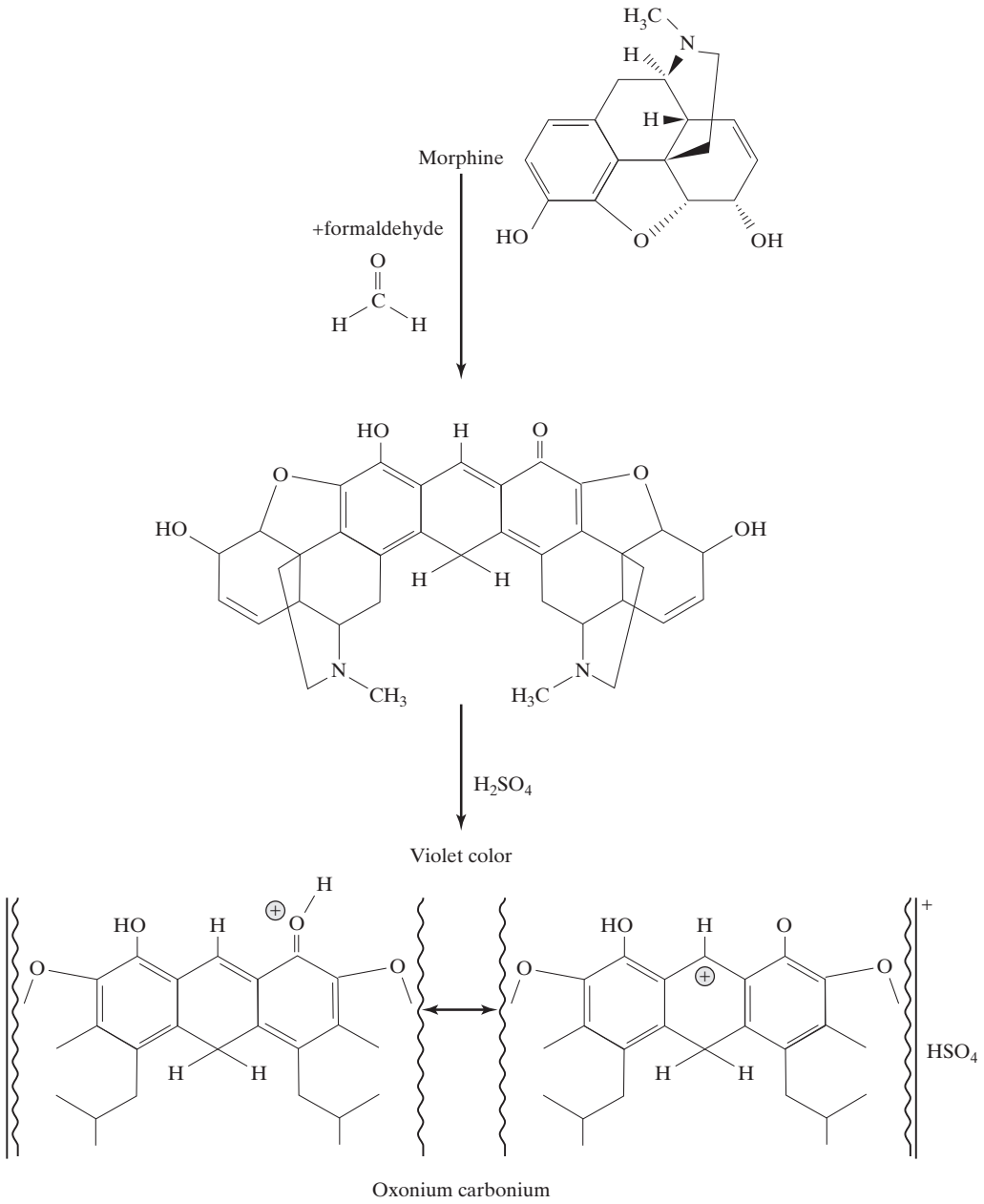


FIGURE 18 The likely steps involved in the reaction of morphine with the Marquis reagent leading to formation of a colored product. Adapted from Auterhoff, H. and D. Braun. "Die Farbreaktion des Morphins nach E. Marquis." *Arch. Pharrmaz. (Weinheim)*, 306 (1973): 866–73.

mechanism involves an attack of the amine on the carbonyl of formaldehyde, although the path from there to the products proposed is debatable. The orange color produced by amphetamine and methamphetamine is distinctive, but to differentiate amphetamine (a primary amine) from methamphetamine (a secondary amine), an additional color test is needed. The Simon test, described shortly, is employed to differentiate the two compounds.

In the case of the Marquis reagent and morphine, a linked oxonium carbonium ion has been suggested as the source of the violet-colored product via an intermediate linked structure.[20] The mechanism is analogous to the amphetamine–methamphetamine pathway: an attack on formaldehyde by the amine, the linkage of two drug molecules, and formation of the colored carbonium ion under acidic conditions.

Ehrlich's Test: Ehrlich's reagent, *p*-dimethylaminobenzaldehyde (*p*-DMAB) in ethanol with HCl, works through the formation of a stable carbocation or ion by a **condensation reaction** (Figure 19).[21–24] This reagent is used principally for the detection of LSD, as well as for **indole** alkaloids, for which the test is highly sensitive. This sensitivity is necessary, given that the individual dosage unit of LSD is in the low microgram range.

The Liebermann Test: The **Liebermann test,** also known as the *Liebermann nitroso test* (Figure 20), is another presumptive test that incorporates a coupling reaction to form

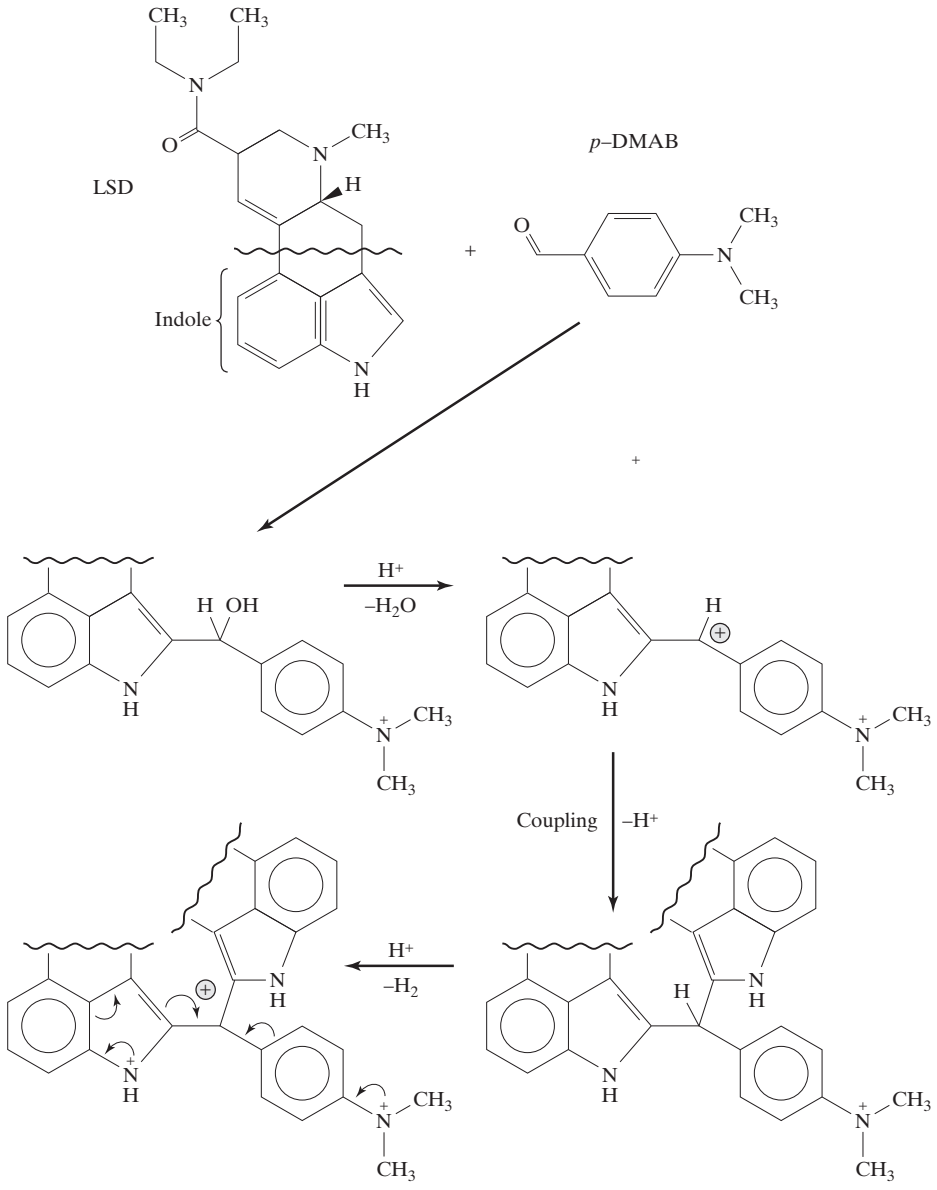


FIGURE 19 The proposed reaction for LSD (an indole) and Ehrlich's reagent. Adapted from "United Nations Scientific and Technical Notes," *Chemistry and Reaction Mechanisms of Rapid Tests for Drugs of Abuse and Precursor Chemicals*, 1989.

the colored product. The Liebermann reagent will react with phenols and amines, both of which are common in drug molecules. The reagent consists of KNO_2 dissolved in sulfuric acid, which forms nitrous acid (HONO). Two steps are involved in color formation: nitration of the substrate drug (step 1 in Figure 20) and coupling to form a quinone imine (step 2). The coupling begins with a dehydration step promoted by the sulfuric acid, followed by diazonium coupling to yield a colored product. The resulting compound possesses characteristics of an indicator, since the color is pH dependent; not surprisingly, many acid–base indicators, such as methyl red and methyl orange, are diazonium based.

The Simon Test: The **Simon test** (Figure 21) is one of the more intriguing color tests that combines elements of dyes (diazonium ions), transition-metal complexes (discussed in detail shortly), and carbocations.[25–27] The Simon test is a variation of the **sodium nitroprusside test** that has been utilized in organic qualitative analysis for decades; the typical formulation used in forensic testing is a nitroprusside solution containing acetaldehyde[13] with a second reagent consisting of 2% Na_2CO_3. This two-step test is used to differentiate methamphetamine from amphetamine, both of which produce a similar orange color when treated with the Marquis reagent. Note that amphetamine is a primary amine of the generic form RNH_2, and methamphetamine is a secondary amine of the form R_2NH.

The generic reaction of nitroprusside with bases such as the amines is[26]

$$[(NC)_5FeNO]^{2-} + B^n \longleftrightarrow [(NC)_5\,FeN(B)O]^{-2+n} \qquad \textbf{(2)}$$

For methamphetamine (a secondary amine), the inclusion of acetaldehyde in the reagent facilitates an addition–condensation reaction to form an enamine and an

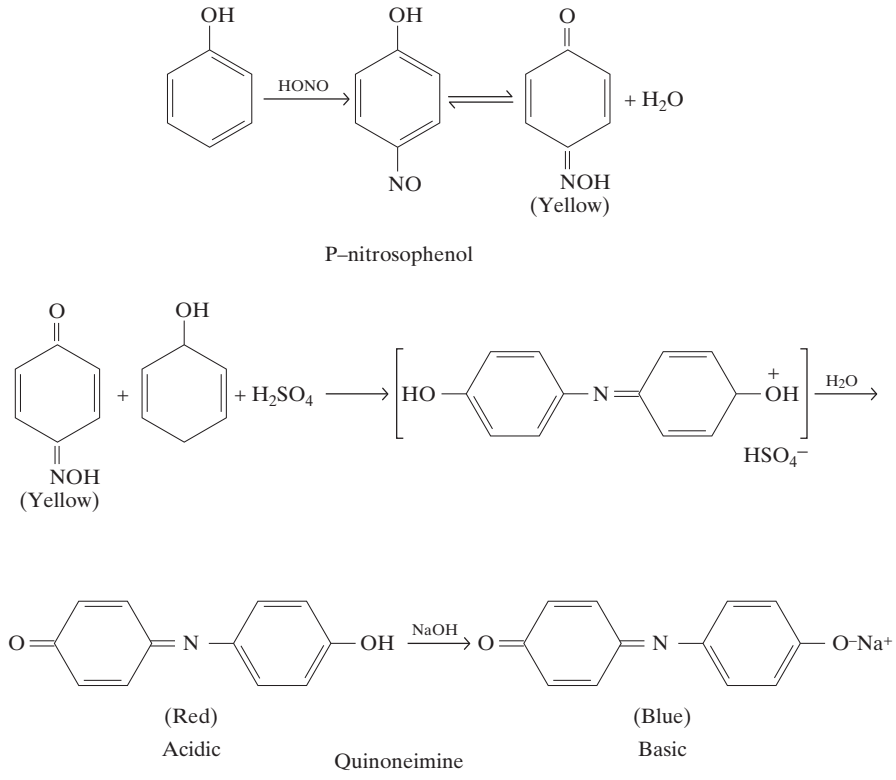


FIGURE 20 The reaction of nitrous acid from the Liebermann reagent with phenol, which results in a pH-sensitive colored product.

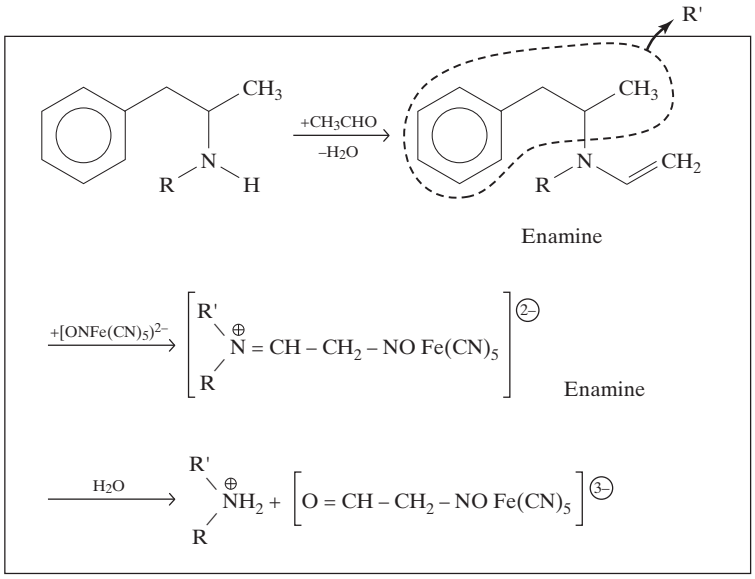


FIGURE 21 Proposed reaction of methamphetamine with Simon's reagent. Adapted from "United Nations Scientific and Technical Notes," *Chemistry and Reaction Mechanisms of Rapid Tests for Drugs of Abuse and Precursor Chemicals,* 1989.

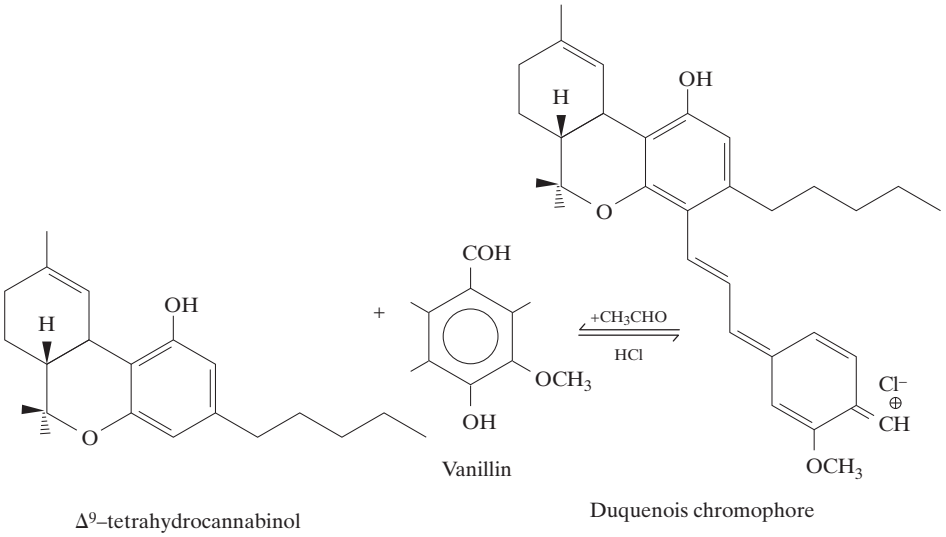


FIGURE 22 The Duquenois reaction for THC in marijuana. The acid drives the condensation of THC with vanillin and acetaldehyde to form a resonance-stabilized and conjugated chromophore. This purple material can be extracted into chloroform. Adapted from Forrester, D. E., *The Duquenois Color Test for Marijuana: Spectroscopic and Chemical Studies*. PhD dissertation, Georgetown University, Washington, DC: 1997.

intermediate that further reacts with water to form a blue complex, as shown in Figure 21.[16] This complex is sometimes referred to as the Simon–Awe complex.[10]

Tests for Marijuana: The Duquenois reagent (Duquenois or Duquenois–Levine test) involves a condensation reaction leading to creation of a purple chromophore (Figure 22) with the active ingredient tetrahydrocannabinol.[28–29]

4.1.4 TRANSITION METALS IN COLOR-BASED PRESUMPTIVE TESTS Related to the Simon test is a family of color-producing reactions based on transition-metal complexes (coordination complexes) and tightly associated ion pairs. **Coordination complexes** arise from a **Lewis acid–base** interaction between a metal cation, such as cobalt, and an atom with unshared electrons, such as water or, in the case of drugs, basic nitrogen found in alkaloids and amines. Metals that have been used in these reagents include copper,

vanadium, bismuth, and cobalt. Cobalt, as part of two common reagents (cobalt thiocyanate and **Dilli-Koppanyi**) is perhaps the most versatile. Cobalt has the electron structure $3d^74s^2$, whereas the cation has a $3d^7(2^+)$ or $3d^6(3^+)$ structure.

In aqueous solution, the cobalt ion typically has a light pink appearance as a result of the complex formed with water (Figure 23). The same is true of copper, which is light blue in solution owing to a water complex. In both cases, the water ligands are arranged in an octahedral pattern around the central metal cation. If a color is observed, electronic transitions are taking place, which can occur only if there is some disparity in the energy of the outermost orbitals containing electrons. In the case of transition-metals, these are the d orbitals, which, under normal conditions, are degenerate (all of equivalent energy). **Ligand field theory (LFT)** allows for an explanation of d-orbital alterations, color, and magnetism of transition-metal complexes. LFT represents a merging of **crystal field theory (CFT)** and molecular orbital theory, invoking principles such as orbital overlap, bonding–nonbonding interactions, and orbital splitting.[30]

To greatly simplify LFT, as a **ligand** approaches and forms an association with a metal ion, it is acted on by two different electron density environments, as illustrated in Figure 24. The approach along an axis will be impeded by two of the five d orbitals, with the electron density repelling the electrons of the approaching ligand. The other three d orbitals have a zero electron density along the axis and present an easier approach for a ligand, since the repulsion is weaker. Because the three orbitals are symmetric, their energy is degenerate and lower than that of the other two. Put another way, an unshared pair of electrons on oxygen that reside in the metal's $d_{x^2-y^2}$ orbital will be repelled significantly more from metal-ion electrons than would the pair of electrons on oxygen residing in the d_{x-z} orbital. Because a gap is created, d electrons can be promoted if the energy of the incoming photons is sufficient. All that is required for promotion is a vacancy in the upper d orbitals.

The degree of d-orbital splitting depends on the relative strength of the ligand, described in the spectrochemical series. This abbreviated series is $CO, CN^- > NO_2^- > NH_3 > H_2O > OH^- > Cl^-$, with NH_3, H_2O, and Cl^- being of most interest in forensic chemistry and presumptive color testing. CO and CN are the strongest ligands and create the largest splitting. Any charge on the metal ion will also affect splitting. All else being equal, an octahedral ammonia complex will have a larger gap than a water complex of the same structure around the same central atom. A larger gap means that light of higher energy (more blue) will be absorbed, leading to a more reddish appearance. However, there are complicating factors that exceed the scope of this discussion. Suffice it to say that there are also square planar and tetrahedral complexes with four ligands ($[CoCl_4]^-$, for example) in which the splitting is not

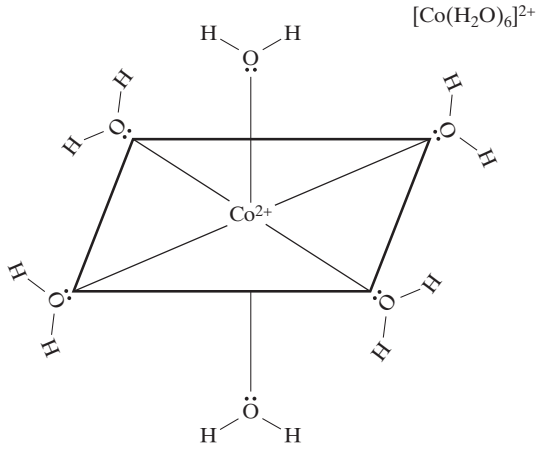


FIGURE 23 Hydrated cobalt ion, a coordination complex with an octahedral structure. The unshared pairs of electrons on the oxygen are attracted to the metal cation via a Lewis acid–base interaction.

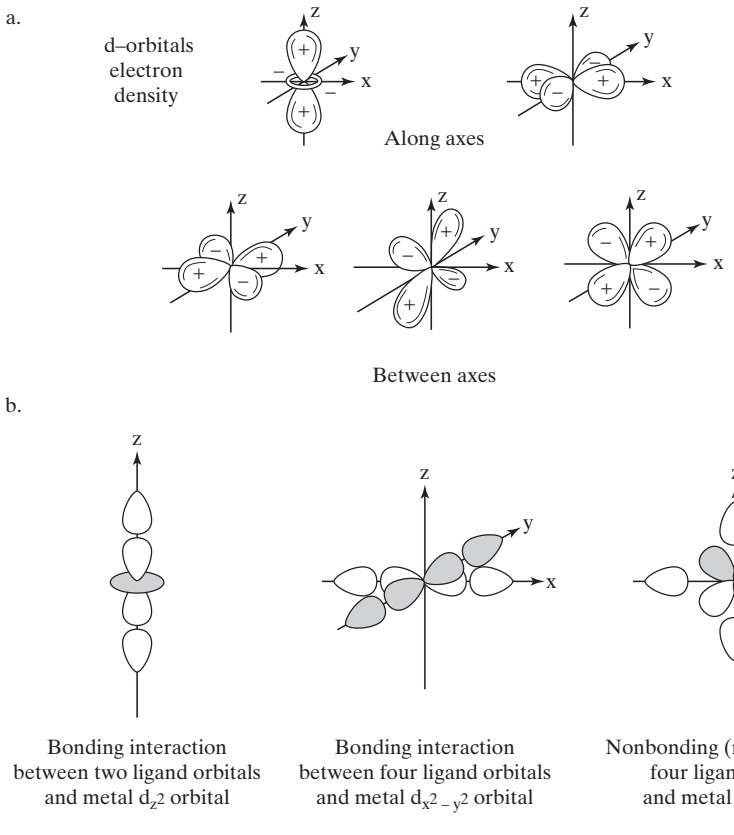


FIGURE 24 (a) Unlike s and p orbitals, d orbitals are not all symmetric. The $d_{xz}-$orbital, for example, has lobes that project out into the x–z plane; there is no electron density along the axes, only between them. (b) Where overlap occurs with the orbitals of ligands, bonding interactions occur; nonbonding interactions occur where there is no electron density and no overlap.

as straightforward. Also, with many d electrons, such as those in cobalt, the distribution of electrons will depend on the gap distance, leading to what are called **high-spin** and **low-spin complexes** (Figure 25). High-spin complexes have unpaired electrons, whereas low-spin complexes have paired electrons.

Key to the current discussion is the ability of transition metals to form charged complexes. As charged and relatively stable entities, complex ions can act as cations or anions and form coordination compounds or tightly associated ion pairs. These ion pairs may be solids, or they may form stable neutral species that can be extracted into an organic solvent such as chloroform. This is the basis of the cobalt thiocyanate test for cocaine, in which a pink solution of aqueous cobalt thiocyanate is added, along with hydrochloric acid, to the unknown powder. If cocaine or a related substance, such as procaine, is present, a blue color results. In one modification of the test, this blue **ion pair** is extracted into a chloroform layer as further evidence for the formation of a tightly associated ion pair.[13, 31–36]

As an alkaloid and a tertiary amine, cocaine is basic, with a pK_a of 8.6. To be extracted into an organic solvent in the base form, the pH of the aqueous layer must be adjusted to approximately 11. The cocaine remains neutral and thus extractable. However, in acidic aqueous solutions, cocaine behaves as any other nitrogenous base and will exist in the protonated form BH^+. Once ionized, the BH^+, equivalent to the protonated cocaine, can form the ion pair according to the reaction

$$2\,NHR_3^+ + [Co(SCN)_4]^{2-}(\text{pink, aq}) \longrightarrow (NHR_3)_2[Co(SCN)_4](\text{blue solid}) \quad \textbf{(3)}$$

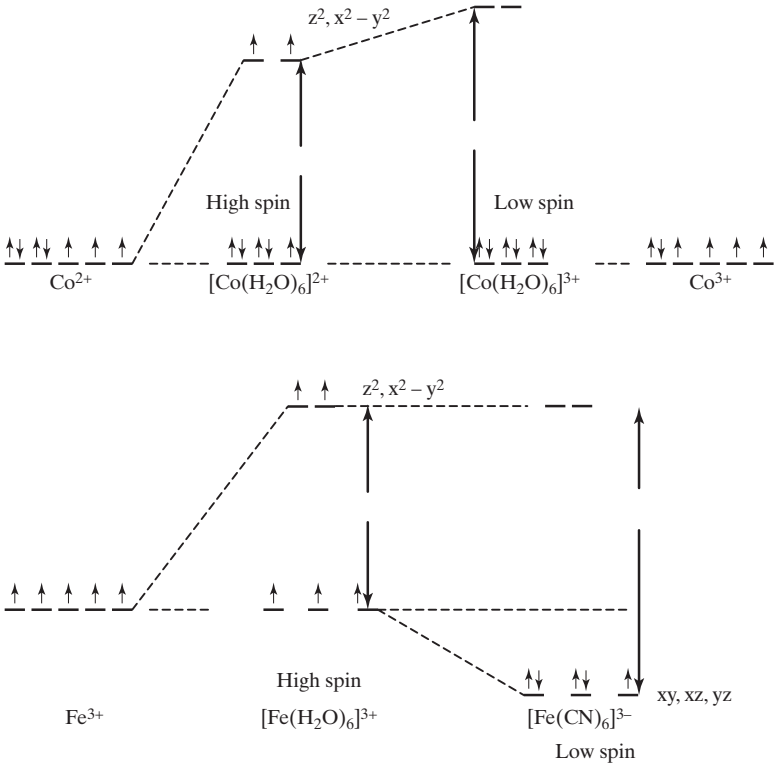


FIGURE 25 Effect of ligand and metal-ion charge. High-spin complexes, with unshared electrons, are favored when weak-field ligands generate smaller energy gaps than strong-field ligands do. Similarly, for the same ligand, increasing the charge on the metal ion increases the energy gap, favoring low-spin complexes.

where the product is a neutral compound that will extract into chloroform. This ion pairing is one of many that can be used for simple color testing, as well as in quantitative assays and atomic absorption spectrophotometry.

As with most complexation and drug solubility situations, pH is a critical variable. Cocaine base is not soluble in water, and if the drug is in this form rather than a soluble salt, no reaction occurs. Acid is needed to ensure that the cocaine is in the water-soluble ionic form to allow for the formation of a complex. The color is the result of an **ion-pair compound** formed from the cationic cocaine and the anionic cobalt complex. As with all amine bases, such as ammonia, the base becomes protonated in acidic solution. The pK_a of the base determines the ratio of the protonated, ionized form to the neutral form. It is possible to add too much HCl, because cobalt forms a water-soluble pink complex with chloride $[CoCl_4]^{2-}$. The pH can also influence the type of complex and ion pair formed. Under acidic conditions, the ion pair favored is $[Co(cocaine)_2](SCN)_2$ (which is pinkish and soluble in water), whereas in the neutral-to-basic ranges, the ion pair is assigned the structure $[cocaine \text{¥} H^+]_2[Co(SCN)_4]$ (which is a blue solid and soluble in chloroform).[37, 38] The important points of the cobalt thiocyanate reaction with cocaine are summarized in Figure 26–28.

The Bottom Line: Drug analysts and toxicologists use many more color tests, far too many to list and discuss in this chapter. Table 4 gives a summary of the more common color tests, and forensic practitioners who use color tests usually adopt a battery of personal favorites. A good laboratory reference for color tests is L. Y. Galichet et al., eds., *Clarke's Analysis of Drugs and Poisons, Vols. 1 and 2* (London: Pharmaceutical Press, 2004). Some references provide flowcharts, but there is no one correct protocol for presumptive color testing. The important point is that analysts understand the reagents and reactions, the methods, and their strengths and limitations.

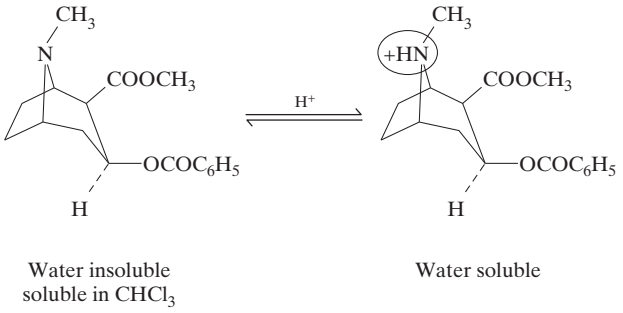
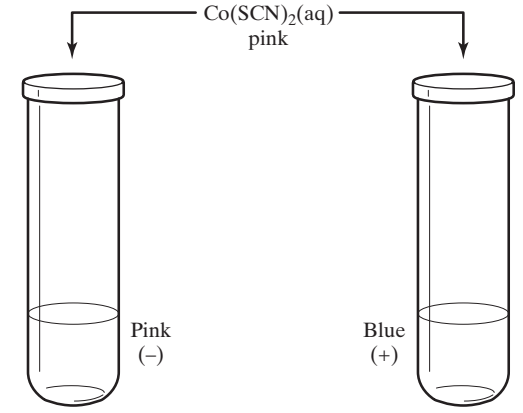


FIGURE 26 An acidic solution is required for the cobalt thiocyanate test, since the cocaine must be soluble.



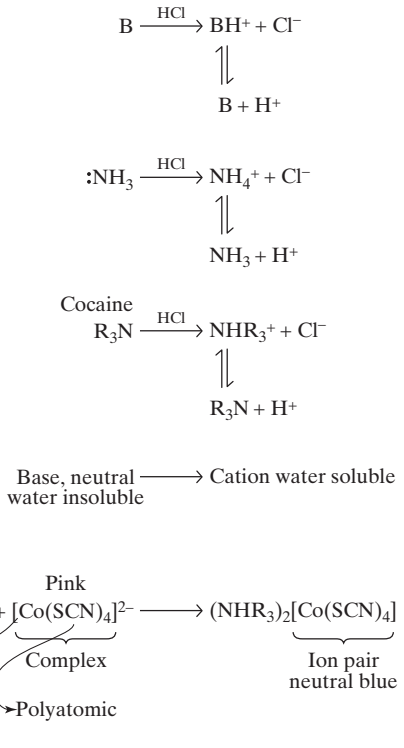


FIGURE 27 The addition of acid ensures that the base is soluble. The overall reaction illustrates several interactions and bonding types.

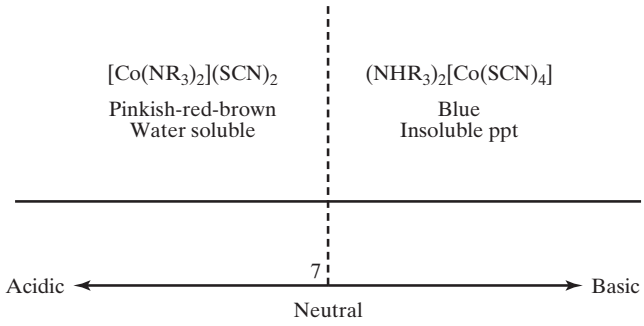


FIGURE 28 Additional pH effects that can occur in the cobalt thiocyanate reaction.

4.2 Microcrystal Tests

Some controversy and debate surround the use of microcrystals (**crystal tests**) in drug analysis, but this shouldn't be the case. Properly employed, crystal tests are powerful extensions of color tests. Specificity is derived chiefly from appearance and behavior of crystals under polarized light. The catch—and there usually is one—is the user's ability to identify crystal forms. However, there is a subjective element in judging colors in all color tests, which is addressed by controls, literature validation,[13] experimentation, and experience. The same is true of crystal tests. In fact, prior to the wide availability of instrumentation, crystal tests were considered to be one of the most specific and selective tests available for many drugs.

To give a refined definition, crystal tests are presumptive precipitation tests in which crystal morphology lends specificity. Consequently, the analyst's experience is an important part of interpreting results of crystal tests. Heroin, cocaine, and amphetamine are the most frequently targeted analytes for crystal tests, and procedures using gold and platinic chlorides and bromides have been standardized by ASTM.[39-41] A recent study supports microcrystal testing as a viable tool for the forensic chemist.[42] The authors note that although a battery of presumptive color tests produced false positives for cocaine among a number of drug powders, the inclusion of crystal tests in the regime allowed for the elimination of false positives that might have been observed during color testing. Microcrystal tests are also valuable for differentiation of isomers such as *d*-amphetamine/*d*,*l*-amphetamine and *d*-cocaine/*l*-cocaine.

Microcrystal tests are simple to perform: A reagent is added to a speck of homogeneous sample on a microscope slide, and the result is observed under transmitted light microscopy. Variations in methodology take advantage of solubility and volatility. For example, the freebase forms of amphetamine and methamphetamine have appreciable vapor pressure at room temperature and can be driven into the vapor phase. The drop of reagent hanging above the sample contains acidified gold chloride in which the characteristic crystals will form if the drugs are present. This simple, but elegant, separation leaves behind diluents such as sugars and starches, making the crystals easier to see and study. Photomicrographs of crystal tests can be found in the color insert to Figure 29.

The basis of most crystal tests for alkaloids involves protonation of basic nitrogen to form a charged cation and precipitation as an ion-pair salt. In fact, many of the most widely used reagents for crystal tests also are or were at one time used in presumptive color tests. Examples include ion pairs formed between the cationic alkaloid base and anion complexes such as BiI_4-bismuth tetraiodide (**Dragendorff test**) or HgI_4^{2-} (an older color test called **Mayer's test**). Currently, the most commonly used forensic microcrystal tests involve complexes incorporating gold, platinum, and halogens, principally chloride and bromide.[39-41] The gold standard for crystal tests is described in a book written by Fulton and published in 1969.[43] Unfortunately, this reference is now difficult to obtain.

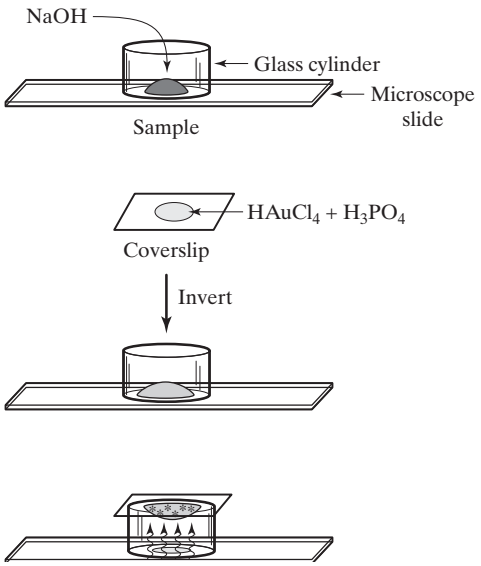


FIGURE 29 A volatility-based separation and crystal test for amphetamine and methamphetamine. The precipitating reagent is placed on a coverslip and inverted over a small portion of the sample to which a strong base is added. Amphetamine and methamphetamine in the freebase form have sufficient vapor pressure to evaporate and condense in the reagent. Nonvolatiles are effectively removed from the sample. See the color insert for photos of this test.

TABLE 4 Common Color (Presumptive) Tests and TLC Developers for Drugs

Name	Other Names or Variants	Reactive Species	Use or Targets	Likely Mechanism
Dragendorff	Kraut's	BiI_4^-, bismuth tetraiodide anion.	Alkaloids, cholines, TLC developer	Ion pair with BiI^{4-}, bismuth tetraiodide complex
Dilli-Koppanyi		Co^{2+}	Barbiturates	Colored complex
Ferric chloride		Fe^{3+}	Salicylates (aspirin family), phenols	Colored complex
Froedhe	Frodhe (older literature)	Mo^{3+} acidic	Alkaloids	Colored complex
Iodoplatinate		I^-, Pt	Alkaloids, amines, general developer for TLC	Colored complex
Mandelin's	Ammonium vanadate	V cation	Steroids, alkaloids, aspirin	Colored complex
Zwikker		Cu^{2+}, pyridine in chloroform	Barbiturates	Colored complex

4.3 What Is Definitive Identification?

Before moving on into specific drug classes, it is worth reflecting on a uniquely forensic question: What constitutes a definitive identification? How much data and what combination of tests are adequate to identify a white powder as cocaine or as something else? This is both a scientific and a legal question and, for any forensic chemist, not a trivial one. When M.B. Orfila analyzed the exhumed remains of Charles Lafarge, his analysis was at the cutting edge of analytical chemistry in 1840, but it is unlikely that his results and testimony would be accepted today. This is not Orfila's fault but the inevitable result of the evolution of chemical science, instrument technology, and legal precedent. Less than a hundred years ago, forensic chemists identified drugs on the basis of their color and microcrystal tests; there were no mass or infrared spectrometers readily available, as they are today.

As instrument capability and availability have improved, courts have come to expect instrumental confirmation of identification in drug cases. The one common exception is marijuana (discussed shortly), but for nearly every other type of controlled substance, instrumental identification is expected, be it confirmation of other testing, such as presumptive and screening tests, or as stand-alone data. The specific requirements for a complete analysis vary. A given laboratory may utilize color tests, TLC with standards, and GC-MS. At the other end of this spectrum, another laboratory may employ only GC-MS and IR analyses. Both protocols, if performed with the appropriate controls and procedures, can provide definitive identification and exceed what would be required in many other contexts. For example, environmental analysis for most organic pollutants relies on GC-MS; such data also are subject to legal scrutiny.

Analytical chemists usually accept chromatographic retention-time data combined with mass spectral data as sufficient to identify most compounds as long as the proper validated standards are analyzed as well. The legal question involves the degree of certainty of such identification. Could there conceivably be a compound that has the exact same retention time as cocaine on a given chromatographic column and exactly the same mass spectrum? Of course there could; the question is, How likely is it that such a compound exists? This question is unanswerable and is addressed by adding confirmatory testing. The more testing added to the protocol, the less likely a false positive becomes. Is it conceivable that a compound has *identical* (not just similar) behavior and responses to color testing, crystal testing, TLC using multiple solvent systems and standards, gas chromatography, mass spectrometry, and infrared spectrophotometry? Conceivable perhaps, but the probability is vanishingly small. It is in this context that a forensic chemist operates.

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) has put forth recommendations regarding selection of analytical methods to ensure that any drug identifications made are reasonable, defensible, and trustworthy. Here, analytical methods are categorized based on their relative discrimination power. The document recommends that at a minimum, results from a category A method should be supported by results from at least one other technique from any of the three categories. If a category A method is not used, at least three uncorrelated category B methods should be used. Here, uncorrelated means that the analytical methodology does not rely on the same fundamental chemical properties or characteristics. For example, GC, TLC, and LC are all separation methods based on selective partitioning. If sufficient care is not taken in the design of assays based on these three methods, correlation can occur. For example, if TLC and LC methods are devised, but both use polar mobile phases and nonpolar stationary phases, these are correlated techniques. A better selection would be a combination of LC and CE for example; typical CE separation is based on size-to-charge and is uncorrelated with typical LC methods. Additionally, hyphenated methods can be considered as two separate methods if data from both are used. Thus, a GC-MS analysis would be considered to combine category B and A as long as the retention time obtained for the analyte was compared with that of a validated reference standard. Of course, the underlying assumption is that validated methods are used, and any limitations with a given method are known and accounted for.

One final question merits attention: At what concentration is a drug considered to be present in a sample? The LOD and LOQ of any validated analytical protocol are known and documented. Suppose, for example, that a solid-phase extraction/GC-MS analysis of a resinous powder is capable of reliably detecting a heroin concentration of 12 ppb $\pm$ 2%. This concentration may extrapolate to only a few micrograms of heroin in a powder, a value that may in turn represent a weight percentage of far less than 1% in the original material. If the method was properly performed and all other data remained consistent, the analyst can indeed be confident that heroin was present. Is this amount of heroin, even

though it is detectable, a sufficient amount to warrant judicial action? This is where analytical chemistry ends and legal debate begins.

The issues raised in this discussion are as much philosophical questions as they are legal and scientific ones. There are no single correct answers, but there are accepted scientific and legal guidelines used to frame them. For example, color tests are not, by themselves, sufficient to identify a drug. This is why such tests are called presumptive. Arguments continue as to whether color tests combined with crystal tests can provide unambiguous identification. The question is less important in the instrumentation age, but it raises yet another question: Is GC-MS or IR alone sufficient to identify a substance? The discussion has come full circle, and the forensic community has generally taken the position that if there is any reasonable doubt as to identification, additional confirmatory testing is added to the protocol. This is the spirit in which the SWGDRUG recommendations were formulated. At what concentration levels is a drug considered present in the eyes of the law? That varies; all the chemist can do is follow laboratory protocols, be aware of jurisdictional standards, and report the best scientific data possible.

TABLE 5 Discriminating Power of Various Analytical Methods

Category A (highest discrimination power)	Category B	Category C (lowest discrimination power)
Infrared spectroscopy (IR)	Capillary electrophoresis (CE)	Color tests
Mass spectrometry (MS)	Gas chromatography (GC)	Fluorescence spectroscopy
Nuclear magnetic resonance spectroscopy (NMR)	Ion mobility spectrometry (IMS)	Immunoassay
Raman spectroscopy	Liquid chromatography (LC)	Melting point
X-ray diffraction (XRD)	Microcrystalline tests	Ultraviolet spectroscopy
	Pharmaceutical identifiers	
	Thin layer chromatography	

EXAMPLE PROBLEM 4

Devise an analytical method for the qualitative identification of methamphetamine (as a powder) using only category B techniques.

Answer: Because the exhibit is a powder, we cannot take advantage of any characteristic markings or dimensions (pharmaceutical identifiers). The challenge is to combine three uncorrelated techniques for the identification. A reasonable combination would be GC-FID, a microcrystal test, and ion mobility spectrometry. These are three uncorrelated techniques that when executed using validated methods and reliable standards would meet the SWGDRUG recommendations.

Key Terms and Concepts

Achromatic
Adulterant
Alkaloid
Anabolic steroids
Analgesics
Anti–Drug Abuse Act
Aryl group
Auxochrome
Azo dye

Azo/aza linkages
Barbiturates
Bathochromic shift
Carbocation
Central nervous system (CNS)
Chemical Diversion and Trafficking Act (CDTA)
Chromatic
Chromophore
Classification
Cobalt thiocyanate test
Color test
Colorant
Condensation reaction
Conjugation (conjugated system)
Controlled Substances Act
Coordination complex
Crystal field theory (CFT)
Crystal test
Cutting agent
Depressants
DFSA (drug facilitated sexual assault)
Diazonium salt
Dilli-Koppanyi
Diluent
Dragendorff test
Dronabinol
Drug
Duquenois–Levine test
Dye
Excipients
Exhibits
Five P's
Free-radical mechanism
Functional group
Hallucinogens
High-spin complex
Hyperchromic shift
Hypochromic shift
Hypsochromic shift
Impurities
Indole
Inhalants
Ion pair
Ion-pair compound
Lewis acid–base
Liebermann reagent or test
Ligand
Ligand field theory (LFT)
Low-spin complex
Marquis test
Mayer's test
Medicine
Methamphetamine Anti–Proliferation Act (MAPA)
Microcrystal test
Molecular orbitals
Narcotics
Natural drug or product
NSAIDs
Paracetamol
Paraphernalia
p-DMAB test
Physician's Desk Reference (PDR)
Pills
Pigment
Plant matter
Powders
Precursors
Predator drugs
Presumptive test
Profiling
Semi-synthetic
Simon test
Sodium nitroprusside test
Spot plate
Spot test
Stable isotope ratio
Stimulants
Synthetic
Thinner

Problems

FROM THE CHAPTER

1. Why is it becoming increasingly difficult to classify drugs as natural, semi-synthetic, or synthetic?
2. From a regulatory and analytical perspective, why would immediate precursors be of more concern than distant precursors?
3. For over-the-counter preparations, inactive ingredients are called *fillers.* Are these the equivalent of diluents, adulterants, impurities, contaminants, or thinners? Why or why not?
4. Would a profile based on residual solvents be useful in locating the geographic origin of a sample? Why or why not?
5. Summarize the mechanisms by which color can be created with a color-test reagent.
6. Why are reagents based on the formation of diazonium ions and salts used almost exclusively for alkaloids and bases?
7. What would happen if the Liebermann reagent was added to a sample of cocaine HCl? Why? Give a defensible chemical explanation.
8. The nitroprusside reaction will not work (i.e., it will not produce colored products) with primary and secondary amines if the pH is acidic. Why?
9. With regard to the previous question, why would NaOH be a poor choice for the base, as opposed to sodium carbonate?
10. In reference 31, the following statement is made: "When a chloroform solution of indole is treated with dilute acid (up to approximately 12 percent) and Ehrlich's reagent the color remains in the chloroform, but if the test is made with stronger hydrochloric acid the color is transferred to the aqueous phase. If the acid is too concentrated, the color may be inhibited or destroyed." Explain this statement.
11. Name the functional group(s) in THC. What presumptive color or crystal tests could be useful, aside from the Duquenois–Levine test? Why are crystal tests of limited use with plant extracts?
12. Resorcinol gives a false positive with the Duquenois–Levine test. Why?

13. Name a presumptive test or test series that could distinguish between testosterone and estradiol. Justify your selection.

14. Explain what is meant by the term "undetectable" in referring to a designer steroid. Obviously, it is a misnomer if taken literally, since such compounds have been identified.

15. The following colors are obtained in presumptive testing:

Steroid	Liebermannn's	Mandelin's
Testosterone	Light violet	Orange red
Testosterone cypionate	Orange brown	Dark brown
Testosterone enanthate	No reaction	Orange red
Testosterone propionate	Orange brown	Orange brown

Explain or justify these observations.

Source: Chiong, D. M., et al. "The Analysis and Identification of Steroids." *Journal of Forensic Sciences* 37 (1992): 488–502.

16. For the following drugs, complete the following table and classify the drugs based on their acid/base/neutral characteristics, physiological effect, and Schedule on the CSA if applicable.

Compound	Structure (Cite source)	A/B/N pK_a	Water solubility (mg/L)	Effect	log P	Schedule
4-Methoxyamphetamine						
Fentanyl						
Secobarbital						
Lidocaine						

17. For the compounds located and data gathered in the preceding question, complete the following table. Work with the drug only, not any salt forms.

Compound	FW	A/B/N pK_a	Water solubility (mg/L)	S_0 (M)	pH of a 0.01 M solution	% ionization at pH 7.4
4-Methoxyamphetamine						
Fentanyl						
Secobarbital						
Lidocaine						

INTEGRATIVE

1. Calculate the minimum and maximum frequencies and energies of a photon capable of promoting an electron with UV/VIS radiation.

2. Set up a spreadsheet and use it to determine the ratio of protonated to unprotonated cocaine at the following pH values: 1.0 (as if concentrated HCl were used), 2.0, 4.0, 6.0 (typical of laboratory-distilled water used in preparing reagents), 6.6 ($pK_a - 2.0$), 7.0, 8.6 9.0, 10.0, 10.6, ($pK_a + 2.0$), and 12. Make the spreadsheet as generic as possible so that other compounds and other values can be substituted. Graph the results showing the concentration of the two species as a function of pH.

Using the preceding data, information from the chapter, and supplementary information as needed, answer or explain the following:

a. A "one-well" approach is a variation of the cobalt thiocyanate test, as follows: the cobalt thiocyanate reagent is dissolved in 98 mL of water to which 2 mL of concentrated HCl is added. When the solution is first prepared, it turns blue, but no precipitate forms. The color eventually reverts to pink. Explain and discuss the implications of this test for field-test kits.
Source: Deakin, A. L. "A Study of Acids Used for the Acidified Cobalt Thiocyanate Test for Cocaine Base." *Microgram Journal* 1 (2003): 40–44.

b. In an experiment similar to that described in part a, it was observed that if 0.1 N HCl was used instead of concentrated HCl, no transitory color change was observed, and the test worked as expected with cocaine. Explain this observation.
Source: Deakin, A. L. "A Study of Acids Used for the Acidified Cobalt Thiocyanate Test for Cocaine Base." *Microgram Journal* 1 (2003): 40–44.

c. Cocaine can hydrolyze in solution when exposed to strong acids or bases, but the process typically requires hours. Give equations or reactions for the hydrolysis under both conditions, and state the likely products.

d. The Scott variant of the cobalt thiocyanate consists of a cobalt thiocyanate solution that is prepared in 1:1 water and glycerin. When this solution is added to a sample of cocaine, the characteristic blue precipitate forms. Next, HCl is added until a precipitate-free pink solution is observed. The solution is extracted with chloroform. The bottom layer is blue, the top pink. Explain. What is the likely function of the glycerin?
Source: Schlesinger, H. L. "Topics in the Chemistry of Cocaine." *United Nations Office on Drugs and Crime: Bulletin on Narcotics* (1985): 63–85.

e. What other transition metals might work as ion-pair reagents? List two or three, and discuss the considerations and experiments that would be needed to use them.

3. One colored species proposed for some stable carbocation color change reactions is a tropylium ion. Why would a tropylium ion be expected to be colored a different color than the starting material?

FOOD FOR THOUGHT

1. Is caffeine an addictive substance? If so, is it psychologically addictive, physiologically addictive, or both? What about chocolate? Bubble gum? How are such distinctions made?

2. A common scene in movies and TV programs shows the detective tasting a suspected drug powder to determine its identity. In addition to being poor laboratory practice, it is a really bad idea. Discuss and explain.

3. How important is it for a forensic chemist to understand why heroin turns the Marquis reagent purple or why cocaine forms a blue precipitate with the cobalt thiocyanate reagent?

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Forensic Drug Analysis: Selected Drug Classes

OVERVIEW AND ORIENTATION

In this chapter we will examine some of the drugs and drug families frequently encountered in seized-drug analysis. We stick with the acid/base/neutral classification scheme but will now add additional information regarding chemical analysis. Once we have a grasp on these drugs as physical evidence, we will move into toxicology and study how these drugs behave when ingested.

Acids and lipophilic neutrals will be the first drug classes described in detail. Although relatively small by comparison, the acidic drug group includes arguably the star of the forensic chemistry show: marijuana. Exhibits of marijuana, of drugs suspected to be marijuana, or of drugs related to marijuana often make up the largest caseload of forensic laboratories that have drug sections. Indeed, marijuana exhibits make up the largest single category of forensic science casework. Chemically, the active ingredient THC is atypical in that it is a nonalkaloid plant extract that is acidic and lipophilic. Another interesting acidic drug, gamma hydroxybutyrate (GHB), is also one of the smallest in size. In addition, given its propensity for interconversion between an open and a closed molecular form, it is one of the more difficult to extract and to analyze. The basic drugs are a much larger group and consist of several families, including the opiates, tropanes, and phenethylamines. We will look at each and also discuss the clandestine synthesis of methamphetamine.

1 ACIDIC DRUGS

1.1 GHB and GBL

Gamma hydroxybutyric acid (**GHB**) and gamma butyrolactone (**GBL**, Figure 1) are among the most analytically challenging abused drugs. GHB is a small, polar, water-soluble molecule capable of extensive hydrogen bonding. It is a club and predator drug originally designed to treat narcolepsy; now it is encountered principally in cases of

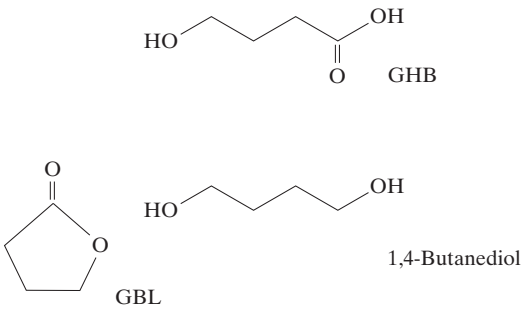


FIGURE 1 Structures of 1,4-butanediol, GHB, and GBL, the corresponding lactone. Conversion between GHB and GBL depends primarily on pH.

drug-facilitated sexual assaults (DFSAs). Analogs of GHB relevant in forensic analysis are 1,4-butanediol and GBL.

GHB has been used as a hypnotic and as an anesthetic agent; during the 1980s, it became popular as a bodybuilding supplement. It was banned by the Food and Drug Administration in 1990, around the time it began being used as a club and predator drug. In 2000, GHB was added to Schedule I. Subsequently, GBL and 1,4-butanediol found increasing use, since these substances are converted to GHB in the body. As a predator drug, GHB is typically spiked surreptitiously into a beverage.

The effects and toxicology of GHB are somewhat unusual. Ingested GHB is converted to the lactone and quickly eliminated via urine. The peak plasma concentration occurs within an hour and induces sedative effects. A dose of approximately 50 mg/kg is required to induce unconsciousness. Ethanol has a synergistic effect, increasing the anesthesia. The half-life of GHB is 20–60 minutes, and nearly all the compound is converted to GBL; less than 5% of the unchanged drug is found in the urine.[2] As a consequence of the short half-life, traces of the drug are undetectable in blood within 8 hours of dosing and undetectable in urine within 12 hours of ingestion.[2,3] Unfortunately, one of the effects of GHB is a loss of short-term memory, and as a result, victims of sexual assault may not seek medical attention until after the drug has been cleared from their system. In the worst cases, they do not seek help at all. Adding further to the complexity, GHB is found naturally in the body in concentration ranges of 5 mg/L, although this concentration increases significantly postmortem.[2,3]

Applying the Science 1 GHB as an Anesthetic

The anesthetic power of GHB was illustrated in a 2004 case reported in the *Journal of Forensic Sciences*. When paramedics arrived at the home of a couple, they discovered the woman bleeding from the mouth. Both residents were chronic abusers of GHB. At the home, the responders found 18 teeth that had been removed from the woman's mouth with a pair of bloody pliers also found at the scene. Later interviews were unable to confirm who had done the extraction, but both individuals reported that they believed the female had been possessed by a demon. At the hospital, the wounds were sutured. Fortunately, despite the lack of dental training by either party, the extraction was done with no additional damage to bone. The male was charged with assault, but was acquitted when the jury was unable to decide which party had done the actual extraction.

Source: Reprinted, with permission, from Journal of Forensic Sciences, copyright ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428.

Exhibits suspected of containing GHB or related substances are often received as a liquid, either as a suspect beverage or in concentrated form. Powders may also be encountered. Given the chemistry of the substance, the submission of liquid samples presents significant analytical challenges.[4–10]

Analytical Approach and Interconversion: Under mildly acidic conditions, GHB undergoes an internal condensation via Fischer esterification to produce a stable **lactone**. This reaction involves two steps: cyclization, followed by the elimination of water. The reverse reaction, the hydrolysis of GBL to GHB, can be driven in the presence of a strong base, which is the method used clandestinely to make GHB (Figure 2). The reverse reaction can occur in solution when GHB is spiked into a drink, many of which are slightly acidic.[6] However, once equilibrium between the acid and the lactone form has been reached, no significant losses of GHB appear in typical exhibits over the time frame of days or weeks.[1]

The starting points for GHB analysis are presumptive tests, including many of the color tests based on acid–base indicators such as phenol red or the formation of a colored complex with cobalt.[3] Crystal tests using copper and silver nitrates have also been reported,[4–6] and TLC has been employed as well. The significant analytical challenges arise at the stage of instrumental confirmation, owing to the tendency toward interconversion and the polarity of the molecule.

One obvious analytical approach would involve driving the equilibrium to one extreme or the other by adjusting the pH, thereby driving the equilibrium to strongly favor a single form. Two problems arise: first, the analysis of the sample will not reflect the ratio of GHB to GBL, and second, driving the equilibrium to either extreme does not solve the fundamental analytical problem related to polarity. If the pH is set to 12, hydrolysis is favored, and the lactone is rapidly and completely converted to the free acid form. However, sample preparation and cleanup are complicated, since a liquid–liquid extraction is difficult.[8–10] The extracting solvent must be sufficiently polar to extract the polar GHB, yet the solvent itself cannot be soluble in water. A solvent such as *n*-butanol is a possibility, but the low vapor pressure of this alcohol makes it difficult to concentrate extracts.[10] Ethyl acetate has been recommended,[10] because it can extract the free acid form in sufficient purity to obtain an IR spectrum. However, problems with water and hydrogen bonding persist.

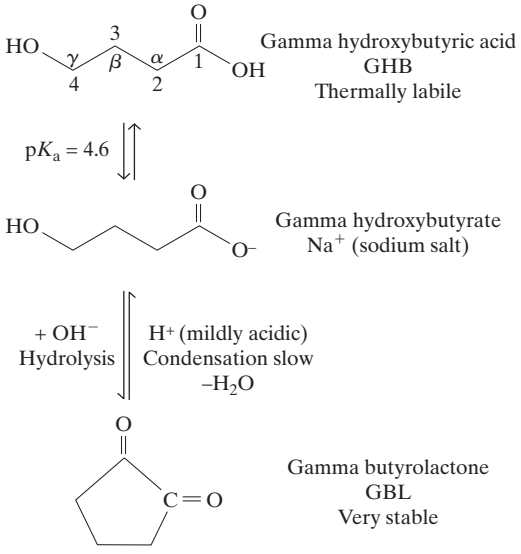


FIGURE 2 Interconversion of GHB and GBL.

The alternative method, setting the pH to acidic conditions, might seem a better option, but also fails. At a pH of 2, the esterification of GHB takes hours or longer, only to reach equilibrium with GBL rather than complete conversion.[8] Even though the lactone form can be extracted with an organic solvent, the link to the original sample concentration is further muddied, with no discernible gain for the trouble. Direct injection of the sample into a GC-MS is not an option, because GHB is thermally labile. HPLC is an alternative, but unless the system is equipped with a mass spectrometer, definitive identification is not possible. One method used successfully in some laboratories is derivatization of GHB using silanizing agents followed by GC or GC-MS.[11–13] Derivatization using **TMS** (tetramethylsilane) and an on-fiber SPME approach has also been reported.[7,8] A 2004 article described a screening method utilizing micellar electrokinetic chromatography applicable to spiked beverages.[1]

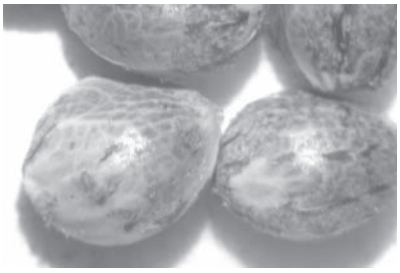
1.2 Tetrahydrocannabinols: Marijuana and Hashish

In terms of caseloads, marijuana represents the bulk of the work in most forensic chemistry sections and the largest number of case submissions to most full-service forensic laboratories. It is also the most widely available, most widely used illegal drug in the United States. Marijuana (also spelled "marihuana") is usually defined as all parts of the plant *Cannabis sativa* L., excluding the stalk and sterilized seeds (Figures 3–5). *Cannabis sativa* is a weed also known as **hemp**, cultivated for use as a fiber. Some jurisdictions use a more generic definition that lists any plants of the genus *Cannabis*. This approach circumvents legal questions about species that have been raised in the past. **Hashish** and hash oil are derivative products of the marijuana plant. Hashish is the resinous material derived from the flowering tops, and the oil is a potent solvent-extracted variant (Figure 6). Sinsemilla is a particularly potent form of marijuana plant in terms of its psychoactive ingredients.

The active ingredients of marijuana and its derivatives are the cannabinoids, summarized in Table 1. Two naming conventions (Figure 7) can be used for these compounds, so names can be a source of confusion. The dibenzofuran method is more common in the forensic context and will be used throughout this text. All cannabinoids are oily and insoluble in water but soluble in solvents such as chloroform and petroleum ether. They are unusual among plant-derived controlled substances in that none contain nitrogen; thus, none are alkaloids. However, marijuana plant and extracts do contain a variety of alkaloid bases, as is typical of any plant extract.[14]

Oklahoma State Bureau of Investigation

FIGURE 3 A leaf of a marijuana plant showing the distinctive serrated leaves. Image courtesy of the Oklahoma State Bureau of Investigation.



Aaron Brudenell, Crime Laboratory

FIGURE 4 Marijuana plant seeds. Note the distinctive mottled appearance. Image courtesy of Aaron Brudenell, Tucson Police Department Crime Laboratory.

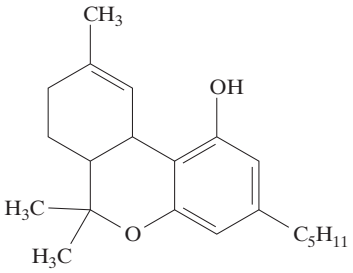
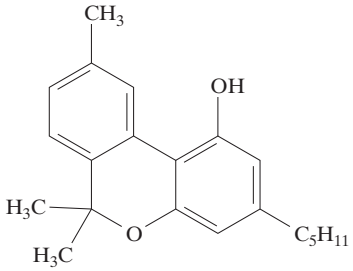
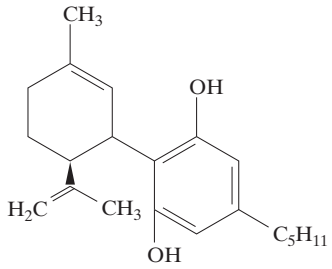
Oklahoma State Bureau of Investigation

FIGURE 5 A roach (hand-rolled cigarette) of marijuana, along with plant matter and seeds. Image courtesy of the Oklahoma State Bureau of Investigation.

Heather Campbell, Idaho State Police Forensic Services

FIGURE 6 Hashish. This batch has a dry, taffylike consistency, but hash can be much oilier. Note the distinctive jaguar imprint. Image courtesy of Heather Campbell, Idaho State Patrol Forensic Laboratory.

TABLE 1 Cannabinoids

Name[a]	Structure
Δ^9-Tetrahydrocannabinol, also generically referred to as tetrahydrocannabinol or THC	
Cannabinol (CBN)	
Cannabidiol (CBD)	

[a]Using the dibenzopyran–dibenzofuran naming convention.

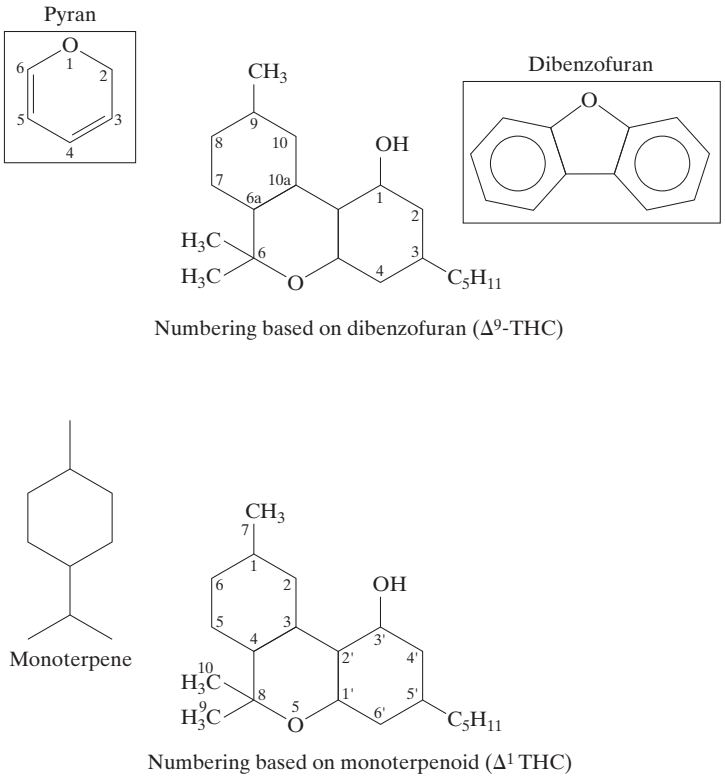


FIGURE 7 Two naming conventions used for the cannabinols. The top frame shows the dibenzofuran–dibenzopyran method that is more common in forensic usages, although the monoterpenoid system (bottom frame) is also encountered. Δ^9-THC in the dibenzofuran method is the equivalent of Δ^1-THC in the monoterpenoid system, which is based on a skeleton of 10 carbon atoms, as shown.

The principal active ingredient of concern in marijuana is **Δ^9-tetrahydrocannabinol** (Δ^9-THC, or just THC). THC is acidic and has an ionizable center and pK_a of 10.6. THC also has a log P value of 6.97, which reflects the insolubility of oils in water. The typical concentration of THC in the leaves is between 1% and 5%, and much higher values are found in the oily resin on the flowering tops. Marijuana growers have also taken the lessons of selective breeding to heart. The concentration of THC has increased steadily since 1980. Hashish and hash oil are much more potent, with a THC content ranging from 2% to 30%.[15] Δ^8-THC is found in much smaller quantities, whereas cannabidiol (**CBD**) and cannabinol (**CBN**) are found in larger amounts; all are less psychoactive than THC.

Acceptable, but limited, medical uses have been found for THC and we have entered a period where state marijuana laws are changing. The pharmaceutical company Unimed manufactures Marinol, currently the only manufactured drug containing a synthetic isomer of THC (dronabinol) in its formulation. Marinol (also called Dronabinol) is listed on Schedule III. The medicine is used to stimulate the appetite in AIDS patients and to treat nausea and vomiting associated with chemotherapy.[21] The synthesis of THC and cannabinols is difficult and complex;[14] thus, there is little reason to expect clandestine synthesis to be a factor in the restricted legal use of marijuana.

The last few years have seen an influx of consumer products manufactured from hemp. Because the plant is hardy and grows well in many types of soil, marijuana is easy to cultivate. Products made from hemp include fiber, clothing, rope, paper, and consumer products such as shampoo, which contains oil extracted from seeds. These legitimate uses of hemp products led the Drug Enforcement Administration (DEA) to

issue clarifications to existing regulations governing marijuana. In essence, the clarifications state that such products are exempt from provisions of the Controlled Substances Act if the products are in a form in which the THC is unavailable, unsuitable, or unlikely to be used for human consumption and abuse. For example, it is hard to image anyone attempting to get high on hemp-derived shampoo; the THC content is minute, and any extraction would not be worth the time or the effort (or the purchase of gallons of shampoo). In many countries, varieties of hemp that are low in THC content are grown for purposes of manufacturing hemp products. Another recent phenomena is the introduction of THC mimics or **cannabimimetics**. These synthetic substances mimic THC effects to some extent.

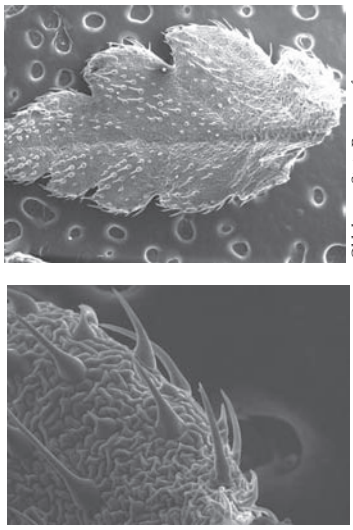


FIGURE 8 Scanning electron micrographs of the marijuana plant, showing the cystolithic hairs from the side (bottom frame) and above (top frame). Image courtesy of the Oklahoma State Bureau of Investigation.

Analytical Approach: The analysis of marijuana begins with an examination of the plant's morphology. A microscopic examination is also required, as the leaves have characteristic (but not definitive) features. The most important of these are "**bear claws,**" or **cystolithic hairs**, found on the dorsal leaf surface. These features are seen in Figure 8. The shape is distinctive, and the information to be gained is enhanced by adding a small amount of a dilute acid to the sample and observing the results under the microscope. The $CaCO_3$ that makes up the **cystolith** will dissolve and produce bubbles. Bear claws are actually one of a group of surface features referred to as **trichromes**. The others are unicellular trichromes and glandular trichromes. Unicellular trichromes are thin hairs found on the ventral leaf surface, and glandular hairs are topped with a bulb. Even in ground material or hashish, trichromes may be microscopically identifiable.

The presumptive color test for marijuana is the Duquenois–Levine test described at length in the previous chapter. A portion of the sample is extracted with petroleum ether and transferred to a test tube. The Duquenois reagent is added, followed by hydrochloric acid. A bluish purple color is indicative of cannabinoids. Several drops of chloroform are added to the test tube, and it is shaken until the purple color is extracted into the chloroform layer. Depending on the concentration of cannabinoids, this transfer can be complete, leaving the top layer in the test tube nearly clear. Potential false positives occur in the presence of some coffees and other oils, but, as with most color-based presumptive tests, an experienced analyst will often recognize a false positive as being subtly different from a true positive. See the color insert for photos of the Duquenois test.

In some laboratories, the analysis stops with the Duquenois–Levine test, but many add TLC with standards as the final step, using the dye Fast Blue B (which is carcinogenic), or Fast Blue BB or the Duquenois reagent as a developer. Fast Blue B gives the constituents distinctive colors: Δ^9-THC turns red, CBN purple, and CBD orange. The combination of microscopy, the Duquenois–Levine test, and TLC with standards is usually considered conclusive identification for marijuana. Quantitation of THC is currently not required in the United States.

On the Stand (Legal Issues and Questions): Because the severity of the crime and applicable penalties depend on the weight of the marijuana seized, the issue of total weight (aggregate weight) is central. Threshold weights in the context of uncertainty estimation are the types of cases where weighing and uncertainty estimations become critical. The stalks of the marijuana plant are not always controlled; definitions vary by state. Similarly, wet or rotting plant matter will have a higher weight than the same

material fresh or dried. Laboratory policy and procedures (including proper notation in laboratory notes and reports as appropriate) tempered by applicable local statutes, if any, should be followed.

Applying the Science 2 The Importance of the Visual Examination

At first glance, the candy bar might seem innocent, but closer inspection of the label and contents reveal that this is no ordinary confection. Note the label "For medicinal use only."

Oklahoma State Bureau of Investigation

Oklahoma State Bureau of Investigation

Images courtesy of the Oklahoma State Bureau of Investigation.

Another question posed to chemists during their testimony deals with the lack of an MS or IR identification of THC. The issue is raised in part because many attorneys who work on drug cases are familiar with those techniques and are accustomed to seeing some form of instrumental data to accompany visual, color, and TLC testing. A proper and defensible reply to such challenges is that to a reasonable degree of scientific certainty, there is no plant save marijuana that possesses the combination of microscopic morphology, response to the Duquenois–Levine test, and TLC behavior documented, assuming that a validated method has been used and that proper appropriate quality control procedures are followed. Finally, the limited acceptable medical use of marijuana has been employed as a defense. Again, the forensic chemist should testify only to his or her area of expertise: The *reason* for possession or use is not related to analytical chemistry.

1.3 Human-Performance Drugs: Anabolic Steroids

The analysis of drugs abused in sports—particularly toxicological analysis—has evolved into a discipline of its own. Because many of the banned substances are not controlled (**caffeine** above a threshold concentration is a violation, for example), the chemistry and analysis are unique. Once **anabolic steroids** and other performance-enhancing substances were added to the Controlled Substances Act in 1991, what had principally been a toxicological concern became a drug analysis issue as well, since physical evidence could now be seized and submitted to crime laboratories (Figure 9 and Table 2).

Steroids are a class of biological compounds that include hormones. Steroids contain large fused ring structures classified as simple fats, which means that, unlike other

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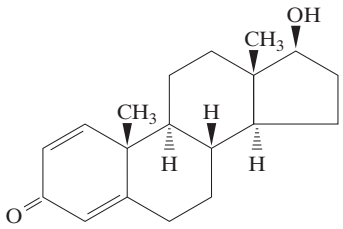
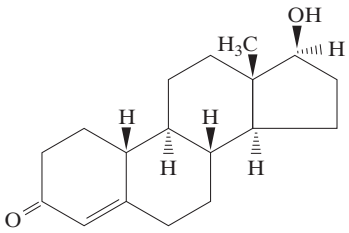
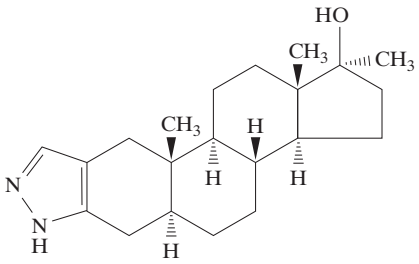
FIGURE 9 A syringe exhibit containing mestanolone testosterone propionate. Image courtesy of the Oklahoma State Bureau of Investigation.

fats, they will not undergo hydrolysis. The fused ring structure is grossly similar to that of THC, in that there are no nitrogen groups, and both are oily substances. Some subgroups of steroids are not of forensic concern. The corticoids (andrenocorticals), for example, secreted by the adrenal cortex, are referred to as the cortisones or simply "cortisone." These substances are used to treat itching and inflammation, and pain. Aspirin also reduces inflammation but is classified as a nonsteroidal anti-inflammatory drug (NSAID); cortisone is a steroidal anti-inflammatory drug. Other drugs in this category include prednisone and hydrocortisone.

The bodybuilding steroids include testosterone and related anabolic steroids. Anabolic substances promote secondary male sex characteristics that improve athletic

TABLE 2 Selected Steroids Encountered as Physical Evidence

Compound	Structure
Boldenone	
Nandrolone	
Stanozolol	

performance by increasing muscle mass and promoting a fast recovery between workouts. Illicit steroids can be synthesized or diverted and are available via mail order on the Internet (Figures 10 and 11). Because these substances are sex hormones, abuse can lead to excessive aggression (**'roid rage**), the development of male characteristics in females, liver damage, sterility, acne, and increased cholesterol levels (Figures 12 and 13).

The analysis of steroids is complicated by the similarity of the drugs' structures but can be achieved with standard forensic analysis tools.[17–18] Color tests with ammonium

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FIGURE 10 Tablets containing methandrostanolone. Image courtesy of the Oklahoma State Bureau of Investigation.

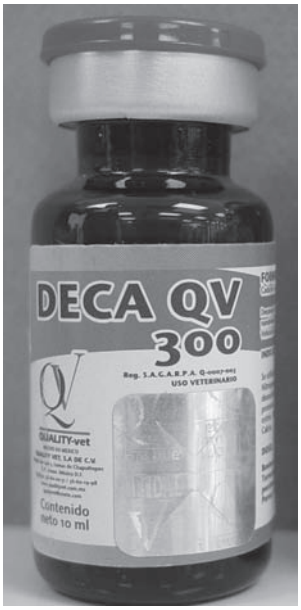

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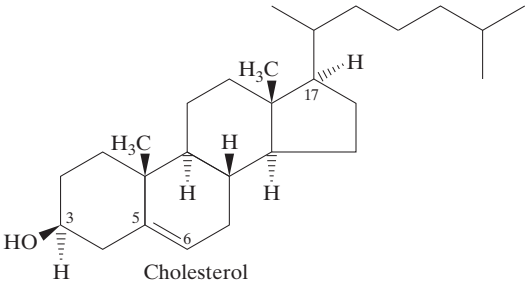
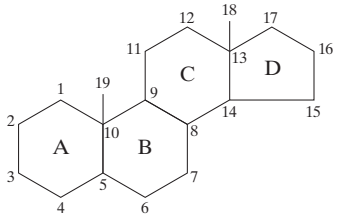
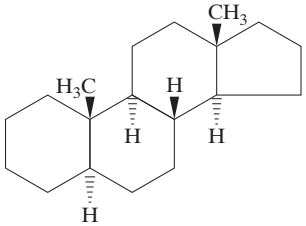
FIGURE 11 Nandrolone decanoate solution, an example of a diverted drug. This solution is a veterinary preparation likely from Mexico. Image courtesy of the Oklahoma State Bureau of Investigation.



Cholesterol





Androstane

FIGURE 12 Steroids are biologically derived from cholesterol, and the ring-numbering scheme used to describe these compounds is shown. Androstane is an example of a steroid molecule with a methyl group at C19, an important position for classification.

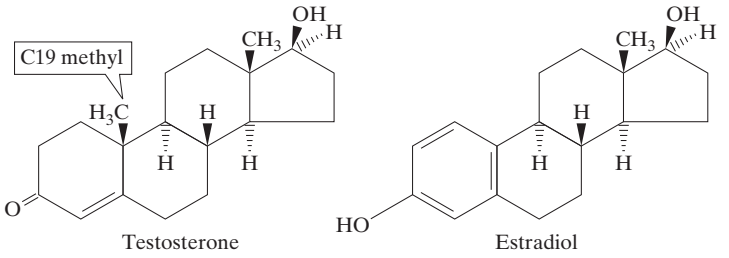


FIGURE 13 The male sex hormone testosterone and the female sex hormone estradiol. Bodybuilders use testosterone or other steroids that encourage male secondary sex characteristics, such as increased muscle mass.

vanadate (Mandelin's reagent) has been reported to be the most versatile,[18] but the form of the exhibit and the matrix, such as an oil or a cream, can complicate preliminary testing. A hexane–methanol extraction of the oil-based steroidal preparations has been reported to be a useful method of sample preparation.[18]

1.4 Other Acidics of Note

Had this text been written 25 years ago, an entire section would have been devoted to the **barbiturates,** which are sedatives and hypnotics derived from barbituric acid (Figure 14). However, the introduction of benzodiazepines, tricyclic antidepressants (TCAs), and SSRIs such as Paxil® and Prozac® has reduced the use and abuse of the barbiturates. The other class of acids the forensic chemist can expect to see includes **salicylates** (aspirin) and other analgesics with acid functionality, such as ibuprofen. These are not controlled substances but are encountered as fakes and adulterants.

2 BASIC DRUGS

Basic drugs elicit a variety of physiological effects. Stimulants such as methamphetamine can act as hallucinogens at higher doses, whereas the opiate alkaloids are analgesic and promote general depression of the central nervous system. Many natural products, such as coca leaves, **peyote**, and **khat** leaves, have been used for hundreds of years. Some—for example, LSD and mescaline—are associated with religious explorations or celebrations.

A large contingent of the basic drug family consists of stimulants, which humans have been using for centuries. Their desirable effects include the ability to work longer and harder with increased alertness. Many would give just those reasons for drinking their morning cup(s) of coffee or tea, and what student has not pulled an all-nighter fueled by some form of caffeinated beverage? Like caffeine, many stimulants have a history of accepted use, and some, such as khat, are integrated into cultures. Structurally, stimulants such as the amphetamines, mescaline, and the **tryptamines** (psilocin and psilocybin) are similar to the **neurotransmitters** dopamine and serotonin. Many stimulants produce a continuum of effects, beginning with increased alertness, passing through elevated heart rates and agitation, and ending in hallucinations. Some, such as methamphetamine and related compounds, are easily synthesized by amateur chemists; hence, we will delve in detail into how clandestine chemists prepare these substances and their precursors.

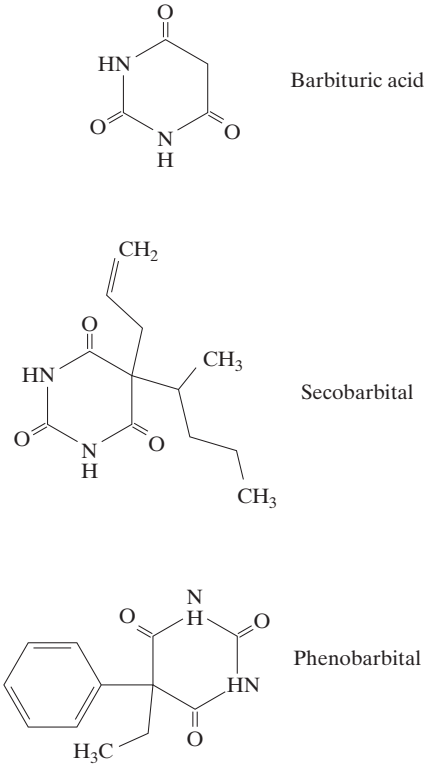


FIGURE 14 Barbituric acid forms the skeleton of the acid barbiturates. Also shown are secobarbital and phenobarbital.

EXHIBIT A

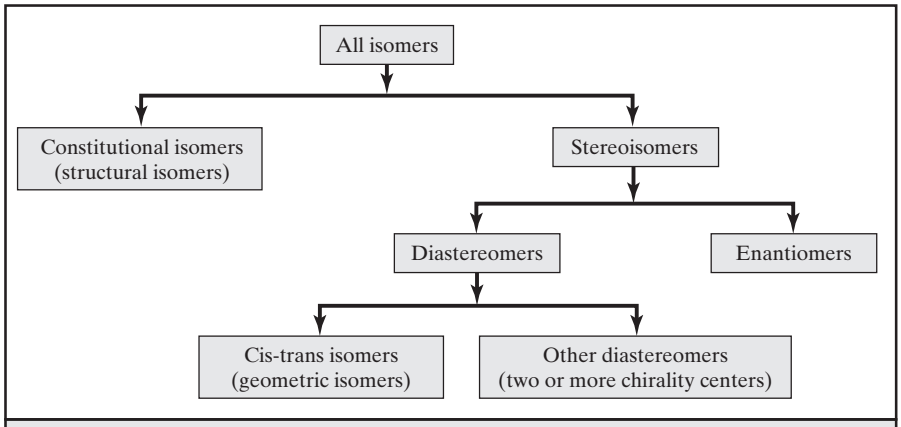
"One Person's Coffee Is Another's Dope"

The definition of a dangerous drug depends as much on social, political, and economic factors as it does on pharmacology and chemistry. One recent example is the drug khat. Khat is a leaf that is chewed, much as coca leaves (the source of cocaine) once were, to provide energy. In parts of Africa and the Middle East, khat is socially accepted just as caffeine is accepted in the West. Both caffeine and khat can be abused; however, at present, coffee has not been placed on the list of controlled substances, whereas the ingredients of khat are on the list. As different nationalities and ethnic groups migrate to the West, substances that are illegal and rarely encountered now will become more common as they filter out of migrant communities and into the general population. Thus, khat, a negligible problem 20 years ago in the United States, is now a controlled substance of concern. An analogy would be for an American to emigrate to a country where coffee and tea are illegal. The situation with khat emphasizes the social and cultural aspects of drug control.

Andrew McConnell/Alamy

There are several categories of basic drugs in which the stereochemistry is important. Knowledge of the stereochemistry of basic drugs, particularly the tropane alkaloids and the methamphetamine family, is essential to understanding synthesis, precursors, and legal questions. A summary of terms is given in Figure 15; for a detailed review, refer to a textbook on organic chemistry, such as any of those listed in "Further Reading" at the end of the chapter.

An asymmetric carbon (also called a *chiral* carbon) is a carbon atom bonded to four different groups. The presence of an asymmetric carbon lends a molecule chirality, and the asymmetric carbon is referred to as the chiral center. Nitrogen atoms, which are key atoms in basic drugs, can also be chiral centers. To describe the arrangement of groups around a chiral atom, the Cahn–Ingold–Prelog convention assigns priorities to the groups bonded to the chiral atom. These rules can be found in most organic textbooks. The lowest-priority group is oriented so that it points away from the viewer (Figure 16), and the direction of a curved arrow drawn through the groups determines the rotation of the group. If the direction of rotation is clockwise, the chiral center is assigned the *R* (Latin *rectus*, for "right") designation. If the rotation is counterclockwise, then the chiral center is designated *S* (Latin *sinister*, for left).



Isomers are different compounds with the same molecular formula.
Constitutional isomers are isomers that differ in the order in which atoms are bonded together. Constitutional isomers are sometimes called **structual isomers** because they have different connections among their atoms.
Stereoisomers are isomers that differ only in the orientation of the atoms in space.
Enantiomers are mirror-image isomers.
Diastereomers are stereoisomers that are not mirror images of each other.
Cis-trans isomers (geometric isomers) are diastereomers that differ in their cis-trans arrangement on a ring or double bond.

FIGURE 15 Definitions and flowchart for stereochemistry.

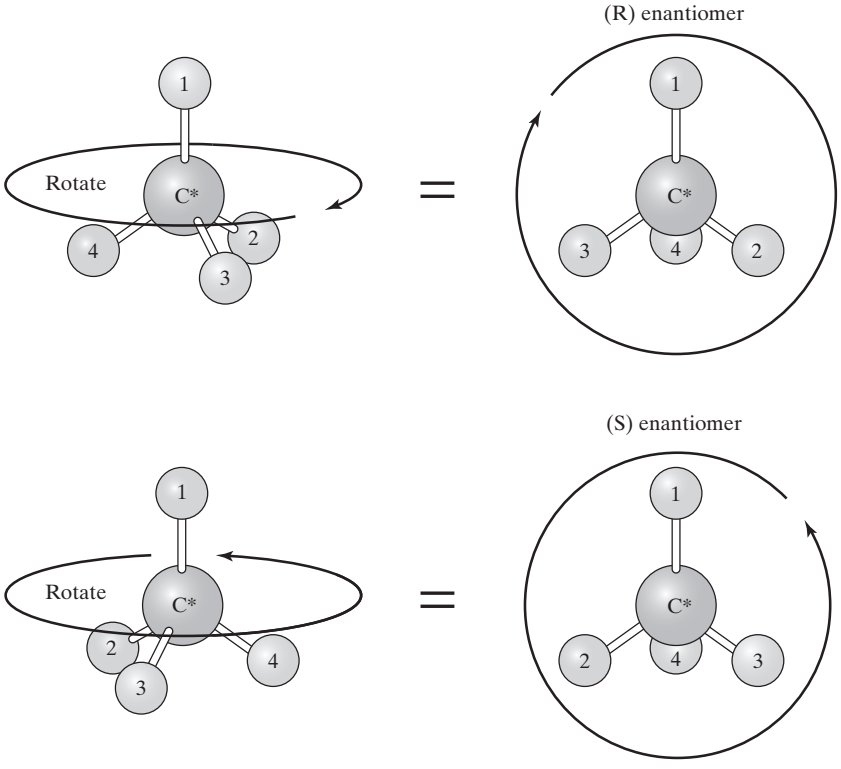
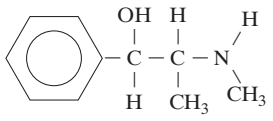


FIGURE 16 The *R*, *S* naming conventions, shown in three dimensions. The numbers refer to the priority of the substituent.

The *R* and *S* nomenclature is related to, but not synonymous with, two complementary nomenclatures used to describe chiral centers. When polarized light propagates through media containing chiral atoms, the plane of rotation shifts slightly. **Enantiomers** of the same compound will shift the rotation in different directions, a property that provides a laboratory technique for differentiating among enantiomers. For this reason, enantiomers are referred to as **optical isomers**. Apolarimeter is used to measure the direction of rotation, which is designated as *d* or + for dextrorotatory (to the right) or as *l* or − for levorotatory (to the left) enantiomers. Many syntheses produce mixtures of *d* and *l* isomers, which are referred to as racemic mixtures. It is common for optical isomers of drugs to have different activities and effects. For example, the **dissociative anesthetic** ketamine, discussed in a later section, has an *R* and an *S* form; the *R* isomer tends to cause hallucinations, whereas the *S* form has anesthetic properties.

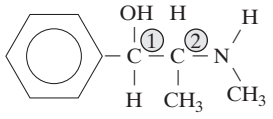
EXAMPLE PROBLEM 1

Ephedrine, a precursor to methamphetamine, has the following structure:



Answer. How many stereoisomers are possible?

First, identify the chiral centers:



Carbons 1 and 2 each have four different groups attached.

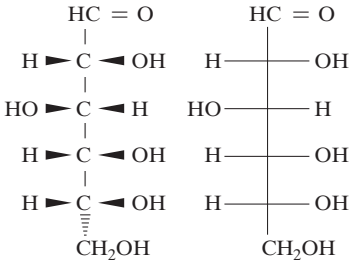
Each chiral center has two configurations, R or S, so the possible combinations are:

①	②
R	R
S	S

(R, R), (R, S), (S, R), (S, S)
four stereoisomers

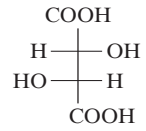
One other notational convention is used in describing enantiomers: the D,L convention, which is distinct from the *dl* system just presented. The D,L convention describes the configuration of groups around a chiral center relative to another molecule, rather than in absolute terms as is done in the *R*,*S* system. The D,L system is still used to describe sugars (carbohydrates) and amino acids. Most natural sugars are of the D

form, and most amino acids are of the L form. Briefly, to determine the D,L configuration of a carbohydrate or an amino acid, the molecule is drawn by means of the Fischer projection method (Figure 17) with the terminal carbonyl group oriented upward. The D,L designation is assigned on the basis of how substituents are arranged when the molecule is drawn in accordance with the Fischer projection method. An example is shown in Figure 18.

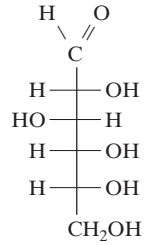


Wedge-and-dash structure | Fischer projection

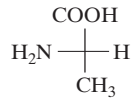
FIGURE 17 The "wedge-and-dash" representation of the groups around a chiral carbon indicates where each group points in space. The Fischer projection is used to orient a carbohydrate molecule such as a sugar. Both projections can be used to derive the *R*,*S* configuration.



L−(+)-tartaric acid
(2R, 3R)-tartaric acid



D−(+)-glucose

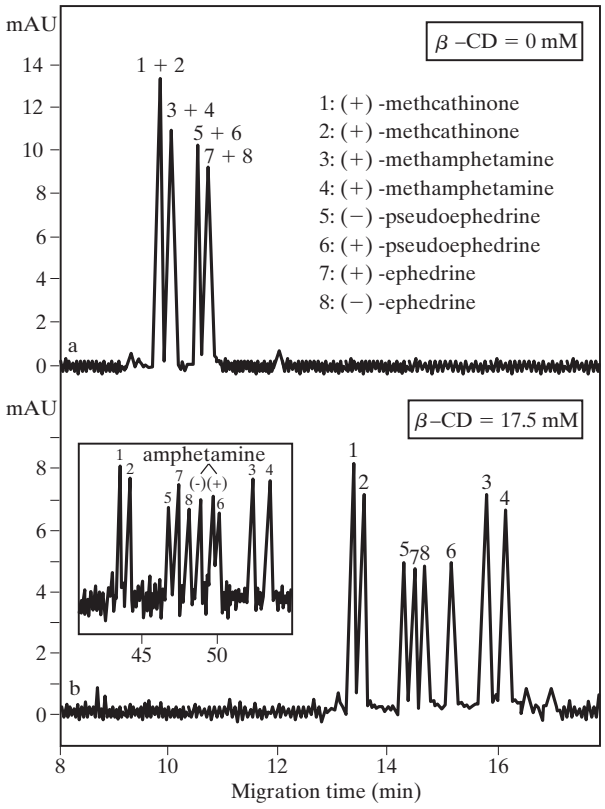


L−(+)-alanine

FIGURE 18 Examples of compounds drawn as Fischer projections and indicating *R*,*S*, D,L, and *d*,*l* (+, –) directionality. Note that the *R*,*S* configuration does not dictate how a molecule will rotate plane- α polarized light.

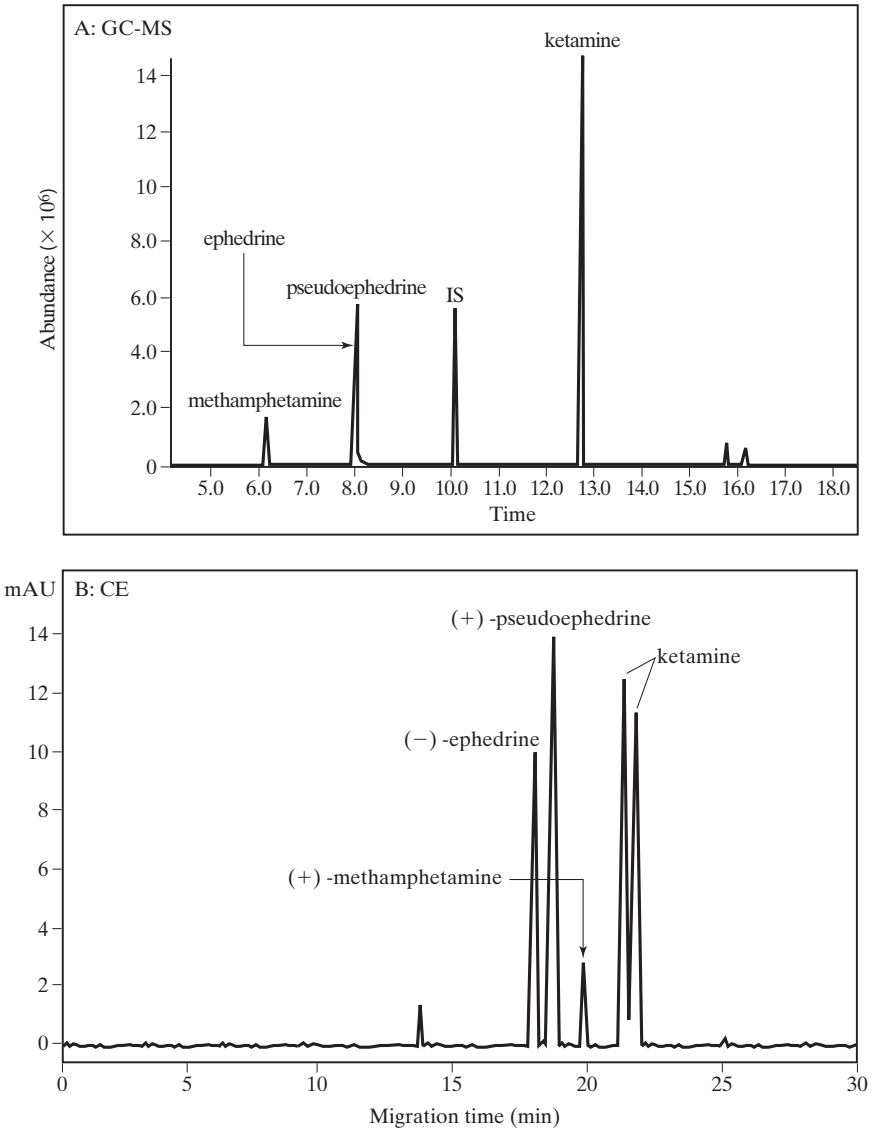
Applying the Science 3 Separation of Enantiomers

The stereochemistry of drugs and precursors can be vital in determining synthetic routes. However, since enantiomers are nearly chemically identical, standard chromatographic methods cannot separate them. To effect a chromatographic separation of enantiomers,



UV electropherograms of (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine and (±)-pseudoephedrine standards. The sample concentrations were 25 ppm. CE conditions: capillary, 58.5 cm (50 cm to detector); detection wavelength, λ_{ab} = 210 nm. Running buffers: (a) 150 mM H3PO4; water:acetonitrile = 95:5 (v/v); (b) the same buffer as described earlier and the addition of 17.5 mM b-CD. The inset shows the separation of (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine, (±)-pseudoephedrine, and (±)-amphetamine standards when an 80-cm capillary was used. Reprinted from reference 3 cited below, copyright 2003, with permission from Elsevier.

(*Continued*)



(A) GC-MS chromatograph of a tablet extract; (B) CE electropherogram of the same tablet extract. Reproduced with permission from reference 3 in sources; copyright 2003 Elsevier Science.

stereospecific interactions have to be incorporated. The use of chiral stationary phases is one method of discriminating enantiomers; micellar electrokinetic chromatography (MEKC) using chiral cyclodextrins is another. Both of these protocols have been applied to methamphetamine and related compounds and precursors, substances that will be discussed in detail later in the chapter.

Cyclodextrin is a chiral molecule with a small cavity into which molecules partition as the mobile phase moves. The degree of partitioning is partially controlled by chirality, so enantiomers can be separated as long as there is sufficient contact time between analytes and the cyclodextrin and as long as the concentration of cyclodextrin is optimized. Although the separation via MEKC is impressive, the problem of detection remains. The great advantage of

GC-MS is its ability to separate and provide definitive identification, a capability that is absent in traditional MEKC detection devices. Combining data from both methods is possible, but MEKC has yet to make significant inroads into forensic laboratories.

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Note that the configuration around a chiral atom (in *R,S* or D,L notation) does not define the direction in which polarized light will be rotated. In other words, a D configuration does not necessarily correlate with *d* (or +) rotation of polarized light. The direction of rotation is a physical property, not a description of the bonding around the chiral center. Molecules may have more than one chiral center, complicating both the notation and the interaction of the molecule with polarized light. Finally, stereochemical aspects of syntheses can be important in forensic chemistry, as will be emphasized when we discuss the methamphetamine family. An example is shown in Figure 19.

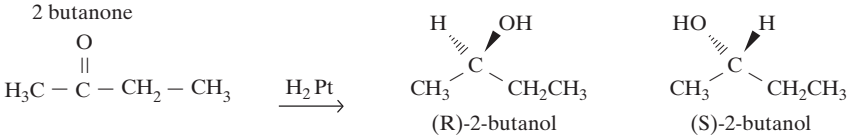
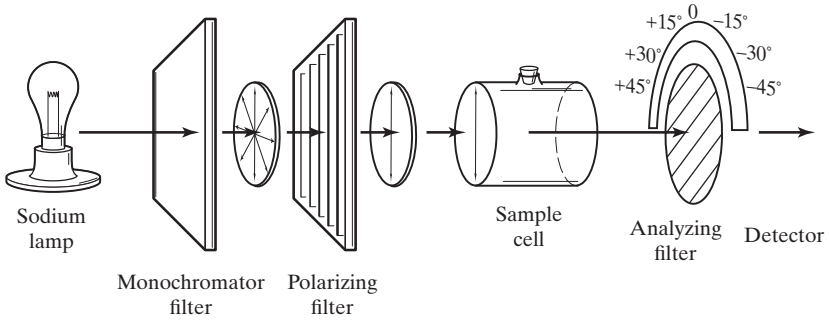


FIGURE 19 Example of how a reaction can create a racemic mixture.

EXHIBIT B

Polarimetry

The direction in which a substance rotates plane polarized light is a physical property that can distinguish isomers from one another. The rotation is measured by a device called a polarimeter, which is based on the relation to polarized light microscopy. A polarimeter uses an intense light source, such as a sodium lamp. The light is polarized with a polarizing filter (corresponding to the polarizer in a PLM scope) that orients the light so that it vibrates in only one plane. The polarized light interacts with the sample, and the plane of rotation may be altered to the left or right. An analyzing polarized filter, analogous to the analyzer of a polarizing light microscope, is rotated such that the light signal is the same as the original polarized light beam, and the angle of rotation is read. If there is no interaction with the sample, the plate does not have to be turned at all.

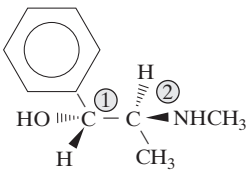


Operation of a polarimeter.

EXAMPLE PROBLEM 2

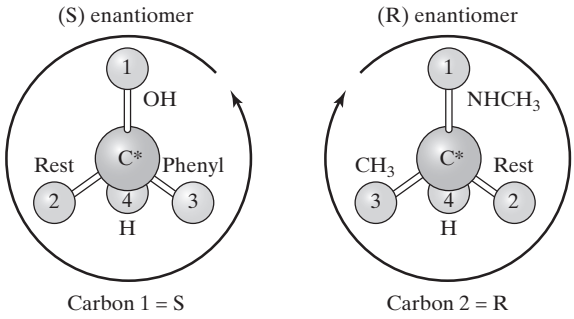
Below is the pharmacologically active stereoisomer of ephedrine.

What is its designation?



Answer

Around each chiral carbon, assign priorities to attached groups using the Cahn-Ingold-Prelog convention. H is the lowest priority in both cases.



Active form is thus: (S, R)-ephedrine

3 ALKALOIDS

3.1 Opiate Alkaloids and Heroin

The **opiate alkaloids** are a mix of semisynthetic and synthetic compounds derived from or related to the extract of the unripe seed pods of the opium poppy *Papaver somniferum* (Figure 20). Specifically, the alkaloids are obtained from the **latex**, or milky, exudates of the seed pod that appear when it is cut or sliced. The liquid contains about 10% morphine and about 1.5% codeine, with various amounts (~0.2%–8%) of papaverine, thebaine, and noscapine. The majority of the family has the character of both a base and a phenol, leading to interesting and complex chemistry (Figure 21). The hydroxide associated with carbon 3 is more active than that associated with carbon 6, a property that is useful in profiling, synthesis, and degradation studies.[19] The poppy plant is also the source of poppy seeds used in baking.

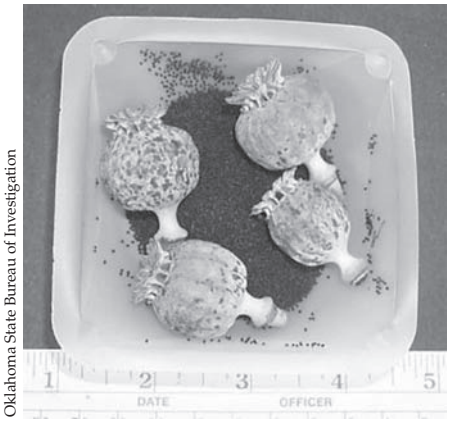


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FIGURE 20 Opium poppies and poppy seeds. Image courtesy of the Oklahoma State Bureau of Investigation.

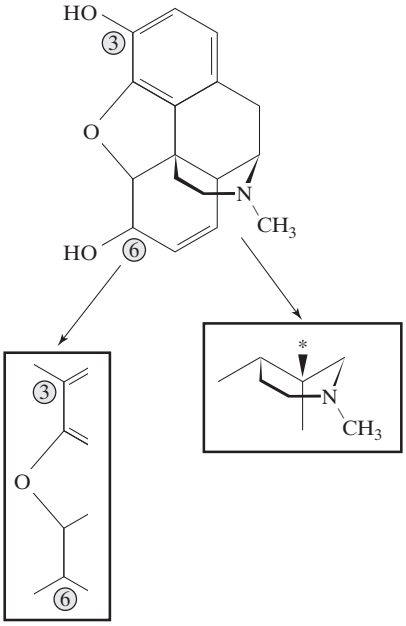
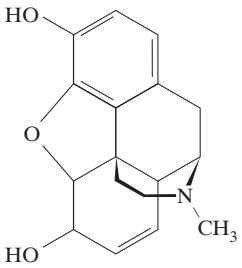
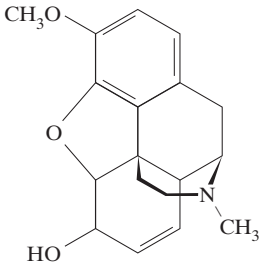


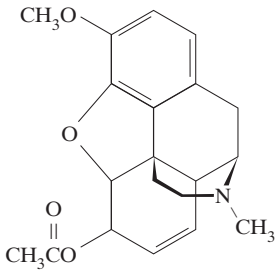
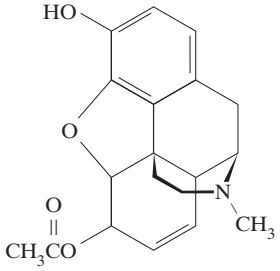
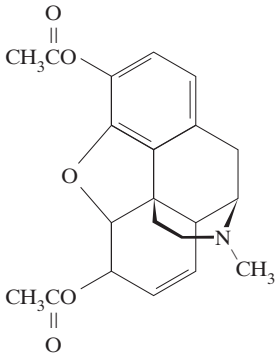
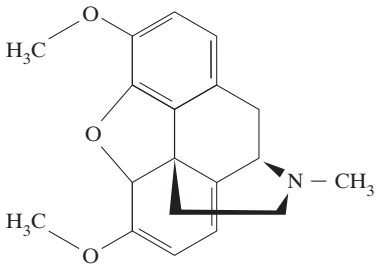
FIGURE 21 The functional groups and atoms of opiate alkaloids. The number three carbon 3 is more important in terms of chemical reactivity than the carbon 6 and the carbon with the asterisk is the site that differentiates oxycodone from other family members.

TABLE 3 Opiate Alkaloids

Compound	Structure
Morphine	
Codeine	

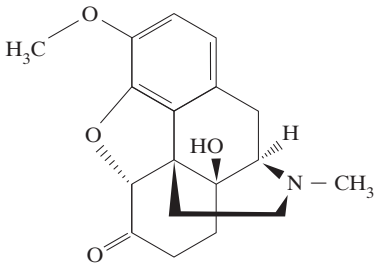
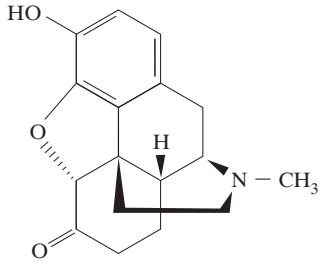
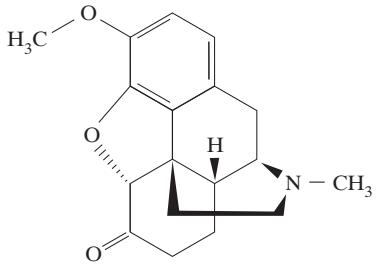
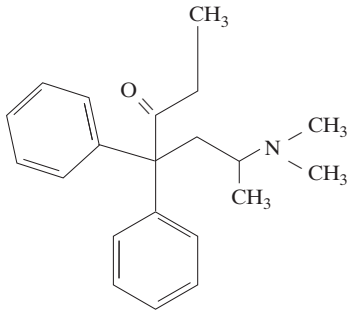
(continued)

TABLE 3 Opiate Alkaloids *(continued)*

Compound	Structure
Acetylcodeine	
6-O-Monoacetyl morphine (6-MAM) 3-O-MAM is analogous	
Diacetylmorphine (heroin)	
Thebaine	

(continued)

TABLE 3 Opiate Alkaloids *(continued)*

Compound	Structure
Oxycodone	
Hydromorphone	
Hydrocodone	
Methadone	

Morphine has a long history of use and abuse. It was the first so-called vegetable base drug isolated, an event recorded in 1805.[20] Because morphine is basic, an early quantitation method was titration with an acid. Morphine is subject to oxidation, so the morphine content of an extract can decrease after harvest. **Codeine**, the 3-methyl ether of morphine, whose first extraction was recorded in 1832, is also found in the raw

opium extracts.[21] Opiates are used principally for pain relief; **thebaine** is the oddball of the opiate alkaloid family, classified as a stimulant. The primary use of thebaine is as a starting point for the synthesis of oxycodone and other related semisynthetic compounds. Thebaine was also used as a poison.

The opiate alkaloids of the greatest forensic interest are **heroin** (**diacetylmorphine** or **diamorphine**, Schedule I), codeine (II and IV), morphine (II), **hydrocodone** (Lortab®/Vicodin®, II), **hydromorphone** (Diluadid®, II), and oxycodone (Percocet®, OxyContin®, II). Thebaine (II) is a precursor to these substances. As a group, the opiate alkaloids are analgesics (painkillers), depressants, and narcotics; all are addictive to varying degrees. Their mechanism of action is related to their chemical similarity to the class of endogenous compounds known as **endorphins**, *endo*genous mo*rphin*es. Endorphins are large molecules compared with the opiate alkaloids and proteins involved in the transmission of nerve impulses (neuropeptides). Unlike aspirin and related analgesics, the opiate alkaloids do not act on the cause of the pain but rather interfere with the transmission of the pain impulses to the brain.

Morphine is one of the strongest analgesics known and is used to treat otherwise intractable pain, such as that associated with cancer. As a manifestation of its depressant characteristics, morphine overdoses cause death by interfering with breathing. Heroin has no acceptable medical uses in the United States, whereas codeine is used as an analgesic and cough suppressant. Formulations of codeine and analgesics are widely prescribed for short-term pain relief. The synthetic opiate alkaloids hydrocodone, hydromorphone, and oxycodone are also considered strong pain relievers; a time-release formulation of **oxycodone** (**OxyContin**, Purdue Pharma) has become one of the most commonly abused prescription drugs. Abusers crush the tablets to obtain a large dose immediately, which can be fatal. The opiate alkaloids are commonly available as sulfate or hydrochloride salts. Among the controlled opiate alkaloids, heroin is typically the most commonly encountered as physical evidence, morphine the least, a fact that is likely due to the relative simplicity of the conversion of morphine to heroin.

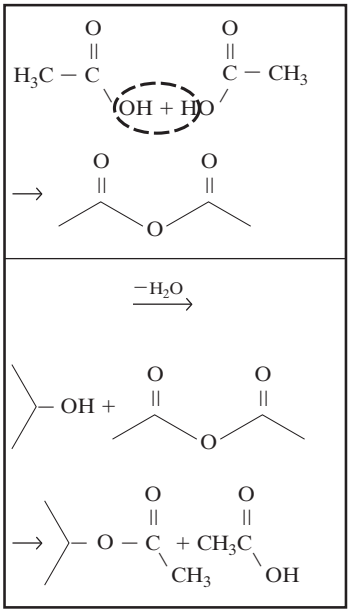


FIGURE 22 Acetic anhydride is formed when two acetic acid molecules combine. Water is lost in the reaction. Acetic anhydride will esterify alcohol functional groups as shown, producing acetic acid.

Chemistry: The first step toward making heroin is extracting morphine from the opium plant extract. Morphine base is the immediate precursor of heroin. The conversion of morphine to heroin involves acetylation of the hydroxyl groups on carbons 3 and 6. The most common method uses acetic anhydride and heating for this purpose (Figure 22).[21]

Acetic anhydride is produced by the linkage of two acetic acid molecules, with water lost during the reaction (Figure 22). Anhydrides are versatile synthetic reagents that will react with alcohols and —OH groups, such as those found in morphine, to form an ester and acetic acid. Once the heroin is produced, the solution is made basic to precipitate heroin base. A series of recrystallizations and purification steps, coupled with the addition of HCl, yields the hydrochloride salt of heroin.

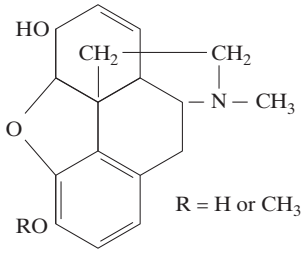
The —OH group attached to the carbon in the 3-position is more reactive than that associated with the carbon in the 6-position. Accordingly, the C3 reacts first. By contrast, when degradation occurs, it generally occurs at C6.[19] Another site of variation is central in the molecule; an OH group in this position is found in oxycodone. The 3- and 6-positions typically follow the chemistry of alcohols.

Starting from morphine, heroin can be made in good yields (typically 50% or greater) by acetylation, using acetic anhydride and heat. The process is illustrated in Figure 23. Acetylation can be accomplished by **refluxing**

EXHIBIT C

Morphine

The name of the drug morphine is derived from *Morpheus*, the God of dreams in Greek mythology. Morpheus was the son of *Hypnos*, the God of sleep, who is the namesake of the hypnotic drugs. Morphine was isolated around 1805, but as late as 1947 the molecular structure was still not clear. The debate in those years related to the group at C6. The question was settled in 1952, with the first reported synthesis of morphine.



The structure of morphine as understood in 1947.

Source: Holmes, H. L., and C. C. Lee. "A Possible Route to the Location of the Nitrogen Atom in Morphine I." *Journal of the American Chemical Society* 69 (1947): 1996–97.

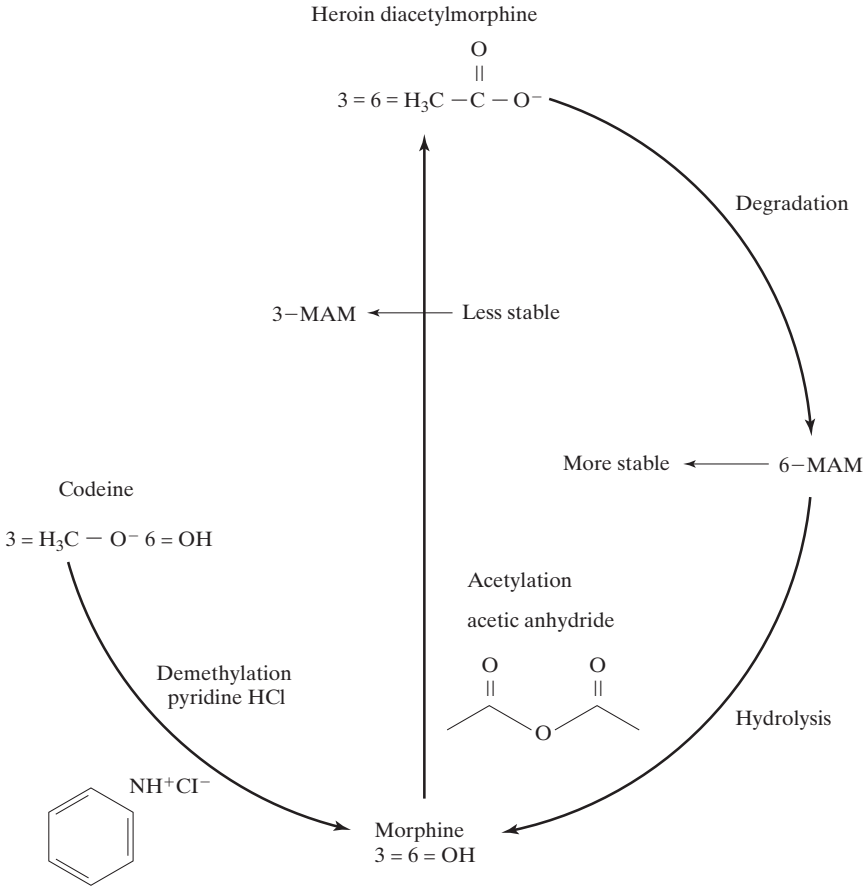


FIGURE 23 The chemistry of morphine–codeine–heroin, showing synthesis and degradation paths. The numbering is the same as in Figure 21.

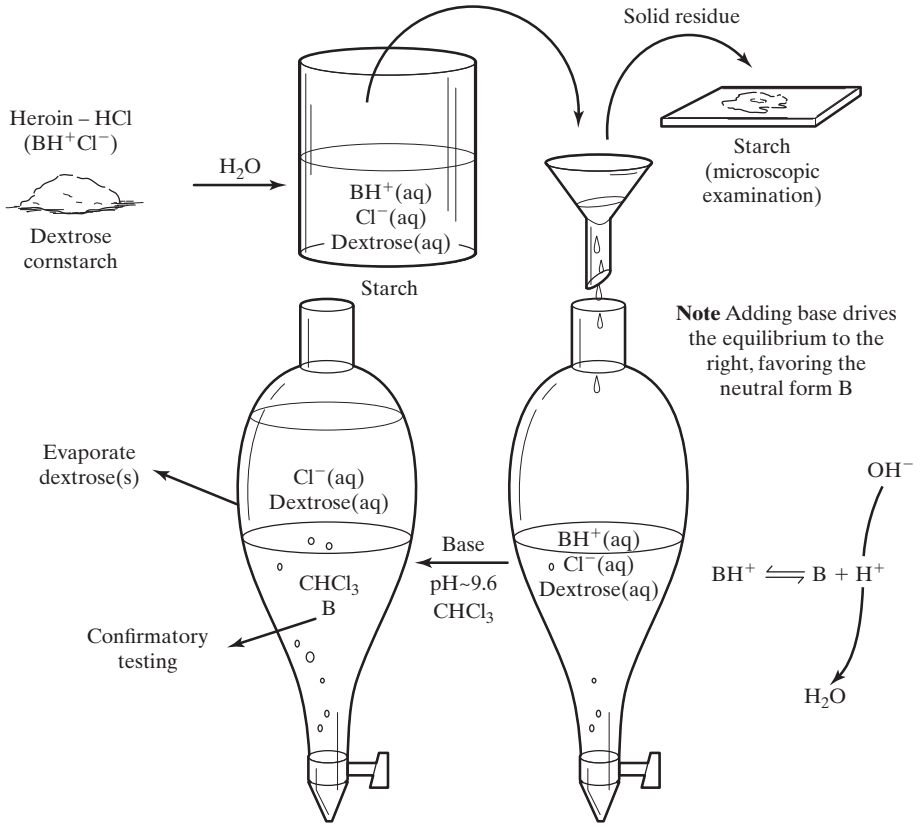
or direct ignition.[19] The hydroxyl group on C3 is preferentially acetylated, yielding 3-O-monoacetylmorphine, which is usually found in heroin manufactured this way. Also present will be residuals of codeine, acetylcodeine, papaverine, noscapine, thebaine, and acetylthebaol.[21,22] Once the heroin has been made, a small portion will partially degrade via deacetylation to form 6-O-monoacetylmorphine (**MAM**).[22] Codeine has also been used as a starting point to make heroin.[23–26] For illicit purposes, the starting point is typically medicines combining codeine and such analgesics as aspirin or acetaminophen.

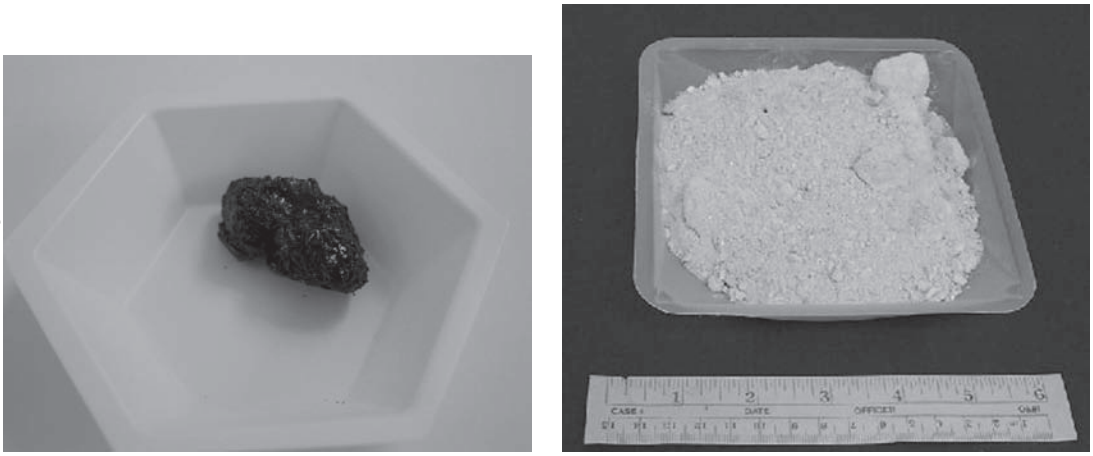
Although heroin is often injected, another mode of ingestion is inhalation, accomplished by placing powder on a piece of foil and heating it until vapors are released.[26] Under these conditions, most of the heroin is degraded to 6-MAM and associated by-products, with 6-MAM being fairly stable and 3-MAM unstable.[21,26] This behavior mirrors the natural degradation of heroin that can occur even when exhibits are refrigerated, a situation that merits attention when quantitative analysis is needed (Figure 24).

EXAMPLE PROBLEM 3

Explain how to separate a mixture containing heroin as the hydrochloride salt, dextrose (glucose), and cornstarch.

Answer:





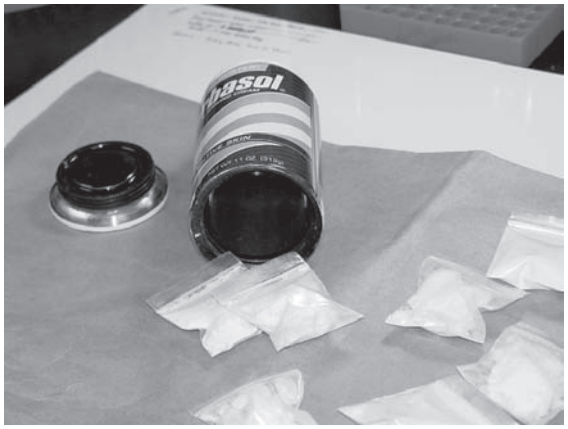
Oklahoma State Bureau of Investigation

FIGURE 24 Two heroin exhibits showing the extremes of solid forms. Image courtesy of the Oklahoma State Bureau of Investigation.

Analytical Approach: Heroin samples follow the typical flowchart approach from presumptive tests to confirmation by instrument, usually by GC-MS, since the presence of other alkaloids makes it difficult to purify the sample sufficiently for IR. Thin-layer chromatography can be particularly useful in separating components of a complex heroin sample and, coupled with the appropriate standards and developers, can assist in identification of specific compounds. Marquis and Dragendorff reagents are commonly used for the purpose.

3.2 Tropane Alkaloids and Cocaine

Cocaine is a **tropane alkaloid** (characterized by a bridge structure) obtained by extraction from leaves of the coca plant. It is an addictive CNS stimulant listed on Schedule II. Coca leaves have been chewed by people for thousands of years, and cocaine was considered to have therapeutic benefits until early in the 1900s. Although the drug can be synthesized, the overwhelming majority of cocaine that is seized is obtained by extraction from **coca paste**. Like other alkaloids, cocaine exists as the freebase, which ranges from a colorless solid to a tan to off-white and slightly oily material. The drug also exists as a salt, usually the hydrochloride. Cocaine HCl is a white powder, known on the street as "snow," among many other monikers. "Crack," or crack cocaine, usually refers to the freebase, smokable form of cocaine often sold in small quantities packaged in vials. Recent estimates are that about 1–2% of the U.S. population uses cocaine.[27] Mexico and Colombia are the principal sources of the drug (Figures 25 and 26).



Oklahoma State Bureau of Investigation

FIGURE 25 Cocaine powder found inside a shaving cream can. Image courtesy of the Oklahoma State Bureau of Investigation.

Oklahoma State Bureau of Investigation

FIGURE 26 Large bricks of cocaine. Image courtesy of the Oklahoma State Bureau of Investigation.

Cocaine is extracted from coca leaves by crushing the leaves into a mush, making the solution basic, and extracting the drug with an organic solvent such as kerosene. Bubbling HCl through the solution is an effective method for converting the base to the hydrochloride salt. The salt can be converted back to the base, to be sold as crack cocaine. Pretreatment of the leaves to convert related alkaloids to cocaine has also been noted.[28,29] One possibility is treating the leaf extract with acid to hydrolyze it to ecgonine. Esterification yields ecgonine methyl ester, which can be further treated to yield cocaine (Figures 27 and 28).

Cocaine seized in dosage-size units is typically cut with diluents such as caffeine, sugars, **procaine,** or **lidocaine**. Like cocaine, the latter are topical anesthetics and produce a numbing sensation on the tongue and mucous membranes. In fact, cocaine is listed on Schedule II because it is still used as a local anesthetic. Other cutting agents frequently seen are sugars (glucose, mannitol, inositol, etc.); starches, including flour and baking soda; lactose; and caffeine.[30]

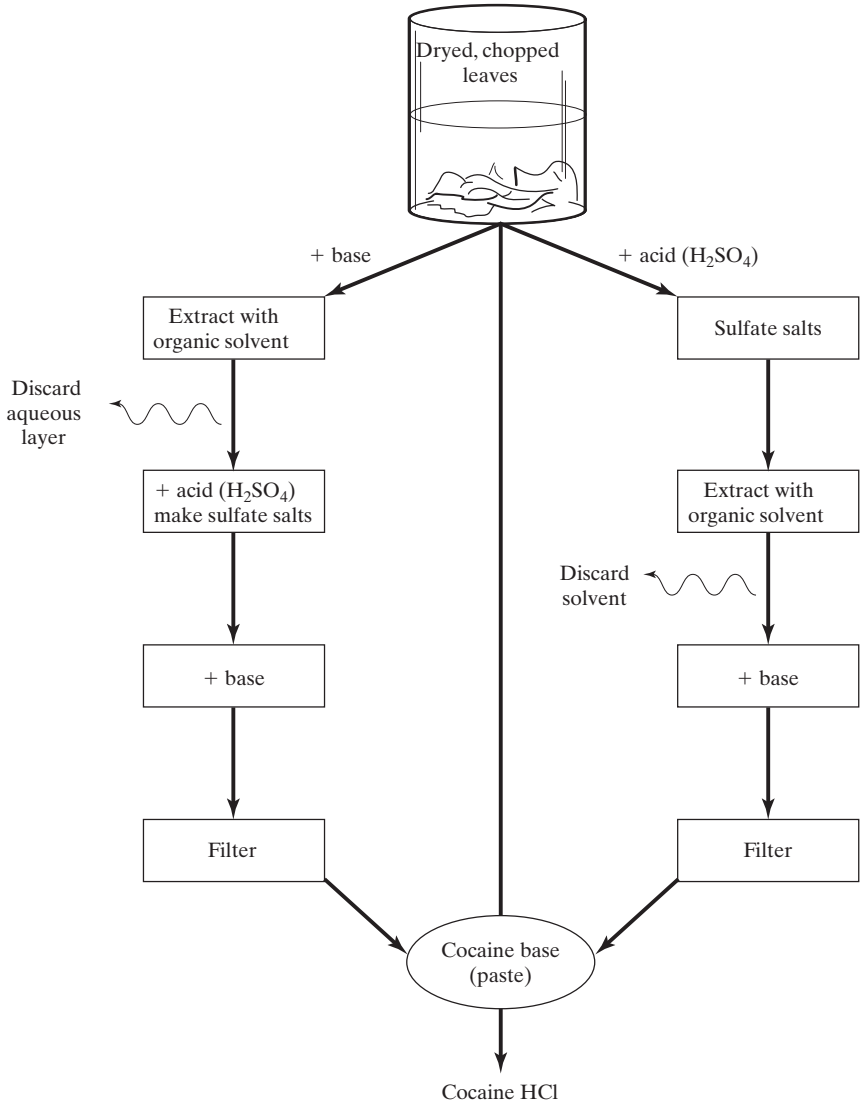


FIGURE 27 Two methods used to extract cocaine from coca leaves.

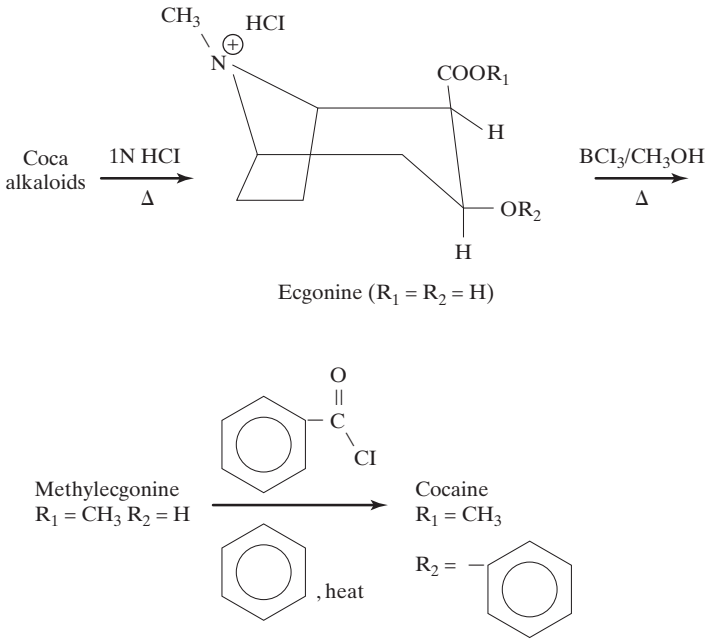


FIGURE 28 Yields of cocaine-content paste-like plant extract can be increased by treating it with hydrochloric acid to hydrolyze the extract to ecgonine. Treatment with boron chloride in methanol produces methyl ecgonine, which is converted to cocaine by the addition of benzoyl chloride and benzene.

Cocaine can be profiled with some success for categorization and **provenance** determinations.[30] Cutting agents and other trace chemical constituents, such as residual extraction solvents, impurities, contaminants, cutting agents, and a chemical profile of the plant material, can all be used for this purpose. **Bleaching agents** added to improve the coloration are also useful in profiling. One recent report described finding predominantly ethyl acetate and *n*-propyl acetate, a mixture consistent with commercial paint thinners.[30]

EXAMPLE PROBLEM 4

Assume a sample is delivered to the lab that is suspected of containing cocaine, lidocaine, benzocaine, and procaine. What would be the best method of sample extraction and preparation?

Answer:

Actually, little sample preparation is needed. All four compounds are related and have similar structures, so a LLE or SPE extraction to separate them from each other is neither feasible nor necessary. A rudimentary sample preparation, such as a dry extract, would be sufficient to allow for TLC with standards and GC-MS.

This problem is a reminder that sample preparation does not have to separate every analyte from every other one. Rather, sample preparation should extract the analytes from the matrix. If GC-MS or other hyphenated techniques will separate the analytes, that capability should be exploited. Exhaustive isolation should be undertaken only when necessary.

Analytical Approach: The analysis of cocaine exhibits is straightforward and employs color tests and instrumental methods. Cocaine is amenable to crystal tests[31] using gold and platinic chloride and can be analyzed using a number of TLC systems. Because the penalty in some jurisdictions is based on purity, cocaine samples are often quantitated. The compound and related species chromatograph well on

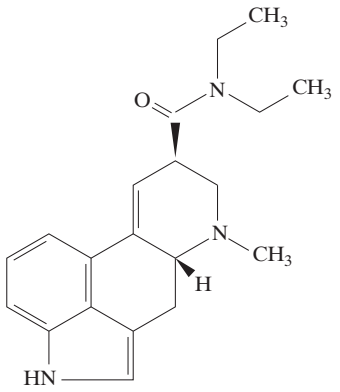
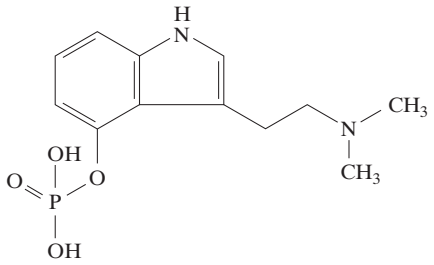
nonpolar or slightly polar stationary phases. GC-MS, along with appropriate standards, provides definitive identification of cocaine; IR spectra can also be used for the purpose. Because many cocaine samples are quantitated, GC-MS is more widely used, as it can accomplish both tasks.

On the Stand (Legal Issues and Questions): In the 1970s and 1980s, questions often arose concerning the isomers and diastereoisomers of cocaine. However, legislative wording has generally been changed to include all (optical and geometric) isomers, enantiomers, and salts thereof.

3.3 Ergot and Tryptamine Alkaloids and Hallucinogens

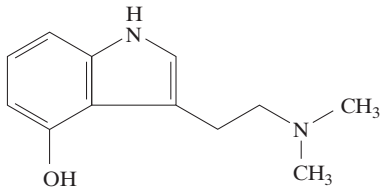
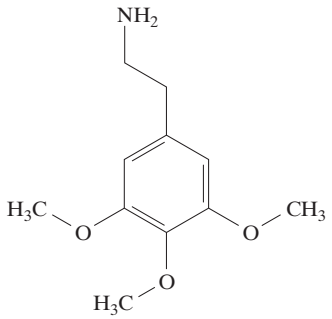
The hallucinogens are among the most dangerous of abused drugs (Table 4). Some, such as PCP ("angel dust"), are synthetic; others, such as **lysergic acid diethylamide (LSD)**, are semisynthetic; and the remainder are derived from plants. The dissociative drugs, such as **ketamine** and **PCP**, are not hallucinogenic in the same sense as LSD but create dissociative feelings described by some as "out-of-body" experiences. These two compounds are or have been used as anesthetics for this reason. Recently, MDMA (Ecstasy) has joined the ranks of widely abused hallucinogens. (See Section 4.1, which covers the amphetamine family.) LSD, although no longer a common form of hallucinogen, is arguably the most infamous. It was synthesized by Albert Hoffmann in 1938.

TABLE 4 Hallucinogens and Precursors

Compound (all Schedule I)	Structure
Lysergic acid diethylamide (LSD)	
Psilocybin	

(continued)

TABLE 4 Hallucinogens and Precursors *(continued)*

Compound (all Schedule I)	Structure
Psilocyn (or psilocin)	
3,4,5-Trimethoxyphenethylamine (mescaline)	

LSD is a derivative of a class of compounds called **ergot alkaloids**. These chemicals are compounds that are extracted from a fungus (*Clavica purpurea*) that grows on cereal grain plants, principally rye. The fungus is the source of toxins as well as the hallucinogens. The effects of **"ergotism"** (ergots as poisons) have been documented since the tenth century.[32] The immediate precursor of LSD is lysergic acid, the synthesis of which is a complex, multistep procedure; thus, LSD is much more difficult to synthesize than methamphetamine. Because of the difficulty of the synthesis, LSD laboratories tend to be larger, more centralized, and harder to find, compared with those that produce methamphetamine.

In addition to marijuana, another form of plant-based drug exhibit seen in crime labs contains **mescaline**. This hallucinogen is obtained from the peyote (peyotl) cactus (*Lophophora williamsii*). Psilocyn (**psilocin** is also an accepted spelling) and **psilocybin (psilocybine)** are obtained from the mushroom *Psilocybe mexicana* and are members of the indole amine family referred to as tryptamines. The structure of psilocin in particular is similar to that of dopamine.

Peyote is an interesting case in that it is legal for use by members of the Native American Church. Although mescaline can also be prepared synthetically, most evidence submitted to laboratories is in plant form. Psilocin mushrooms have the longest-known history of use; they were first noted as part of religious ceremonies, a common theme for the hallucinogens (Figure 29). Psilocin is the physiologically active ingredient, but the body converts psilocybin to psilocin via dephosphorylation after ingestion.

Oklahoma State Bureau of Investigation

FIGURE 29 Mushrooms (psilocybin and psilocin). Image courtesy of the Oklahoma State Bureau of Investigation.

EXHIBIT E

Purple Haze

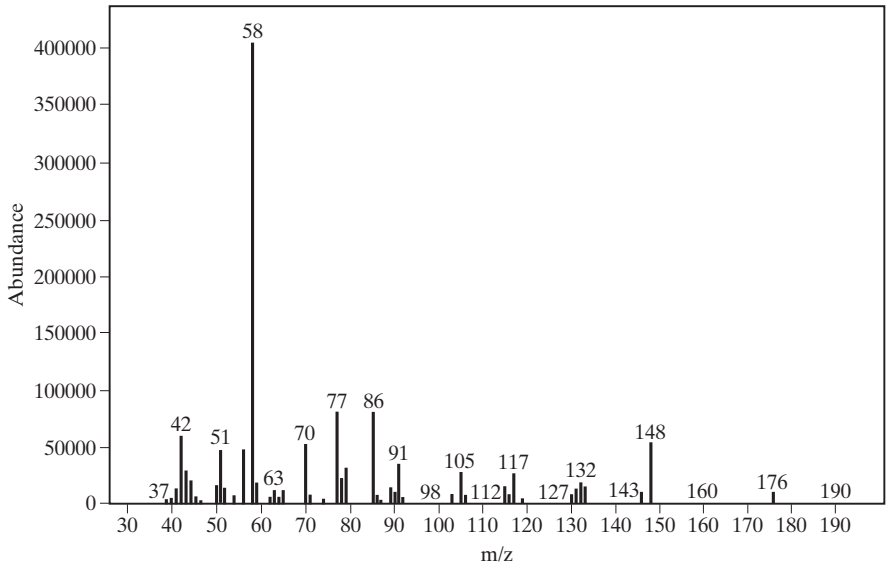
Few drugs have had the impact on popular culture that LSD had in the 1960s and 1970s. Dr. Albert Hoffmann, the discoverer of LSD, inadvertently took the first known "trip" in 1943. Intrigued, he performed a self-experiment, ingesting 250 μg. The typical street dose tops out at around 50 μg. Hoffmann experienced hallucinations and what he termed a "severe crisis." He described out-of-body type experiences and "demonic transformations." He later coined LSD his "problem child." It was subsequently embraced (secretly) by intelligence agencies in the 1950s and early 1960s, but the experiments they performed were eventually abandoned. In the late 1950s, Timothy Leary, a Harvard psychologist, began experimenting with the drug as an aid to therapy. He became its most vocal and, eventually, most famous advocate. The nature of the "trip" was touted to have religious and spiritual overtones much like those taken in older cultures by other psychedelics, such as mescaline. The LSD fad had peaked by the 1970s but left cultural icons in its wake; the songs "Lucy in the Sky with Diamonds" (the Beatles) and "Purple Haze" (Jimi Hendrix) and other works by artists such as the Grateful Dead and Bob Dylan have been linked to the drug, even if speculatively. The novel ***One Flew over the Cuckoo's Nest*** was penned by a man who had been part of a government study on LSD.

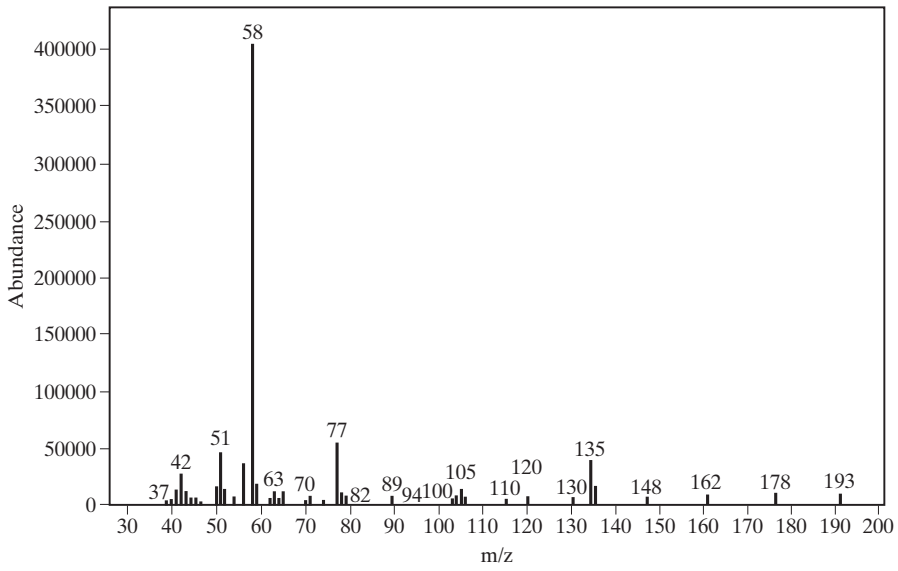
Analytical Approach: LSD as physical evidence is submitted in forms such as blotter papers, gelatin windows, candies, mints, and, less frequently, pills. The potency of the drug creates both analytical challenges and safety concerns, since the effective dose of LSD is quite small. One of the useful characteristics of LSD and related compounds is their strong fluorescence under UV light, which provides a quick presumptive test, as well as a useful property for sample preparation and cleanup. This fluorescence property also makes spectrofluorometry useful, although such instrumentation is not common in forensic labs. Hyphenated methods incorporating fluorescence based on HPLC, capillary electrophoresis (CE), and related methods are appearing in the literature, but it is unlikely that these methods will find routine use in laboratories in the near term.[33,34] Given the low dosage size, HPLC coupled to tandem MS is promising. Ehrlich's reagent provides a good color test, but dyes in the pills or papers may obscure the results. The reagent is useful as a TLC developer for LSD samples. Gas chromatography of LSD is difficult, given low concentrations and reactivity issues, although slow-temperature ramp methods can be employed. Typically, IR is the preferred confirmatory technique when a sufficiently large sample can be extracted to produce a good spectrum.

Applying the Science 4 Good, Not Perfect

GC-MS is central to forensic drug analysis because analytes are separated and definitively identified by the combination of their retention time and mass spectrum. Unfortunately, there are compounds with nearly identical mass spectra. A partial list of compounds of forensic interest includes the following:

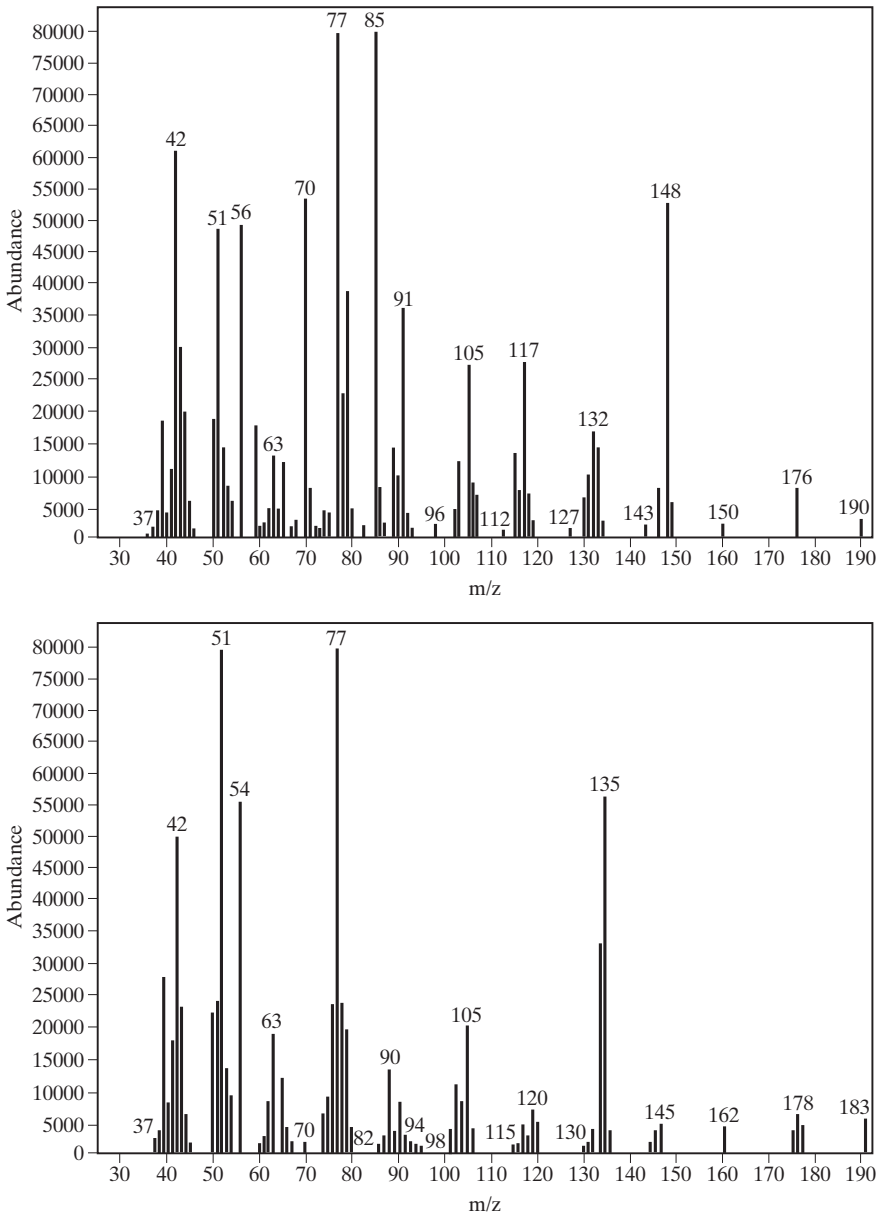
Compound 1	Compound 2	Notes
GHB	GBL	Heat causes GHB→GBL
Psilocin	Psilocybin	Heat causes dephosphorylation
Cathine	Phenylpropanolamine	
Ephedrine	Pseudoephedrine	Diastereoisomers





Original spectra for pseudoephedrine and MDMA. Reproduced with permission from the reference cited, copyright 2004 ASTM International.

As noted in Applying the Science 1, there are separation techniques that will discriminate between enantiomers, but the mass spectra do not definitively differentiate them. As a result, instrumental confirmation of identification can be lengthy and complicated without a definitive mass spectrum. However, there is another option for some compounds, and it has to do with how mass spectra are mathematically processed.



Normalized spectra for pseudoephedrine and MDMA. Reproduced with permission from the reference cited, copyright 2004 ASTM International.

The most abundant ion peak in many compounds in the methamphetamine family is found at 58 amu. Mass spectra are processed by assigning the largest peak (called the base peak) a value of 100 and scaling all other peaks to that value. Consequently, many minor peaks may be all but lost. Removing the base peak and renormalizing the remaining ion peaks has shown promise in visualizing otherwise small differences in related spectra. Although this protocol will not work with enantiomers, in many other cases such renormalization may be useful in distinguishing similar mass spectra that are dominated by the same base-peak ion.

Source: Reprinted, with permission, from Journal of Forensic Sciences, copyright ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428.

IR requires a pure sample, necessitating cleanup and often composite sampling of LSD exhibits. If sufficient papers or pills are available, a cleanup procedure can be used that employs an acid–base extraction scheme to remove excipients and dyes. The final chloroform extract can be placed directly into a mortar and allowed to evaporate before the addition of KBr. This technique also allows for examination of the mortar under UV light; the degree of fluorescence indicates how successful the extraction has been in concentrating LSD. Smaller IR pellets (7–10 mm) are preferred for these applications. Alternatively, the extract may be spread on a KBr surface to allow for micro-ATR IR.

Unlike the situation noted for marijuana, the morphology of mushrooms and peyote is normally not used as part of their identification, owing to the more complex botanical challenge. The plant-matter matrix of peyote and psilocin mushrooms makes the use of color tests impractical, unless the tests are applied to the extract. In most cases, ethanol or methanol is the extraction solvent of choice for dried plant matter. Some laboratories heat the methanol prior to extraction. The peyote button, and sometimes mushrooms, must be ground with coffee grinders or similar equipment or must be macerated prior to extraction. Cleaning these appliances between uses is crucial to prevent cross-contamination. The extracts are then amenable to TLC and instrumental confirmation by GC-MS.

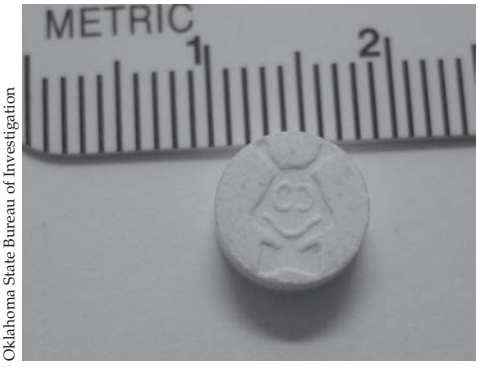
There is one caveat when mushroom extracts are analyzed by GC or GC-MS: in many cases, the injector temperatures are hot enough to facilitate the dephosphorylation of psilocybin to psilocin. This problem can be addressed by reporting the results as "psilocin and/or psilocybin" or by derivatization. LC-MS is an option when psilocin and psilocybin must be differentiated, as are TLC and CE.

On the Stand (Legal Issues and Questions): The primary issue related to LSD analysis relates to the small dosage units, particularly when few are submitted. Instrumental confirmation may require most or all of the exhibits to be consumed, a situation that always demands special consideration, planning, and documentation.

4 NONALKALOIDS

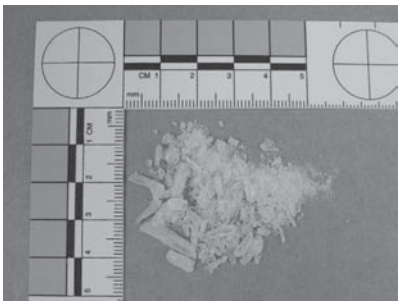
4.1 Phenethylamines: Amphetamine Family

Stimulants related to amphetamine, particularly methamphetamine, have nearly supplanted cocaine as the second most abused controlled substance in the United States (Figures 30 and 31). Methamphetamine is highly addictive and easy to make from readily available materials, a combination that has proven hard to defeat or control. Clandestine synthesis can start from organic chemicals or (semisynthetic) plant extracts. The methamphetamine family (Table 5) consists of phenylalkylamines based on a **phenylethylamine** skeleton.



Oklahoma State Bureau of Investigation

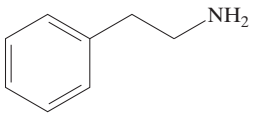
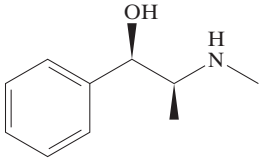
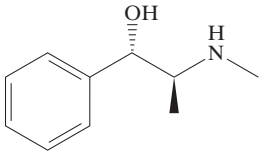
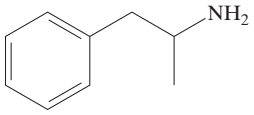
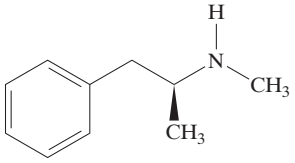
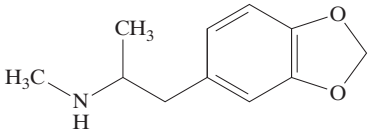
FIGURE 30 A tablet of Ecstasy (MDMA). Image courtesy of the Oklahoma State Bureau of Investigation.



Oklahoma State Bureau of Investigation

FIGURE 31 "Ice," a purified and recrystallized form of methamphetamine. Image courtesy of the Oklahoma State Bureau of Investigation.

TABLE 5 Amphetamine Family

Compound	Structure
Phenylethylamine (phenethylamine)	
Ephedrine (precursor)	
Pseudoephedrine (precursor)	
Amphetamine (Schedule II)	
Methamphetamine (Schedule II)	
Methylenedioxymethamphetamine (MDMA; Ecstasy)	

Amphetamine was synthesized in Germany in 1887 and later marketed under the trade name Benzedrine®, the origin of the older street slang term "bennies." **Methamphetamine** (trade name Methedrine®) was synthesized in Japan in 1919 and was used as a decongestant, much as **pseudoephedrine** is now. Amphetamine and methamphetamine were once widely prescribed as appetite suppressants and antisleep medications. The military issued methamphetamine to soldiers during the Second World War and amphetamines to troops in Vietnam. Long-haul truckers and athletes started using methedrine, causing the drug to increase in both popularity and abuse. Methamphetamine was listed on the CSA in 1970. Large-scale domestic clandestine production of methamphetamine is most prominent in California, but small laboratories are ubiquitous. Most of the smuggled supply originates in Mexico.

Methamphetamine is found as pills and powders and commonly, as a hydrochloride salt. Ingestion is via swallowing, injection, snorting, or smoking. **"Ice"** is a potent form of methamphetamine named for its appearance. Its crystalline appearance is the result of purification and recrystallization. Smoking produces the quickest

absorption and effects, which can last for several hours. Users may stay awake for days, followed by days of sleeping to recover. Methamphetamine is physically and psychologically addictive.

There are many other controlled drugs based on the same phenylalkylamine skeleton. Of greatest current concern is MDMA **(Ecstasy)** which has become a significant problem principally because of its use as a "club drug" or a party drug. As with most stimulants, MDMA has hallucinogenic properties. In acute high doses, the drug can produce serious psychotic events. One insidious side effect of MDMA is that it can cause users to ignore symptoms of dehydration that may arise during marathon parties. This, combined with high-energy activities associated with CNS stimulants, has led to several deaths by dehydration and hyperthermia. Conversely, users who are knowledgeable about this issue may drink far too much water and may even die as a result. MDMA has no legitimate medical uses and is listed on Schedule I. It is usually supplied in tablet form, often with designs stamped onto the surface. MDMA is typically synthesized in Europe and smuggled into the United States, but domestic seizures of MDMA clandestine laboraties have occurred. Two other drugs closely related to amphetamine and MDMA are MDA (3,4-methylenedioxyamphetamine) and MDEA (3,4-methylenedioxyethylamphetamine).

Precursors and Clandestine Synthesis:[†] Methods of synthesizing methamphetamine have changed in response to the control of precursors. No doubt these methods will continue to evolve, so forensic chemists must work to stay abreast of current developments. All common methods are based on reductions[‡] or reductive amination (the addition of an amine group to) of a molecule having the phenethylamine skeleton. Our discussion will focus on methamphetamine; however, chemical methods for synthesizing the phenylalkylamines are generally analogous. Note that dozens of methods have been and are used in clandestine laboratories, as have dozens of variations of those methods under different experimental conditions. Accordingly, a detailed discussion of all synthetic procedures is beyond the scope of the text. Instead, this section will highlight current methods while providing the basis for further study. The DEA, through its *Microgram* and *Intelligence Brief* publications, provides timely updates. Although a bit dated, an excellent review of these methods is also available.[35]

Clandestine synthesis begins with precursors, either immediate or more distant. An immediate precursor is defined as a precursor that is converted directly to the final product, although that conversion sometimes involves more than one step. Another way to think of it is that a direct precursor can be converted to the controlled substance from readily available items and reagents. As shown in Figure 32, methamphetamine has two immediate precursors: phenyl-2-propanone (also called P2P, phenyl acetone, and benzyl methyl ketone) and ephedrine or pseudoephedrine (which are all isomers related through stereochemistry). Starting with P2P, the clandestine chemist must effect reduction of the carbonyl group, the addition of a methyl group, and the addition of an amine group. The term **reductive amination** describes the process. The route from ephedrine or pseudoephedrine is simpler, requiring only the reduction.

[†]Some may question the wisdom of showing these syntheses in the detail presented. The emphasis here is on the chemistry and on mechanisms, not recipes and instructions. The information is drawn from the open literature and is available to anyone armed with a library card, an Internet connection, and sufficient motivation. No secrets are revealed in these sections.

[‡]Here, oxidation and reduction are best thought of in their organic context, wherein oxidation is the addition of oxygen or loss of hydrogen, and reduction is the gain of hydrogen or loss of oxygen.

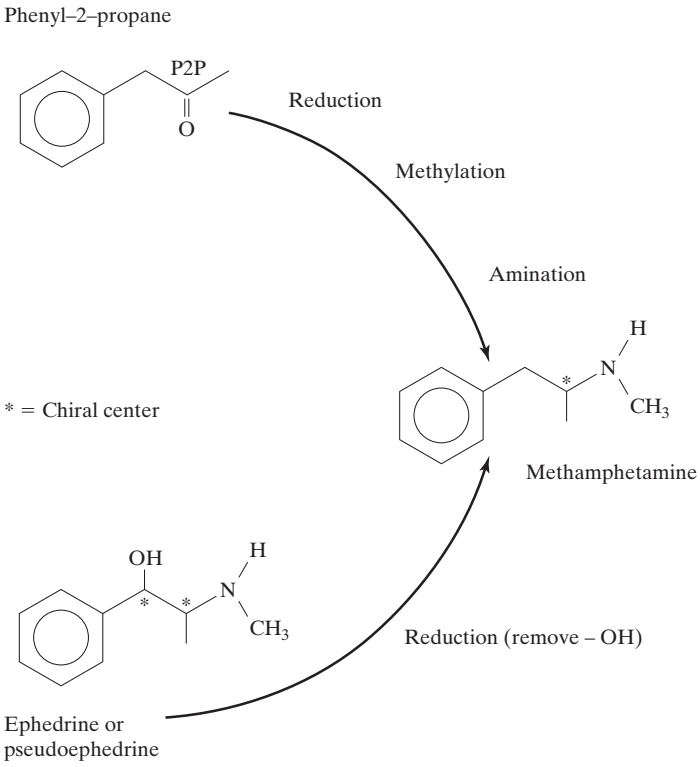


FIGURE 32 Precursors of amphetamine and methamphetamine. Older synthetic methods started with phenyl-2-propanone (P2P), which is reduced to amphetamine or subjected to reductive amination to produce methamphetamine. When P2P became more difficult to obtain, pseudoephedrine, which can be reduced to methamphetamine, became the precursor of choice.

Myriad synthetic routes starting from P2P and ephedrine or pseudoephedrine will yield methamphetamine. Most of these are summarized in Figure 33. Three routes—the Leuckart, red-phosphorus cook (HI), and Birch "Nazi" methods—will be discussed in detail. An analogous situation exists regarding the preparation of amphetamine and related phenylethylamines. The precursor phenylpropanolamine (PPA) used to be a common ingredient in cold remedies, but that is no longer the case. In 2000, the FDA requested that manufacturers reformulate their products to reduce or eliminate PPA after studies showed that the compound could increase the risk of strokes. Immediate precursors such as PPA and P2P can be made from distant precursors. In the case of P2P, several distant precursors are used to make P2P, but as in all syntheses, clandestine or not, the more steps and products produced, the less efficient, more expensive, and time consuming is the process. The P2P/PPA synthetic routes are summarized in Figures 34 and 35.

Up until the 1990s, domestic clandestine methamphetamine laboratories employed P2P, a common and versatile solvent with many legitimate uses. It and related compounds also have potent smells that can betray the location of a clandestine facility. The most common P2P synthesis is based on the **Leuckart reaction**, in which nucleophilic nitrogen attacks a carbonyl carbon, forming an intermediate that is reduced to methamphetamine or amphetamine as shown. The final product is the base, which is converted to the hydrochloride salt by bubbling HCl gas through an organic solution of the product. This synthetic route is illustrated in Figure 35. Variants of the Leuckart method are still used in Europe, starting from other precursors leading to production of P2P.[36]

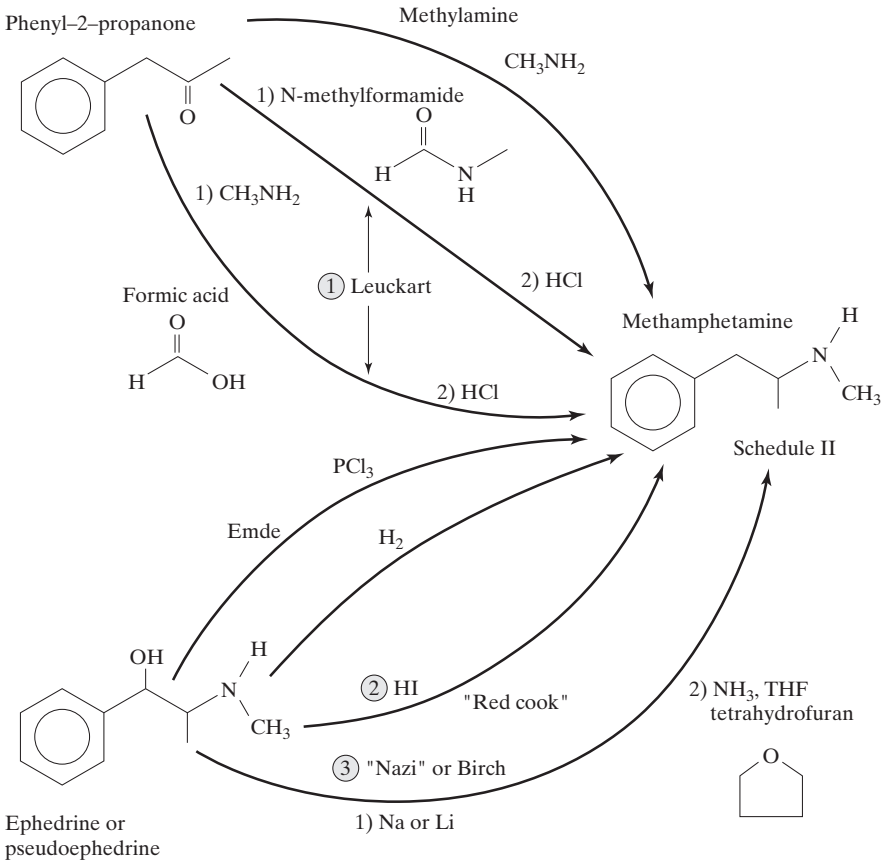


FIGURE 33 A selection of the specific routes to the synthesis of methamphetamine. The Leuckart process (1), HI (2), and Birch methods (3) are described in the text.

In response to the widespread clandestine synthesis of methamphetamine by this method, the DEA added P2P to Schedule II of the controlled substances list as part of the **Chemical Diversion and Trafficking Act** (CDTA) of 1988. The legislation was aimed at impeding clandestine laboratories by cutting off the necessary precursors for methamphetamine as well as other drugs. The act also placed controls on some of the equipment needed for making drugs. For a few years, the number of methamphetamine laboratories that were seized decreased, but the decrease was short lived. Clandestine operations turned to ephedrine or pseudoephedrine, common ingredients in cold and allergy medicines, as a precursor. Many over-the-counter (OTC) products contain only ephedrine as the active ingredient, simplifying the job of the clandestine cook. Soon after the development and dissemination of ephedrine synthetic methods, those medicines were being stolen, diverted, or purchased in large quantities for clandestine laboratories. In 2006, the Combat Methamphetamine Epidemic Act (CMEA) was signed into law in the United States. A primary goal of the law is to regulate OTC sales of products containing ephedrine, pseudoephedrine, and phenylpropanolamine.

Currently, the two favored routes for converting ephedrine to methamphetamine are the **Birch method (Nazi method)** and a **red-phosphorus "cook" method**. The latter is based on reduction of the alcohol group to hydrogen via an alkyl halide, is reasonably quick, produces high yields, and can be carried out via a "one-pot" method. Two variants of this approach are encountered, one involving reflux and another in which the reactant is "cold" or mildly heated. Generally, hydriodic acid is used in conjunction

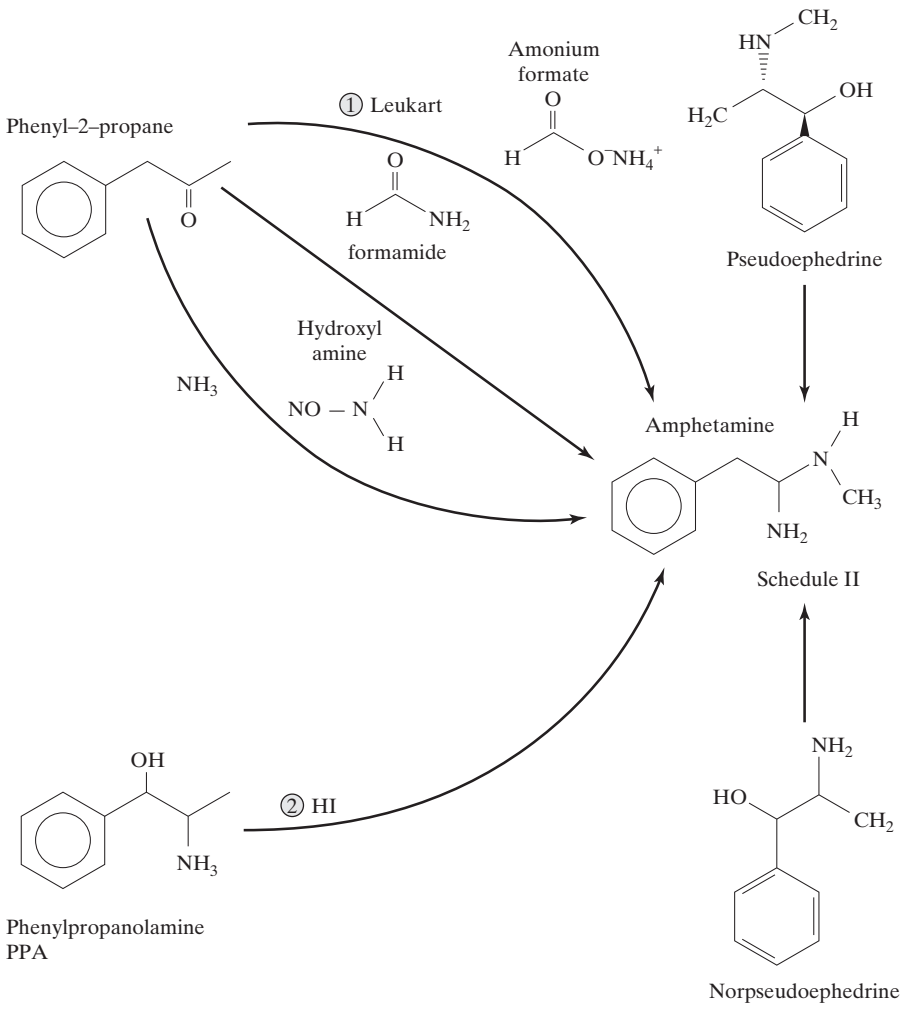


FIGURE 34 The routes leading to the synthesis of amphetamine are analogous to those leading to the synthesis of methamphetamine. Pseudoephedrine and norpseudophedrine can also be used. Although the text focuses on methamphetamine, the principles are easily extrapolated to amphetamine.

with red phosphorus obtained from matches or road flares. The cold-cook method differs in that the reactants are not heated as aggressively (or not at all) and the process uses HI that is generated from iodine (I_2), water, and red phosphorus. With reasonable skill, clandestine cooks can achieve yields of 50–75%.[37] Figures 36 and 37, outline the two cook methods.

The refluxed red-phosphorus method may produce phosphine gas (PH_3), which can be deadly to the cooks or to first responders:[38]

$$3I_2 + 2P \longrightarrow 2PI_3 \quad \textbf{(1)}$$

$$2PI_3 + 6H_2O \longrightarrow 6HI + 2H_3PO_3 \quad \textbf{(2)}$$

$$4H_3PO_3 + O_2 + \text{heat} \longrightarrow 3H_3PO_4 + PH_3 \quad \textbf{(3)}$$

Phosphorous acid (H_3PO_3) is produced as a by-product of the first reaction and under continued heating, is converted to phosphoric acid and phosphine gas.

The second common synthetic route is called the Birch method, Birch reaction, or Birch reduction. The reaction is not a traditional Birch reduction described in organic

① Leuckart reaction pathways

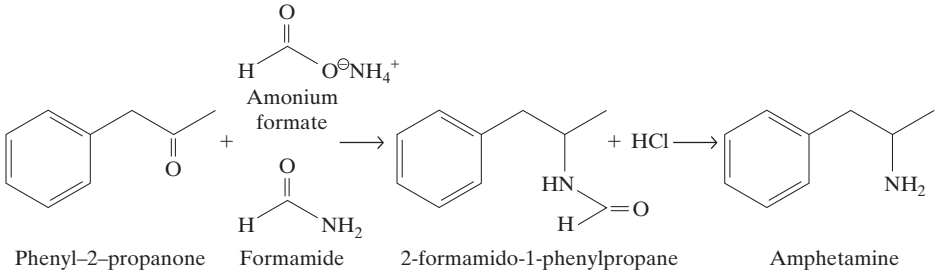
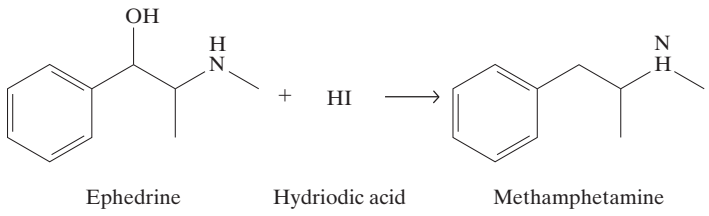


FIGURE 35 The Leuckart method (1 in Figure 33) for synthesizing amphetamine and methamphetamine.

② HI "Red Cook" Method



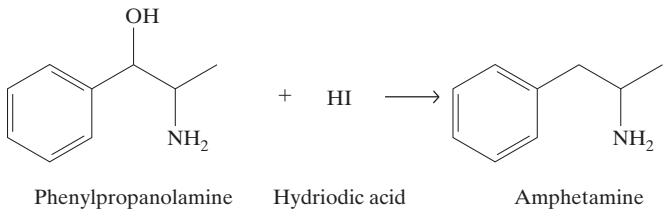


FIGURE 36 Overview of the HI (red-phosphorus cook) method (2 in Figure 33) for producing amphetamine and methamphetamine.

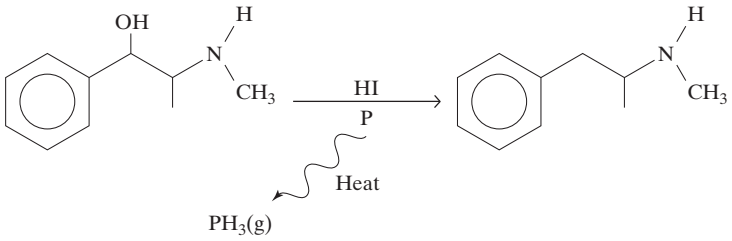


FIGURE 37 A stepwise breakdown of the HI method. The first step displaces the —OH; and replaces it with an I. The reduction follows. If the mixture is heated excessively, phosphine gas can be produced.

text and reference books in which a benzene ring is reduced, resulting in two double bonds rather than three. Rather, the name "Birch" may have arisen from the reactants used in the procedure. The descriptor "Birch reduction" is still seen, but "Birch method" or "Birch reaction" is preferred. The Birch method is also referred to informally as the "Nazi" method, although there are conflicting reports as to why this name has been adopted. Thus, the naming of this synthetic method could not get much more confusing, even if the chemistry is clear (or, rather, blue…).

As shown in Figures 38 through 40, synthesis begins when ephedrine, dissolved in THF, is combined with lithium metal (usually from batteries and anhydrous ammonia, a chemical used as fertilizer). The ammonia can also be generated in situ

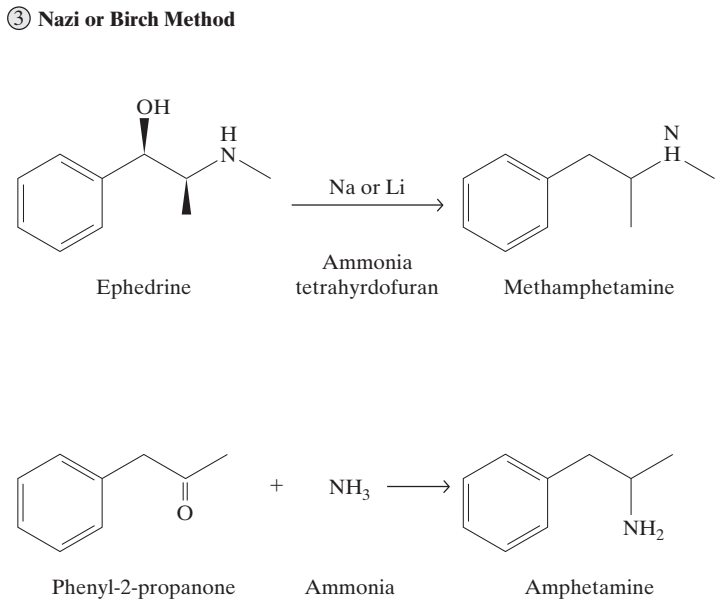


FIGURE 38 Overview of the Birch method (3 in Figure 33) for producing methamphetamine. In an analogous process, P2P can be reduced to amphetamine.

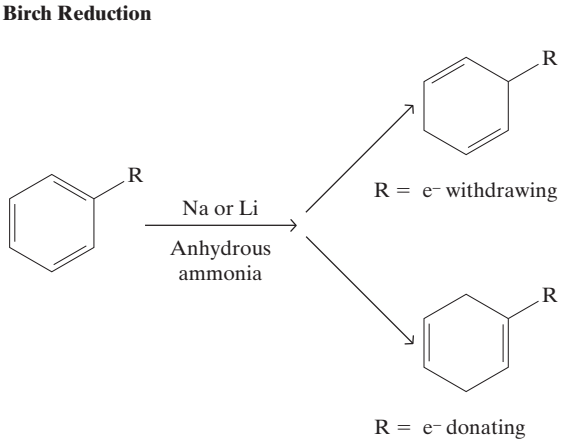


FIGURE 39 The Birch reduction. Anhydrous ammonium and sodium or lithium metal are used in an alcoholic solution to reduce (add hydrogen to) the ring. The electron-donating or -withdrawing nature of R determines where the double bonds will be found. Notice that the Birch method for methamphetamine synthesis does not affect the phenyl ring.

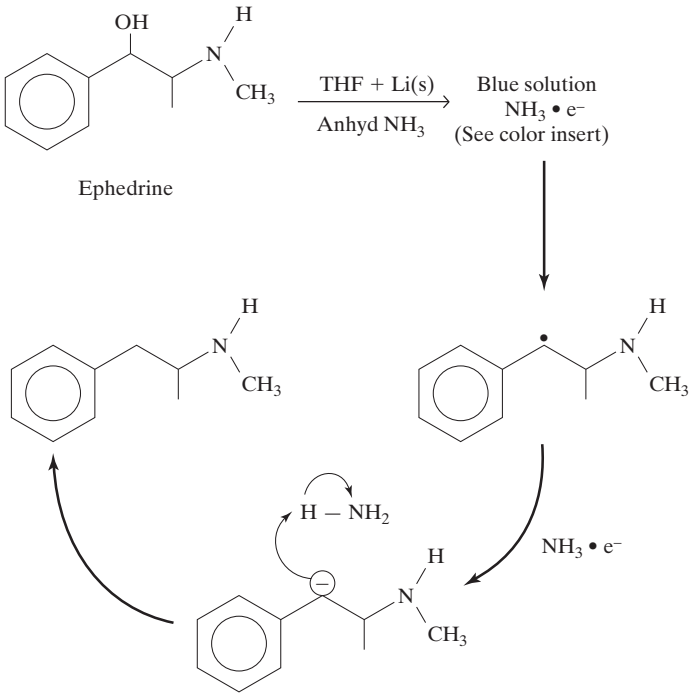


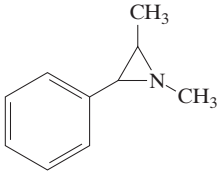
FIGURE 40 The Birch method applied to ephedrine to produce methamphetamine. The progress of the reaction can be monitored by observing the disappearance of the blue color and the appearance of a grayish color associated with the product, methamphetamine. See the color insert for photos of the reaction process.

from lye and ammonia fertilizer products. Once ammonia is present, the solution turns blue due to solvated electrons that are generated by the lithium metal:

$$\mathrm{Li(s)} + \mathrm{NH_3(anhydrous)} \rightarrow \mathrm{Li^+} + \mathrm{e^-(solvated,\ deep\ blue)} \qquad \textbf{(4)}$$

The solution turns a grayish color as the reaction proceeds. The methamphetamine that is generated is converted to its salt by bubbling HCl(g) through the solvent. The advantages of the Birch method are its ability to boil off excess ammonia and its simple decomposition of residual lithium metal by water. The disadvantage is the need for anhydrous ammonia, which can be hazardous, explosive, corrosive, and difficult to handle.

One other synthetic method observed in some clandestine laboratories, the **Emde method,** involves the conversion of ephedrine to the chlorinated analog with the use of $SOCl_2$, PCl_5, $POCl_3$, or PCl_3.[39] This method is sometimes referred to as the Emde synthesis.[40] A by-product is an **aziridine** (Figure 41) that, if found in a sample or in evidence seized from a clandestine facility, is characteristic of the Emde method.



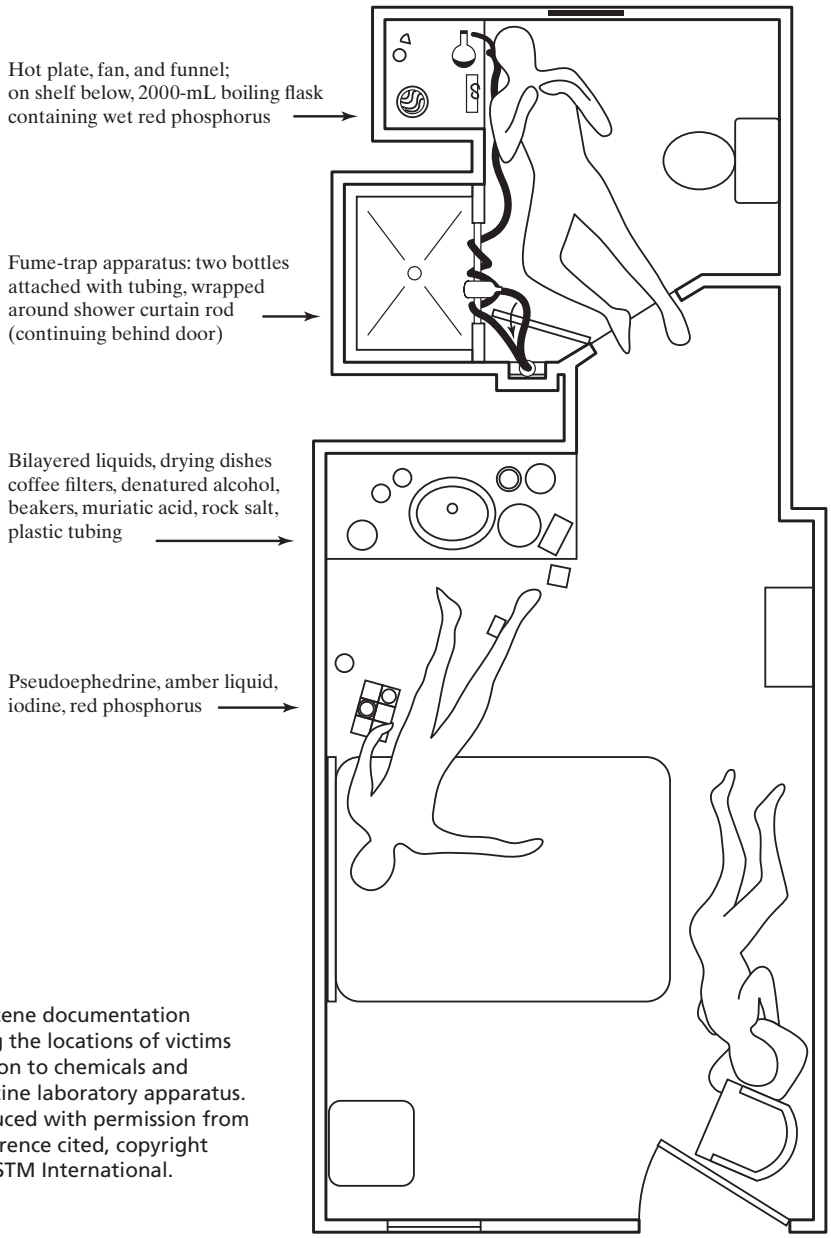
Aziridrine

FIGURE 41 An aziridine that forms as a by-product of the Emde method for methamphetamine production.

Stereochemistry: Because methamphetamine has a chiral carbon at the β position relative to the benzene ring, *d*- and *l*-isomers can be produced. The *d*-form of methamphetamine is the more active, so clandestine syntheses are geared to favor *d*-methamphetamine (also referred to as (+) methamphetamine, the *S* enantiomer). The mechanism used dictates the stereochemistry of the products. The Leuckart method, starting with P2P, yields a racemic mixture of *d*- and *l*- ((+) and (−)) methamphetamine, whereas reductive methods, starting with ephedrine or pseudoephedrine, are controlled by the form of the ephedrine or pseudoephedrine.[41] The Birch and iodide methods yield (+) methamphetamine if the starting

Applying the Science 5 Phosphine Gas Fatalities

Phosphine gas is a highly toxic substance produced by the "red-cook" method of clandestine methamphetamine synthesis. The gas itself is odorless, but associated by-products lend a fishy garlicky odor to the solution, detectable in the concentration range at which toxic effects are manifest. Death has been reported within 30 minutes at concentrations in the range of 400 ppm, with toxic effects noted at much lower concentrations. In one documented report, three individuals died while making methamphetamine; this and similar incidents have led to increased concern and awareness among first responders concerning the dangers of phosphine.



Crime scene documentation showing the locations of victims in relation to chemicals and clandestine laboratory apparatus. Reproduced with permission from the reference cited, copyright 2000, ASTM International.

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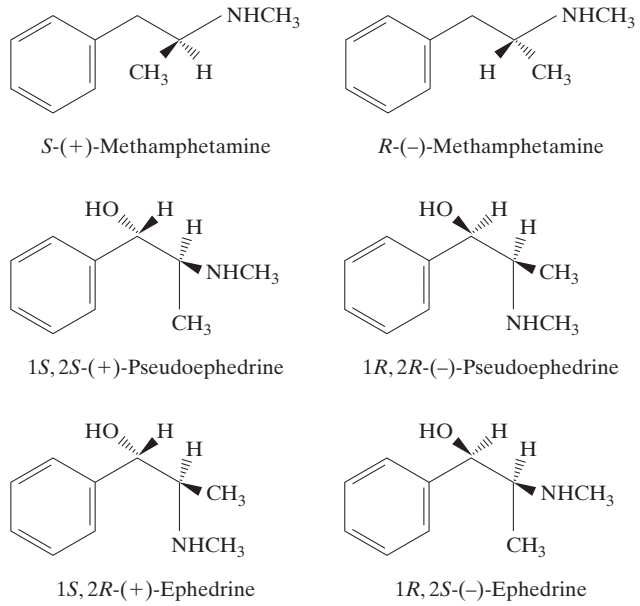
material is either (−) ephedrine or (+) pseudoephedrine.[37,42] The stereochemical aspects of the Emde method are more complex.[39] A few of the stereochemical considerations are summarized in Figure 42.

The plethora of synthetic methods and conditions used to create methamphetamine facilitates profiling investigations. Because of the stereospecificity of the Birch method versus the Leuckart method, stereochemistry can be a valuable profiling aid. A method for profiling in which a basic extraction into ethyl acetate is used has been published by the United Nations;[43] an SPME approach has also received attention and appears to work as well or better.[40] Such extractions often yield a large number of chromatographic peaks and identifications of compounds, not all of which are easily explained. Some may be contaminants of original ingredients, whereas others may be by-products created by less-than-stringent attention to experimental conditions. In one recent study, 1,2-dimethyl-3-phenyl-aziridine (typical of an Emde chloroephedrine intermediate) was identified with the SPME method, as were a number of other compounds, such as caffeine (a diluent) and vanillin, likely added for flavoring.[40]

MDMA (Ecstasy): MDMA is a member of the methamphetamine–amphetamine class of drugs and is unusual in that it is chemically similar to mescaline, a hallucinogen. MDMA has become popular among young adults as a club drug or rave drug. Unlike methamphetamine, it is supplied mostly by foreign laboratories that smuggle their product into the United States.

Analytical Approach: The analysis of the phenethylamine stimulants follows the typical path of color tests to confirmation by instrumental methods, typically IR. Because of the structural similarity among the members of this drug family, special attention has to be paid to distinguish them. Controls are essential, and, in the case of GC-MS,



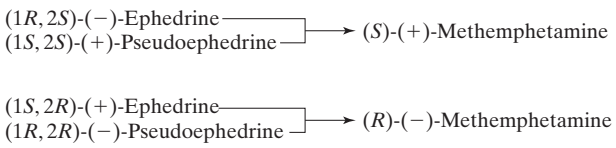


FIGURE 42 The optical isomers of methamphetamine and its precursors. Ephedrine has two chiral carbons—α and β—to the phenyl ring, but the α carbon is reduced to — CH_2 leaving only one chiral center.

retention-time data are a critical element of identification, since the spectra are similar. Longer capillary columns with high theoretical plate counts and high separation efficiency are of value in separating compounds seen in phenethylamine samples. Enantioselective techniques such as derivatization, as well as chiral chromatographic columns based on cyclodextrins, have also been employed to separate the many enantiomers that can be found in this type of evidence.[44]

GC-MS is clearly useful for quantitative purposes and profiling, but IR spectroscopy is often preferred for identification in cases where feasible. Extraction is usually required, although not necessarily a quantitative one. Because methamphetamine and related compounds are amines, they are weak bases and are extracted from a basic solution as the un-ionized amine compound into an organic solvent such as chloroform. Bubbling HCl through the chloroform converts the base to the corresponding salt. Identifying enantiomers is a more difficult challenge. Recent advances in chiral separations using chiral stationary phases allow for enantiomeric differentiation; however, the most promising method for routine use may be capillary electrophoresis (CE), which has the added advantage of allowing for chiral separations using cyclodextrins.[45,46]

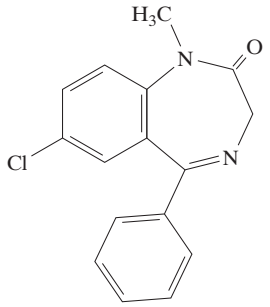
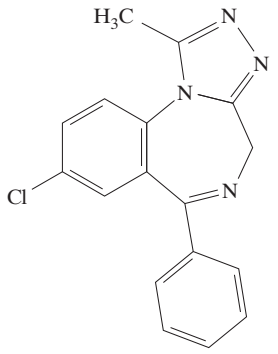
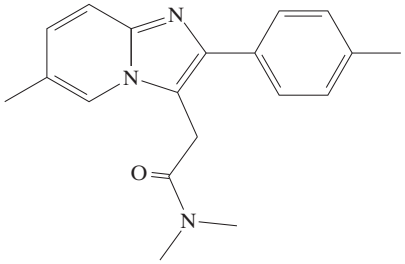
On the Stand (Legal Issues and Questions): Perhaps the most challenging aspect of the analysis of the phenethylamines is their similarity to each other. The electron impact (EI) mass spectra of methamphetamine and its isomers and ephederine and its isomers are all very similar, dominated by a peak at *m/z* 58 amu. In such cases, care must be taken in the gathering and interpretation of mass spectra and the coupling of retention-time data with spectral data. In some cases, derivatization has been used prior to GC-MS analysis[47–53] and in others, a rigorous approach to spectral interpretation.[54] These types of cases illustrate the importance of supporting analytical information.

4.2 Benzodiazepines

The **benzodiazepines** were among the first class of drugs developed as a result of targeted modern drug discovery techniques.[55] Drugs used specifically to treat psychological problems and mental illnesses are relatively new compared with narcotics and stimulants, which trace back to the introduction of lithium carbonate in 1948 as a treatment for bipolar disorder. In the 1950s, monoamine oxidase inhibitors (MAOIs) were introduced. These drugs function by blocking enzymatic processes that degrade neurotransmitters. As a group, they were effective but were plagued by side effects and interactions with other medications and food. To address these limitations, as well as those of barbiturates in treating anxiety and depression, a group at Roche Pharmaceuticals undertook research that led to some of the most widely prescribed drugs in the world.[55]

The first of the benzodiazepines was **Valium** (diazepam), introduced in 1963. A number of related compounds, including Dalmane® (flurazepam) and Ativan® (lorazepam), were developed over the next two decades. More recent entries include Xanax® (alprazolam), an antianxiety drug with additional ring structure. Another group of prescription antidepressants is the tricyclic amines (TCAs), such as Elavil®. Finally, with drug discovery and expanding knowledge of brain chemistry and neurotransmitter function, a new class of antianxiety–antidepressants—the selective serotonin reuptake inhibitors (**SSRIs**)—was produced. This class includes fluoxetine (Prozac®), introduced in 1987, which was followed by many others (e.g., Paxil®) that are consistently among the most widely used and prescribed drugs in the world. Zolpidem (Ambien®), a relatively recent addition to the arsenal of sleeping aids, is becoming more of an abuse concern, particularly when people are driving with the drug in their system.

TABLE 6 Examples of Benzodiazepines and Similar Compounds

Valium® (diazepam)	
Xanax® (alprazolam)	
Ambien® (zolpidem)	

Forensically, these drugs are encountered as diverted pharmaceuticals, simplifying the analysis. In such cases, and in any case in which commercial labeling or marking remains, the first step is usually a check of the *Physician's Desk Reference* (PDR) to determine the likely identity and constituents of the preparation through pharmaceutical identifiers. Rather than identifying a complete unknown, the task then becomes one of confirmation with instrumental techniques. In effect, the PDR or another source of pharmaceutical identifiers becomes a presumptive test. While there are cases in which commercial preparations are faked, such cases are rare. Fortunately, abuse of these new-generation medications is far less prevalent than was abuse of earlier medications, such as the barbiturates.

Summary

This chapter has provided a whirlwind tour of a selection of drugs targeted in seized drug analysis. We are now ready to move into drugs as biological evidence. What we will add is a discussion of how the body metabolizes these drugs and how the analysis of drugs and metabolites differs from the analysis of the drugs as physical evidence.

Key Terms and Concepts

Amphetamine
Anabolic steroids
Aziridine
Barbiturates
Bear claw
Benzodiazepines
Birch method
Bleaching agent
Caffeine
Cannabimimetics
CBD
CBN
Chemical Diversion and Trafficking Act (CDTA)
Coca paste
Cocaine
Codeine
Cystolith
Cystolithic hair
Diacetylmorphine
Diamorphine
Dissociative anesthetics
Ecstasy
Emde method
Enantiomers
Endorphins
Ephedrine
Ergot alkaloid
Ergotism
GBL
GHB
Hashish
Hemp
Heroin
Hydrocodone
Hydromorphone
Ice
Ketamine
Khat
Lactone
Latex
Leuckart reaction
Lidocaine
Lysergic acid diethylamide (LSD)
MAM
Mescaline
Methamphetamine
Morphine
Nazi method
Neurotransmitter
Opiate alkaloids
Optical isomers
Oxycodone
OxyContin
PCP
Peyote
Phenethylamines
Procaine
Provenance
Pseudoephedrine
Psilocin
Psilocybin
Red-phosphorus cook method
Reductive amination
'roid rage
Salicylates
SSRI
Δ^9-Tetrahydrocannabinol (Δ^9-THC)
Thebaine
TMS
Trichrome
Tropane alkaloid
Tryptamines
Valium

Problems

FROM THE CHAPTER

1. Name the functional group(s) in THC. What presumptive color or crystal tests could be useful, aside from the Duquenois–Levine test? Why are crystal tests of limited use with plant extracts?
2. Resorcinol gives a false positive with the Duquenois–Levine test. Why?
3. One test occasionally performed on aqueous solutions containing a mixture of GHB and GBL is a test for elevated concentrations of K^+ or Na^+. Why?
4. Give the specific mechanism of the conversion of GHB to GBL. Would a mix of products be expected?
5. Name a presumptive test or test series that could distinguish between testosterone and estradiol. Justify your selection.
6. Explain what is meant by the term "undetectable" in referring to a designer steroid. Obviously, it is a misnomer if taken literally, since such compounds have been identified.
7. The following colors are obtained in presumptive testing:

Steroid	Liebermannn's	Mandelin's
Testosterone	Light violet	Orange red
Testosterone cypionate	Orange brown	Dark brown
Testosterone enanthate	No reaction	Orange red
Testosterone propionate	Orange brown	Orange brown

Explain or justify these observations.

Source: Chiong, D. M., et al. "The Analysis and Identification of Steroids." *Journal of Forensic Sciences* 37 (1992): 488–502.

8. Would a sample of heroin containing significantly more 3-MAM as opposed to 6-MAM be noteworthy? Why? What could this indicate?
9. For any given molecule of heroin, what percentage of the carbon originates from morphine and what percentage is traceable to acetic anhydride, assuming that is the reagent used in the conversion.
10. Caffeine is an alkaloid base with a bitter taste that is cheap and easy to obtain. It is frequently used as an adulterant of cocaine but is rarely used as an adulterant of heroin. Why?

11. What is a key structural feature seen in the benzodiazepines that is not seen in any other drugs discussed so far?
12. One method used to remove the alkaloids papaverine and noscapine from extracted morphine is to scatter the powder in water and adjust the pH to 6.4. After a time, the pH is made basic (9.0) and extracted with an organic solvent. Explain how and why this method works.
13. The following data are obtained from the PDR regarding an elixir:

Tylenol® with Codeine (Ortho-McNeil)
tablets CIII

(acetaminophen and codeine phosphate)

Contains:

Codeine phosphate	12 mg
Acetaminophen	120 mg

a. Assuming that the elixir is a syrupy aqueous solution, suggest a method to isolate the two active ingredients from the syrup by using SPE.
b. Make the procedure you suggested in part a quantitative for a GC-MS analysis. Assume that the linear dynamic range of the curve for both drugs is 10.0–200.0 ng/mL and that the recovery of your method will be within 95%–105%. Assume further that the injection volume is 1.0 µl. (*Note*: There is more than one correct answer, just as there are many preparations that could work. Select one that is reasonable.)
c. Could this sample be extracted with an organic solvent such as chloroform and directly injected into the GC-MS system as described? Why or why not?

INTEGRATIVE

1. Why are the functional groups attached to carbon 3 in morphine more active than those attached to carbon 6?
2. Postulate a mechanism and explanation for the formation of the aziridines during the Emde method of methamphetamine synthesis.
3. What by-products would you expect if the Birch method is used to prepare methamphetamine from ephedrine?
4. One sign that the Birch or "Nazi" method has been used to synthesize methamphetamine is an empty propane tank like that used for barbecue grills. Such tanks have a blue patina around the valve. What could account for this sheen?
5. On the basis of the fundamental characteristics of abused substances, what are some reasons for the relatively limited abuse of modern antianxiety and antidepressant drugs relative to those they have replaced?
6. Fast Blue B has been used as a spray reagent developer for the TLC of marijuana samples and as a color-test reagent. Owing to carcinogenic properties of Fast Blue B in that form, the Fast Blue BB salt has replaced it in most uses. Find the formula for Fast Blue BB and postulate how it reacts with THC, CBN, and CBD. Why are the colors developed with Fast Blue B different for the three cannabinoids? Which two would you expect to be the most similar?
7. Describe an extraction scheme to separate testosterone and estradiol.
8. Of the compounds shown in Table 2, several are generally provided in solution for injection. What would the solvent likely be and why?

FOOD FOR THOUGHT

1. If designating precursors, such as P2P, as controlled substances or listed chemicals, has been successful in eliminating the method of synthesis, why not add ephedrine to Schedule I of the Controlled Substances Act?

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Drugs in the Body

OVERVIEW AND ORIENTATION

We have examined drugs primarily from the point of view of seized drug analysis and drugs as physical evidence. In this chapter, we begin our explorations of forensic toxicology, in which drugs are now part of biological evidence. When a drug is ingested, it is subjected to metabolism, and the drug and its by-products move about and out of the body by many mechanisms. Because drugs are subject to metabolism, the ingestion of a single substance can lead to the existence of several compounds in different tissues and compartments of the body.

The job of the forensic toxicologist can be thought of as twofold:

1. Obtain the analytical data relating to drugs and metabolites from various types of biological samples.
2. Employ an understanding of metabolism and mechanisms to answer the relevant forensic question(s).

We will spend this chapter developing a basic understanding of metabolism and mechanisms. Keep in mind that forensic toxicology, and toxicology in general, is a large and dynamic field, and the best we can realistically accomplish here is to introduce a few of the basics and to whet your appetite. We will discuss concepts and ideas and present a few types of calculations. These should be taken in the same spirit as what we will describe in the context of combustion and explosives—as basic starting points for study. If your interest is piqued by what you read here, there are a number of excellent books related to forensic toxicology in the "Further Reading" section at the end of the chapter that capture the depth and breadth of the topic.

1 INTRODUCTION

Pharmacology is the study of drugs and medicines. Two aspects of pharmacology are **pharmacokinetics** and **pharmacodynamics**. You will also see the terms **toxicokinetics** and **toxicodynamics**. Often, they are used interchangeably, but strictly speaking, the latter terms apply when the drug dose is high enough to induce toxic effects. Broadly defined, pharmacodynamics is the study of effects of drugs over time and is concerned with the interaction of a drug with its target organ or tissue. Of more immediate concern in forensic applications is pharmacokinetics, the study of the movement of a drug and its metabolic products through the body. The process begins with ingestion and ends with final disposition, be it elimination, storage, or a combination of the two. The process is outlined in Figure 1.

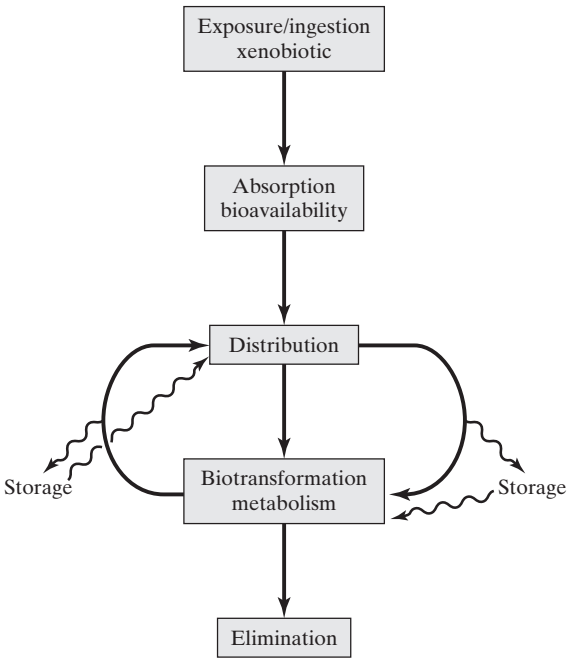


FIGURE 1 The stages of drug movement through the body, beginning with ingestion of the xenobiotic (a substance that is foreign to the body). This depiction simplifies an extraordinarily complex set of physical and chemical interactions and processes that begin with ingestion.

Although a detailed discussion of pharmacokinetics is beyond the scope of this text, basic pharmacokinetics is an important part of forensic chemistry. The foundations of pharmacokinetics are familiar chemical principles of kinetics and equilibrium applied to a biological environment. Pharmacokinetics involves multiple partitioning steps, solubility considerations, protein-bound complexes, and an enzymatically facilitated metabolism that may convert the original drug or toxin into new compounds.

There are several facets of pharmacology that we will not be able to discuss here but that are worthy of mention. For example, metabolic reactions are catalyzed by enzymes, many of which are polymorphic, the structure and activity of which can vary across a population. In such cases, there may be a significant difference in metabolic efficiency from person A to person B and thus can influence the pharmacokinetics of the drug. This subject is referred to as **pharmacogenetics**, and is an emerging topic in forensic toxicology. Similarly, we will not be able to discuss the effects of ingestion of multiple drugs (polypharmacy) in any detail. You should appreciate that complex affects are possible and that ingested substances can alter enzymatic activity both by stimulating (induction) or by slowing (inhibition). As another example, the ingestion

of cocaine along with ethanol can produce a metabolite (cocaethylene) that is not seen when alcohol is not ingested at the same time. These and other topics are discussed in detail in the materials listed in "Further References" at the end of the chapter, and you are encouraged to explore them.

2 ADME

Pharmacokinetics studies the movement of a drug or foreign substance (a **xenobiotic**) by dividing it into the stages of absorption, distribution, metabolism, and elimination, a process referred to by its initials, ADME. The simplified flowchart shown in Figure 1 disguises a complex chain of events that begins with the entry of the drug into the body. The method by which this occurs is the **mode of ingestion** (Figure 2), of which there are several. Therapeutic drugs and drugs of abuse can be swallowed, injected, or snorted (absorbed through the nasal membranes), but there are other routes of exposure, and some of these may be incidental or unintentional. Poisonous gases such as CO and HCN enter the bloodstream by way of inhalation, and many substances can be absorbed through the skin. Drugs delivered by suppository are absorbed in the lower intestine. Injections can be introduced directly into a blood vessel (**intravenous** injection), just below the skin's surface (**subcutaneous** injection), or into a muscle (**intramuscular** injection). The mode of ingestion affects how and when a drug appears in the bloodstream, an event that must occur before the substance can be distributed to various tissues in the body. Although any mode of ingestion is possible in forensic casework, the discussion that follows concentrates on the oral and intravenous routes.

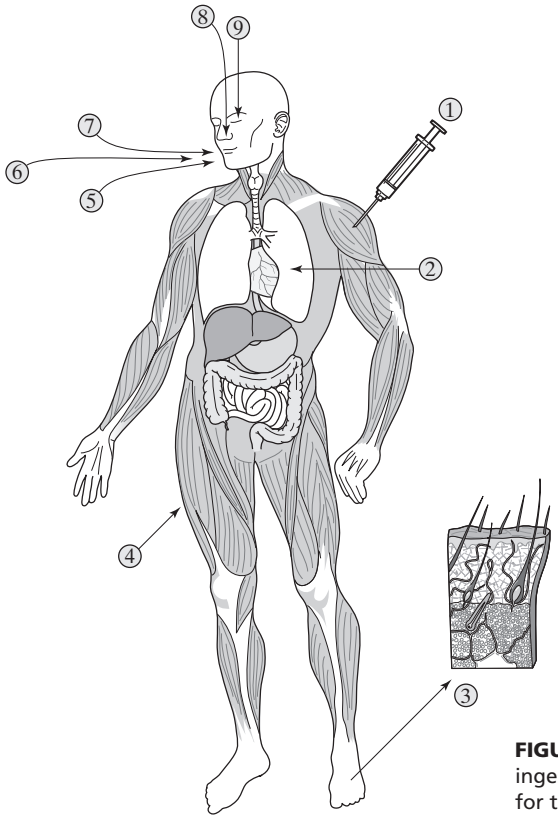


FIGURE 2 Modes of ingestion. (See Table 1 for the legend.)

Pharmacokinetic studies can roughly be divided into two segments, (1) absorption and distribution and (2) metabolism and elimination. The overall path is depicted

TABLE 1 Modes of Ingestion; Numbers Correspond to Notation in Figure 2

Number	Mode
1	Injection into the tissue under the skin
2	Injection into a vein (intravenous)
3	Absorption through the skin (dermal)
4	Injection into a muscle
5	Inhalation or aspiration (inhalation of liquid)
6	Ingestion into the stomach
7	Dissolution below the tongue (sublingual)
8	Absorption through the mucous membranes of the nose
9	Absorption through the eyes

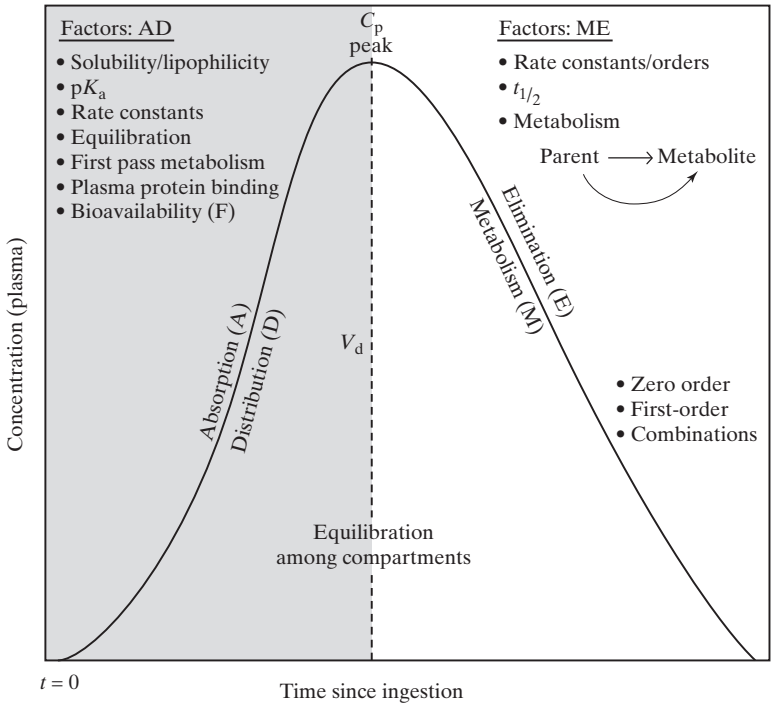


FIGURE 3 The path taken by a xenobiotic substance from ingestion to elimination or clearance; the ADME process. Metabolism and elimination can take place even as concentrations build toward peak values.

in Figure 3. In this approach, the dividing line is that time after ingestion at which the concentration C_p (plasma, specifically) in the bloodstream reaches a maximum.[†] For an intravenously injected drug, this happens quickly, since no phase barriers must be crossed. For an orally ingested drug, the picture is more complex, because a lipophilic membrane is involved. (The particulars of this absorption and distribution process will be discussed shortly.) It is important to note that while we have considered absorption and distribution as essentially ended when the peak plasma concentration is reached, in reality distribution continues for some time after absorption has ended. Also, both metabolism and elimination actually start at t = 0 and continue as long as the drug is in the body. What we have presented here in Figure 3 is a linear simplification of a parallel process that is adequate for an overview approach.

Realize that metabolism is elimination in the sense that the parent molecule is converted to a new substance, causing C_p of the parent to decrease. The rate of removal of the drug, sometimes referred to as the **clearance rate**, usually follows first-order kinetics and is defined mathematically as

$$\text{Cl} = k_{el}/\text{concentration}$$

where Cl is the clearance rate and k_{el} is the rate of elimination. Clearance typically refers to plasma concentrations.

In any first-order metabolic process, the rate of the reaction depends only on the concentration of the drug. The **half-life** is derived by starting with the general equation for a **first-order process**

$$t = \frac{1}{k} \ln \frac{C_0}{C_t} \qquad \textbf{(1)}$$

[†]The *C* is generic and can represent the concentration in any body fluid, such as whole blood, urine, etc. The current discussion focuses on C_p.

EXHIBIT A

Fatal Skin Absorption

Toxicologists played an important role in the investigation of the illness and death of Professor Karen Wetterhan of the Dartmouth College Department of Chemistry in February 1997. Dr. Wetterhan, 48, was using dimethyl mercury (CH_3—Hg—CH_3) in her research. She was working in a fume hood and wearing latex gloves when an exposure occurred. While transferring the dimethyl mercury from one container to another, she apparently spilled a tiny amount (perhaps even a single drop) on the back of one of the gloves. She reported removing the gloves and giving the incident no further thought. Later studies proved that the organic liquid quickly penetrated the latex gloves, delivering a lethal dose via skin absorption. The fume hood likely protected her from incidental inhalation exposure.

Symptoms appeared months later, when she reported numbness, tingling, and difficulty speaking. Given the symptoms and her area of research, mercury poisoning was suspected. The suspicion was confirmed by further testing, including hair analysis. Mercury levels in her urine were recorded at 234 ppb and in her blood at 4000 ppb (4 ppm) months after the exposure. Despite therapy, she died 10 months after the incident. It is thought that the long delay in the appearance of symptoms was due at least in part to the lipophilic nature of dimethyl mercury.

Source: U.S Department of Labor, Occupational Safety and Health Administration Hazard Information Bulletin 19980309. Available online at http://www.osha.gov/dts/hib/hib_data/hib19980309.html. Accessed June 2011.

where k is the elimination rate constant, C_0 is the initial maximum blood concentration of the drug, and C_t is the concentration at some time t. After one half-life, the concentration $C_t = {}^1\!/_2 C_0$ and

$$t_{1/2} = \frac{1}{k} \ln \frac{C_0}{0.5C_0} \quad \textbf{(2)}$$

$$t_{1/2} = \frac{1}{k} \ln 2 \quad \textbf{(3)}$$

$$t_{1/2} = \frac{0.693}{k} \quad \textbf{(4)}$$

The half-life should carry a descriptive label, such as $t_{1/2\ \text{plasma}}$, for clarity. This notation is also important because a decrease in plasma concentration does not necessarily mean that a drug has been removed from the body. A decrease in concentration in the plasma "compartment" may correlate partly or exclusively with a concomitant increase in concentration of the same substance or a metabolite in a different compartment. How those compartments are linked is critical information for the toxicologist.

We can modify our depiction of the ADME of a drug as a process as shown in Figure 4. We still consider the body to be a single compartment, but now we can add and discuss a couple of features. First, the shape of the leading portion of the curve (during the absorption phase) will vary based on the mode of ingestion. If we restrict our discussion to oral ingestion and plasma concentration, there is a lag time between ingestion and the peak plasma concentration. This time varies from substance to substance. However, if the drug is ingested by smoking or intravenous injection (shown at the lower left of Figure 4), the drug is introduced into the bloodstream directly, and the lag time between ingestion and peak plasma concentration is greatly reduced. However, if a drug is absorbed into the body slowly over time, such as via a skin patch, the lag time can be significantly longer than for an oral ingestion. Such factors may be important considerations in interpreting toxicological findings.

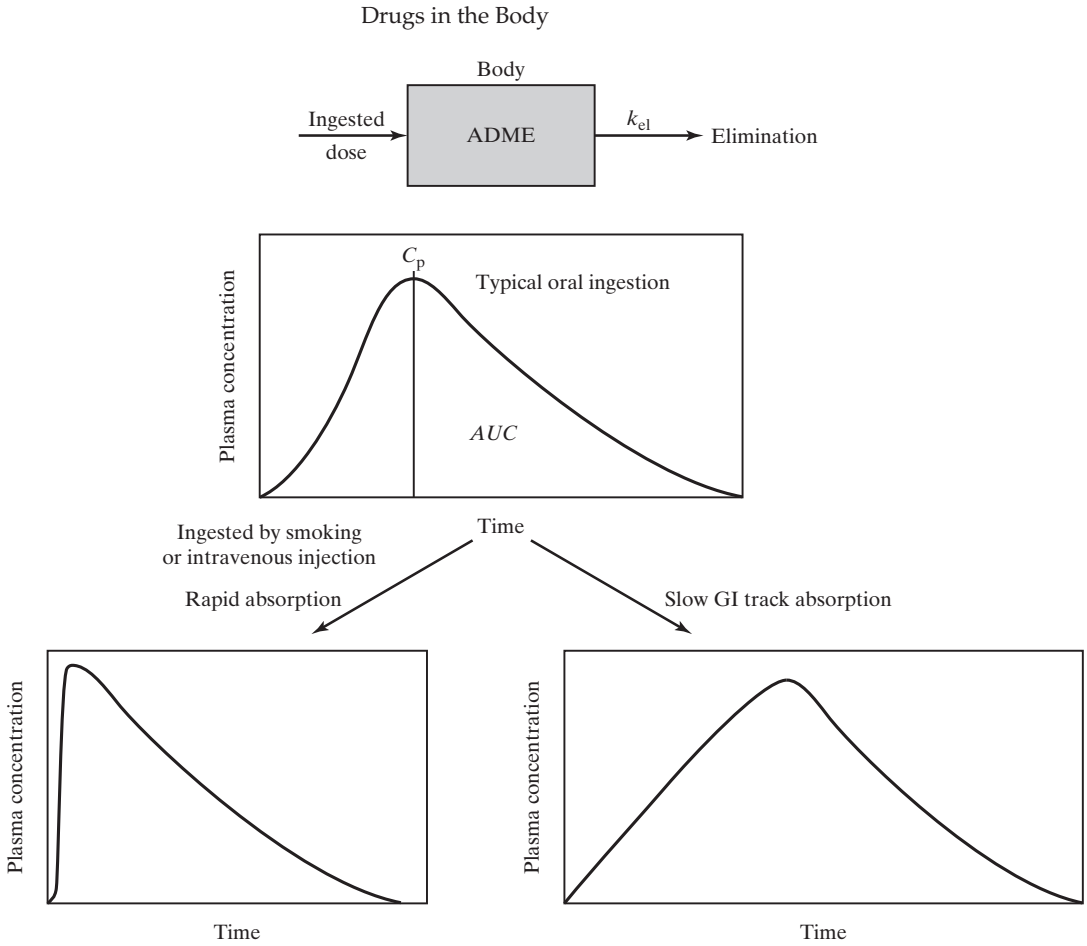


FIGURE 4 Another perspective on the ADME curve. The term **AUC** refers to "area under the curve," a value that can be utilized quantitatively.

EXHIBIT B

Kinetics: A Speedy Review

Kinetics is the study of the speed of a chemical reaction or process and is a critical element in toxicology. Most processes described in the discipline follow zero- or first-order kinetics. Recall that rate laws are employed to determine concentrations as a function of time. Although there are other variables and factors, the elimination of a xenobiotic substance from the body is fundamentally dependent on the kinetics of the reactions involved. Elimination processes often involve catalysts (enzymes), the role of which is central in determining the overall speed of the elimination, since a catalyst reduces the energy of activation, E_a.

Concepts of mechanism and rate-limiting steps apply as well, as can be illustrated with a simplified view of the metabolic processing of ethanol:

1. $C_2H_5OH \longrightarrow C_2H_4O$ k_1 = slow
2. $C_2H_4O \longrightarrow C_2H_3O_2^-$ k_2 = fast

Net: $C_2H_5OH \longrightarrow C_2H_3O_2^-$, rate-limiting step = first

3 COMPARTMENT MODELS

When a drug is ingested orally, the plasma concentration does not peak immediately as time is required for the drug to pass into the intestine, actually transit the intestine, and make its way into the bloodstream. When a drug is injected intravenously, absorption is essentially instantaneous, and the maximum blood concentration is rapidly reached as the drug is distributed throughout the blood and mixed throughout the circulatory system. Further distribution to other tissues in the body, and the elimination processes will result in varying concentrations in different locations in the body. Hence, an understanding of drug concentrations in the body requires that the relative speed of competing processes be taken into account if tissue concentrations are to be used to re-create dosage events. Toxicologists employ compartment models to facilitate such calculations.[1,2] A compartment does not necessarily correlate with a single, isolated place in the body, such as a finger or a lung. A compartment is better thought of as a barrier or partition over which equilibrium must be established before the plasma concentration reaches a steady state.[2]

A one-compartment model (Figure 5) is based on the plasma—also referred to as the *central compartment*. A first-order rate constant k_a determines how fast the drug is absorbed into the bloodstream, and the first-order elimination rate constant k_{el} describes the speed at which the drug is removed. A logarithmic plot of plasma concentration versus time is linear with a slope of $-k_{el}/2.303$. The plasma concentration as a function of time elapsed since the initial dose (C_0) is described by the relationship

$$C_t = C_0 e^{-k_{el}t} \quad \textbf{(5)}$$

Note that this is the same equation used to derive the first-order half-life described in equations 1 through 6. The rate constant is specified as the elimination rate constant k_{el}. The first-order rate law can be restated to allow the calculation of concentration as a function of time:

$$\ln (C_t) = -kt + \ln (C_0) \quad \textbf{(6)}$$

If not given, the rate constant can be derived from the half-life and equation 4. A single-compartment model is based on the assumption that an injected drug is instantaneously distributed in the plasma and tissues.[2] Such a model is a good starting point and applicable in many situations.

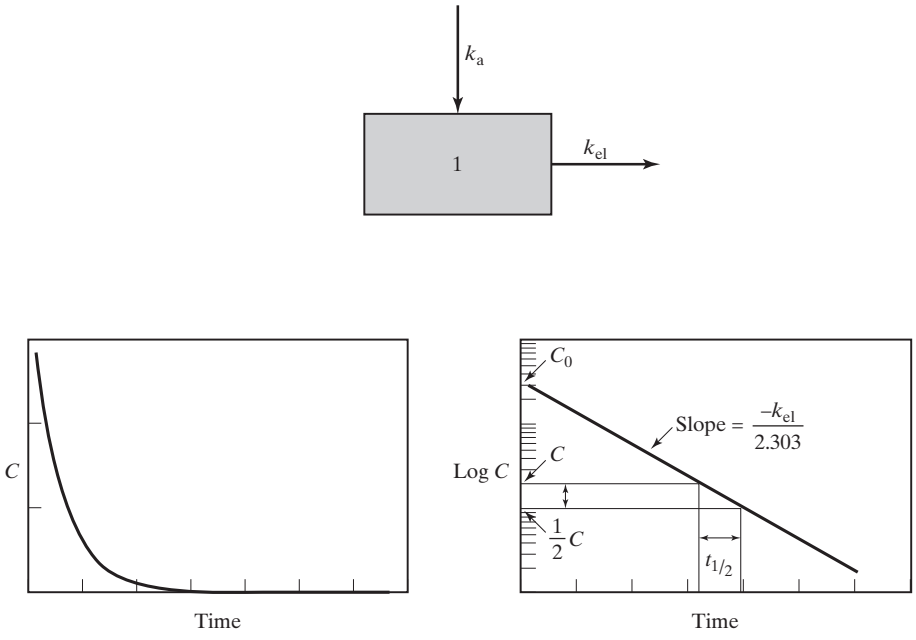


FIGURE 5 A one-compartment model with first-order elimination. The rate constant and half-life can be derived from the logarithmic plot of the concentration as a function of time.

In many situations, the logarithmic plot of concentration versus time is a curve, indicating more than one stage of elimination from the central compartment. In turn, this implies the existence of more than one compartment through which the drug must move. In these situations, a two-compartment model (Figure 6) can be invoked.[1,2] Not surprisingly, even the two-compartment model is sometimes inadequate. To cope with the complexity involved, physiological models are invoked.[2] These are more realistic and flexible but are beyond the scope of the introductory material presented here. Even so, a cursory glance at Figure 7

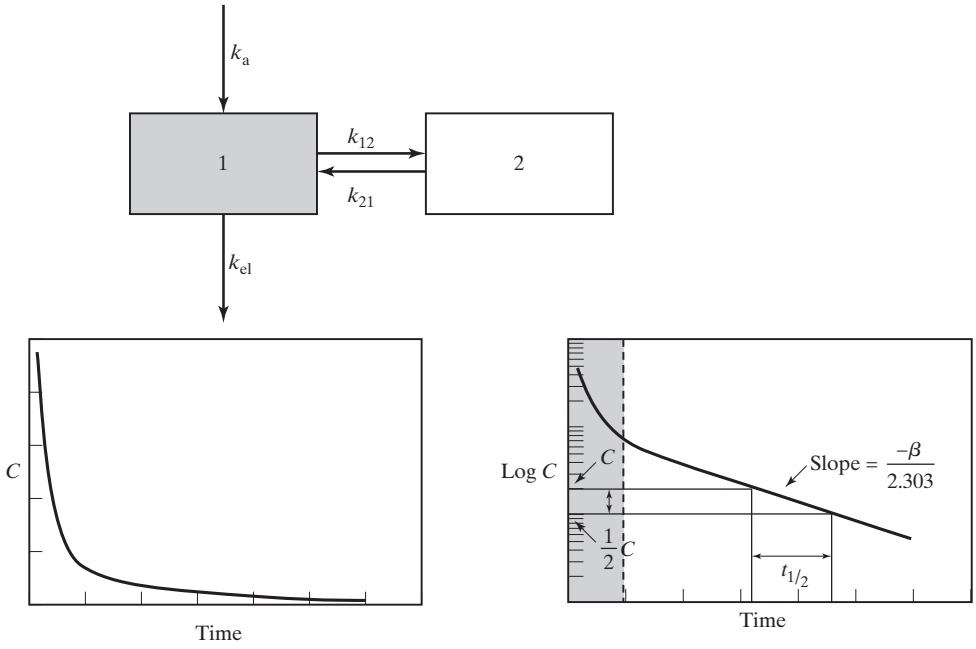


FIGURE 6 A two-compartment model in which the central compartment is linked to another compartment with speed of movement described by new rate constants.

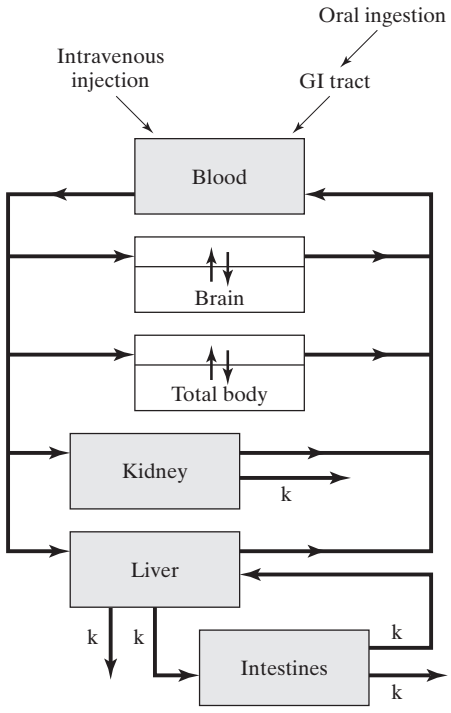


FIGURE 7 An example of a physiological model with many compartments, partitions, equilibrations, and rate constants.

shows that such models retain the components of partitioning, distribution coefficients, and rate constants. These models also are based on the fundamental steps of ADME, which can now be discussed in detail.

When a drug is injected directly into the bloodstream, maximal plasma concentration C_{max} is quickly established. We will assume that C_{max} becomes C_0 in the first-order elimination process (equation 6). When a drug is taken orally, several factors control how long it takes for the compound to establish the peak initial value of C. The absorption of a drug in the gastrointestinal (GI) tract first requires that the drug be soluble, or transportable across the intestinal wall. Solubility of the drug formulation may also be a factor, because time-release formulations or coated tablets are used to control when or where in the GI tract dissolution occurs.

4 ABSORPTION (A)

Once a drug reaches the GI tract, it generally has to be absorbed into the bloodstream to create a physiological effect. Absorption requires that a drug transit through a lipid membrane, so any drug with an ionizable center must be in the neutral state (HA or B) to favor partitioning into the hydrophobic membrane. If a drug is sufficiently lipophilic to partition into the lipid barrier, there still must be some driving force for the process. In most cases, the driving force is the existence of a **concentration gradient** (here, a relatively high concentration in the GI tract and a relatively low concentration in the bloodstream). Diffusion driven by the gradient is referred to as **passive diffusion**.[†] Understanding this allows us to expand on our earlier estimation where in the GI tract drugs are likely to be absorbed.

Perhaps you wondered as you were reading that section about basic drugs such as methamphetamine ($pK_a > 9$) that have relatively high pK_a values. According to our simple model, nowhere in the GI tract would the pH be sufficiently basic for the drug to be in the neutral B form to any significant degree (recall the ± 2 pH guideline). If this were the only factor at work, then basic drugs would be poorly absorbed if at all; yet we know that this absorption is in fact generally efficient and effective. Clearly, there is more to the process than pH and concentration gradients. An important consideration is the structure of the intestinal tract below the stomach. We can think of the intestinal tract as a tube with a combined (small intestine and large intestine) length of about 24 feet. This length translates into a large surface area, which is further increased owing to the ribbed surface of the interior walls of the intestinal tract. This length and corrugated surface provides ample opportunity for a drug to be absorbed, yet structure alone does not explain how basic drugs get across the membrane. No matter how large the surface, ions still do not partition into lipid membranes to any significant degree.

Part of the explanation relies on a familiar concept, Le Châtelier's principle. As seen in Figure 8, we can think of the GI tract/lipid membrane/bloodstream interface as a system attempting to establish equilibrium. For a basic drug with a high pK_a, relatively few molecules will be in the neutral B form at pH values 2 or more pH units

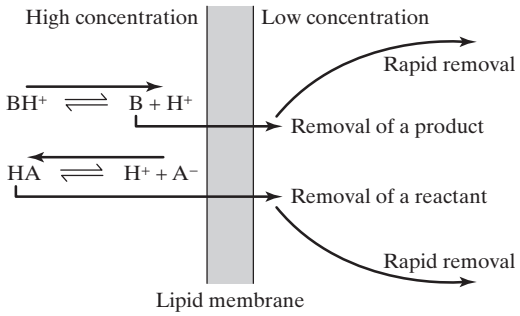


FIGURE 8 To be absorbed, drugs must partition into and out of a lipid membrane. The process is driven by a concentration gradient. Once again, for our introductory purposes, we are not considering active transport mechanisms.

[†]There are also many important active transport mechanisms, but these are beyond the scope of this text. You can find much more about this and related topics in the texts listed as recommended reading.

below the pK_a. A few are enough however, if we recall basic principles of equilibrium. Un-ionized molecules (B) will partition into the lipid membrane and will move into the bloodstream. The gut has a high blood flow, so the few molecules in the B form will be quickly swept away from the site of the absorption. In terms of the equilibrium, we are rapidly removing product and driving the reaction to the right ($BH^+ \rightleftharpoons B + H^+$) for basic drugs or to the left ($HA \rightleftharpoons H^+ + A^-$) for acidic drugs. Thus, if there is sufficient space and opportunity for this process to occur, drugs can successfully be absorbed in the intestinal tract despite the mismatch between the pH and the pK_a. Because there is so much surface area in the intestines, the absorption process is much more efficient than simple pH/pK_a considerations suggest. This model of drug absorption is referred to as the **pH-partition hypothesis**.

In contrast to injected drugs, orally administered drugs pass through the liver before entering the general circulation[2] and are subject to **first-pass metabolism**. These processes occur before the drug is distributed around the body and before pharmacological effects are observed. To account for the changes that occur, the bioavailability (F) can be used. **Bioavailability** is defined as the ratio of drug that would be available after intravenous injection compared with that which is actually available after oral administration. F is expressed as a percentage, and the higher the F value, the smaller is the loss of the active form of the drug to first-pass metabolic changes. The value of F can be determined experimentally by comparing the AUC (Figure 4) that results from an oral dose compared with the AUC obtained from an intravenous injection.

EXAMPLE PROBLEM 1

The bioavailability of morphine is reported to be between 20% and 30%. A common pharmaceutical preparation of morphine is in the form of 40-mg tablets that contain 40 mg of the drug (as opposed to the salt). If a person takes two pills at the same time, how much morphine will be available to enter the general circulation?

Answer:
It is not unusual for pharmacological data such as F and $t_{1/2}$ to be reported as ranges. The first challenge is to decide which value to use. If you have no additional information, it is reasonable to select the middle of the given range, here 25%. The calculation is straightforward:

2 tablets $\times$ 40 mg/tablet = 80 mg $\times$ 0.25 = 20 mg available to enter the general circulation

In such instances, you should always make clear what assumptions were made and why. This allows honest evaluation of your work by other experts (and your instructor!).

Other modes of ingestion follow the same basic steps of ADME, but the specific processes and mechanisms differ. For example, substances introduced intravenously can immediately be distributed, since the drug does not have to cross a barrier to enter the bloodstream. Gases entering the lungs are also nearly in direct contact with the blood. Xenobiotics on the skin must move through seven layers of the outer epidermis before contacting capillaries and blood, making skin a good protective barrier against many toxic agents.[3]

The distribution of a drug in tissues is determined by considerations similar to those governing absorption. Depending on these factors, a drug may preferentially reside in the blood, in fatty tissues, or in other locations, such as the liver. The **volume of distribution** is a quantitative expression of this distribution and is given by

$$V_d = \frac{D}{(C_p \times \text{kg})} \tag{7}$$

where D is the dose of the drug; C_p is the plasma concentration once equilibrium has been reached in the plasma, but before elimination of the drug has begun; and kg is the person's body weight in kilograms. Put another way, the volume of distribution is the volume of fluid in which a given amount of the drug would have to be dissolved to obtain the same concentration as that found in the plasma. Several factors dictate the volume of distribution, including lipophilicity and plasma protein binding. For example, if a large fraction of a drug binds to plasma proteins, the volume of distribution will be smaller; there is less of the drug available to diffuse into tissue and thus less fluid will be needed to achieve the corresponding plasma concentration. Protein binding is illustrated in Figure 9.

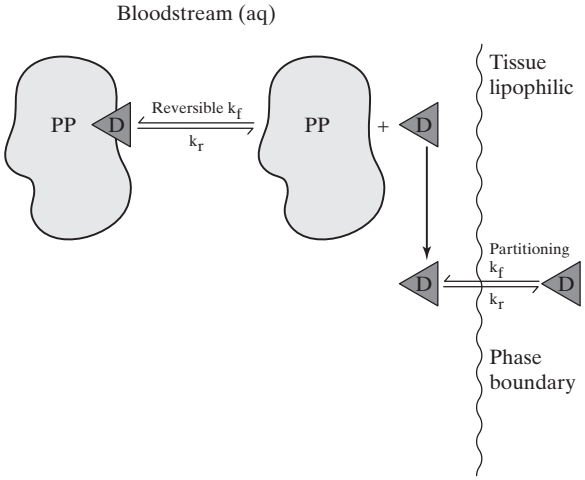


FIGURE 9 Distribution into the tissues. To move into tissue, a drug must be free from any bound plasma proteins (PP). If the protein involved is large (~30kdal or larger), the rate of elimination of the bound species is essentially zero. Only unbound drug is eliminated.

EXAMPLE PROBLEM 2

Return to the data presented in Example Problem 1. Assume a woman who weighs 145 lb takes two tablets of morphine. Use V_d to estimate the peak plasma concentration. The range of V_d is 3–5 L/kg.

Answer:
From the previous example problem, we know that the dose, corrected for bioavailability, is 20 mg. We also need to make an assumption regarding which volume of distribution to use; we select 4 L/kg, since we have no other information available. The only remaining chore is to convert the body weight to kilograms. These types of problems often revolve around the units and unit conversion, so always write these down and use your knowledge of dimensional analysis to check results.

$$V_d = \frac{\text{dose (mg)}}{C_p \times \text{kg}}$$

$$C_p = \frac{\text{dose(mg)}}{V_d \times \text{kg}} = \frac{20 \text{ mg}}{4.0\frac{\text{L}}{\text{kg}} \times 145 \text{ lb} \times \frac{1\text{kg}}{2.2 \text{ lb}}} = 0.076\frac{\text{mg}}{\text{L}} = 76\frac{\mu\text{g}}{\text{L}}$$

We convert to ppb (μg/L) because this is the most convenient unit. However, in medical, pharmaceutical, and toxicological literature, frequently values are reported in mg/L. You have to be aware of the units at all times and use those which make the most sense in the context of the problem or case at hand. Notice also that we are being a bit cavalier about significant figures here. Why? Because we are making assumptions for both V_d and for F. The assumptions are valid and defensible in the context presented, but we do not want to imply greater accuracy than the data support. Our result of 0.076 mg/L for a peak plasma concentration is an estimate, but a reasonable one.

The volume of distribution is not a physical volume but, rather, a quantitative expression calculating how a drug is distributed in compartments of the body. It is used comparatively, but it is not a physicochemical descriptor with a single definable value in the sense that p*K*a is, for example. For this reason, it is referred to as the **apparent volume of distribution.**[2,4] In general, the higher the V_d, the more the drug is distributed into tissues from the blood. This can be related to the lipophilicity of the drug or a reflection of how much of the drug is taken up by transport proteins. Drugs that are widely distributed throughout tissues in the body will have a comparatively higher volume of distribution compared with drugs that tend to remain in the bloodstream and are thus less widely distributed throughout the body. As we noted in the preceding paragraph, plasma protein binding also plays a role. If the drug is administered orally, the expected value of C_p has to be adjusted on the basis of the bioavailability. The value of V_d depends on the lipophilicity of a drug, the degree of ionization pK_a, the association of the drug with proteins, the degree of protein transport, and many other physical and chemical parameters.[5–7] V_d provides a measure of the relative partitioning of the drug between the plasma and other tissues. If two compounds experience roughly the same degree of binding to plasma proteins, the compound with the greater affinity for association with the tissues will have a larger V_d relative to the compound with more affinity for the plasma. Compounds that are lipophilic also tend to have higher V_d values.[7] Note that calculations of V_d combine all tissues together and consider them as one unit.[6,8]

EXAMPLE PROBLEM 3

The desired therapeutic concentration of morphine for a man weighing 180 lb is 0.05 mg/L. Assuming that V_d for morphine is 4 L/kg and the bioavailability is 25%, calculate the oral and injected doses needed to obtain the desired plasma concentration.

Answer:

$$V_d = \frac{D \longrightarrow ?}{C_p\ k}$$

$\frac{4\text{ L}}{\text{kg}}$ $\frac{0.05\text{ mg}}{\text{L}}$ 81.65 kg 180 lb

$$D = \frac{4\ \cancel{\text{L}}}{\cancel{\text{kg}}} \bullet \frac{0.05\text{ mg}}{\cancel{\text{L}}} \bullet 82\ \cancel{\text{kg}} = 16.4\text{ mg} = 16\text{ mg injected}$$

For an oral dose, $F = 25\%$ meaning that
three fourths of the dose is lost to first-pass metabolism.
Thus, the oral dose must be larger:

$$0.25D = \frac{4\ \cancel{\text{L}}}{\cancel{\text{kg}}} \bullet \frac{0.05\text{ mg}}{\cancel{\text{L}}} \bullet 82\ \cancel{\text{kg}}$$

$F = 25\%$ $D = 65.6\text{ mg} = 66\text{ mg oral}$

As mentioned in the previous paragraph, drugs in the bloodstream may bind with plasma proteins such as albumin. This binding is illustrated in Figure 9. The extent of such binding is dependent on many factors, including the concentration of the drug and the relative affinities of the drug and protein for each other, as well as their affinities for other substances found in the plasma. The drug must be in the unbound form to cross into the tissue, so protein binding can be useful in controlled-release applications.

Protein binding affects V_d, since binding prevents crossing into tissues. The more a substance binds with a plasma protein, the more it will tend to stay in the plasma. Forensic toxicologists can use V_d values as a starting point to estimate the amount of ingested drug using equation 7.

5 METABOLISM (M)

The major tissue site of metabolism (outlined in Figure 10) for most drugs is the liver. The biological goal of metabolism is deactivation and elimination of the xenobiotic substance. In general, the more susceptible to oxidation a compound is, the more likely it is to be metabolically altered.[7]

Understanding the mechanism of the metabolism and **biotransformation** of xenobiotic substances such as drugs is essential to forensic toxicology. To estimate the initial dose of a parent drug, the toxicologist must know what biotransformational products are produced and in what relative quantities. Beyond that, it is important to understand these processes and how they work. Therefore, we will delve more deeply into biotransformation and metabolism. We will focus on oral ingestion of drugs and metabolism taking place in the liver, but keep in mind this is not the only way these processes can occur. In oral ingestion (Figure 11), absorbed drugs are transported to the liver via the portal vein. As noted previously, the liver is where first-pass metabolism occurs. With regard to bioavailability, the loss of drug to first-pass metabolism is usually the most important factor contributing to the loss of drug between ingestion and distribution. In some situations, first-pass metabolism is an integral part of the drug design and produces the active form of the drug. In such cases, the parent compound ingested is called a **prodrug**. The prodrug is pharmacologically inactive, but the metabolites are active. A forensic example is heroin, which rapidly metabolizes to morphine, which is the principal pharmacologically active species associated with heroin (the prodrug).

As noted in Figure 10, metabolic transformations are typically discussed in the context of phase 1 and phase 2 reactions. Phase 1 metabolism involves the addition or activation of functional groups with the goal of increasing the water solubility of the product. A few examples are presented in Table 2.

It should not be surprising that phase 1 reactions are oxidative, since adding an oxygen to a molecule is likely to increase water solubility. Oxidation can occur at many different points on the molecule; often, these reactions are classified based on where the oxidation occurs. In the case of

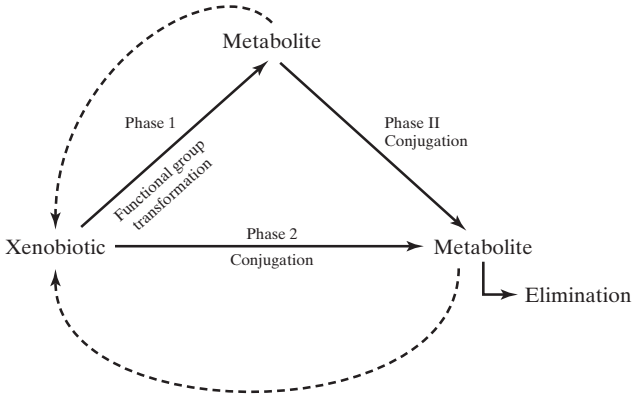


FIGURE 10 General flow of metabolism. Not all metabolic pathways go through Phase 1, and at each step, metabolic by-products may reenter the cycle as new xenobiotics.

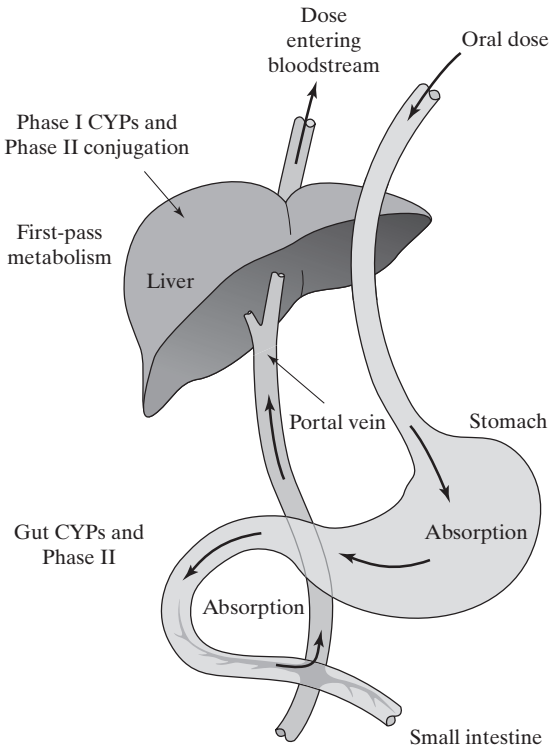
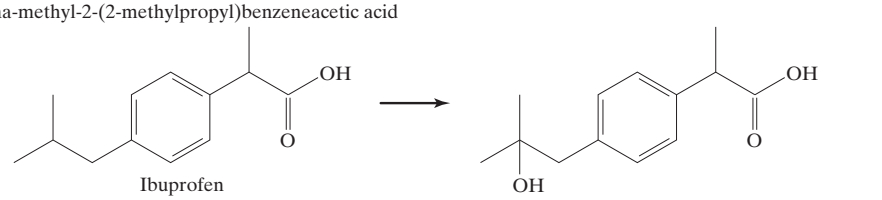
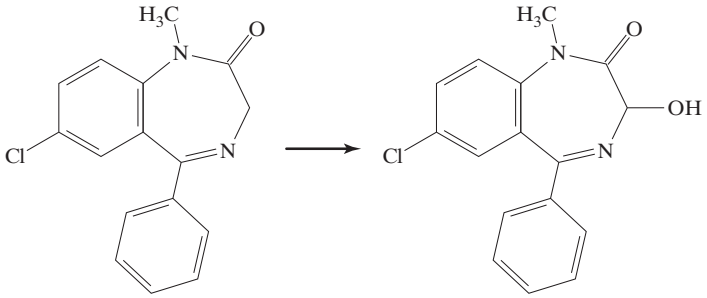
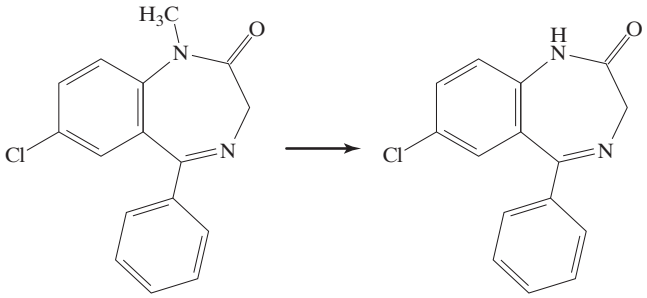


FIGURE 11 Pathway taken by an orally ingested drug.

TABLE 2 Selected Phase 1 Biotransformations

Type (generic)	Example Compound	Reaction
Oxidation	Ethanol	$CH_3CH_2OH \longrightarrow CO_2$
Oxidation	Ibuprofen	alpha-methyl-2-(2-methylpropyl)benzeneacetic acid  Ibuprofen; 2-(4-(2-hydroxy-2-methylpropyl)phenyl)propanoic acid 2-hydroxy metabolite of Ibuprofen
Oxidation/ Hydroxylation	Diazepam	 Diazepam; 3-hydroxylation metabolite
Dealkylation	Diazepam	 Diazepam
Oxidative deamination	Amphetamine	 Amphetamine; Oxidative deamination metabolite (phenylacetone)

ibuprofen, we can classify this reaction as a side-chain oxidation. Hydroxyl groups can also be added to aromatic rings and heterocyclic systems as well as to methyl groups. Similarly, dealklyation can occur at different locations, such as the N-dealkylation shown. Some drugs and their metabolites undergo hydrolysis. Cocaine is among the compounds that are susceptible to these types of conversions. The products of Phase 1 metabolism (the biotransformation products) may or may not be pharmacologically active, and for some xenobiotics, metabolites may be toxic.

EXHIBIT C

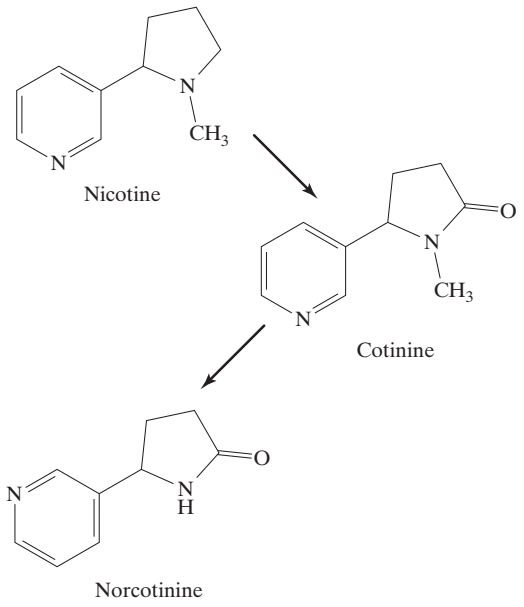
Toxic Metabolites

The metabolite produced from a drug or some other exogenous substance is often pharmacologically active. Such substances are referred to as *active metabolites*. In some cases, active metabolites are responsible for toxic effects that exceed those of the parent substance. Methanol, ethylene glycol, and DDE are examples of active toxic metabolites. Methanol (wood alcohol) can be ingested accidentally or intentionally. Methanol is metabolized similarly to ethanol, via a two-step oxidation to an aldehyde (formaldehyde) that is converted to an acid (formic acid). The acid causes toxic effects such as acidosis and impaired cell respiration, and penetration of methanol into the vitreous humor can lead to blurred vision and blindness. The mechanism is neurotoxicity as a result of formaldehyde in the optic nerve.

Ethylene glycol (antifreeze) has a sweet taste that makes it attractive to animals and children, resulting in frequent accidental poisonings. Although the metabolic process is more complicated, active toxic metabolites of the corresponding aldehyde and acids are produced. Particularly insidious is the formation of crystals of calcium oxalate (K_{sp} (hydrate), $\sim 10^{-9}$), which physically damages the kidney and may be detected as crystals in the urine.

EXAMPLE PROBLEM 4

Nicotine can be metabolized to cotinine and norcotinine. Describe each step in terms of the type of Phase 1 metabolic transition involved.



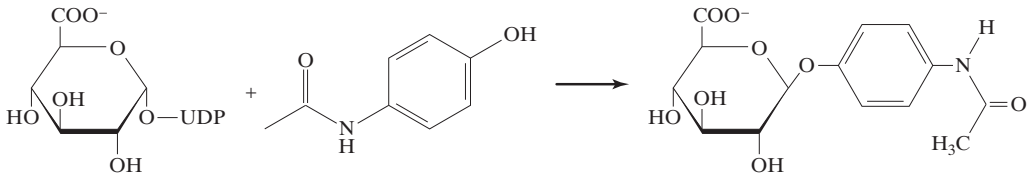
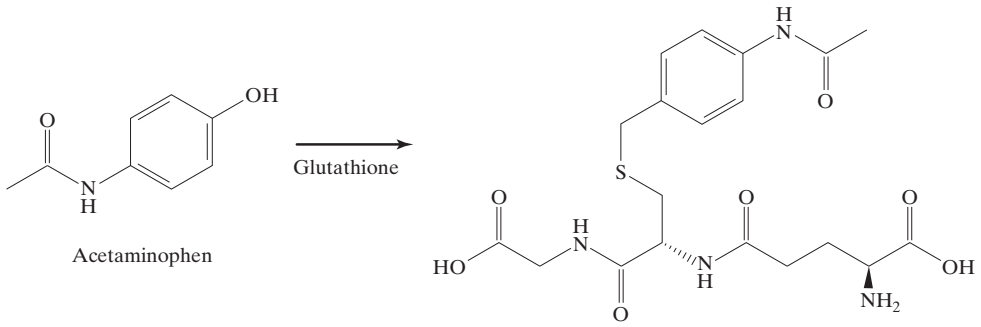
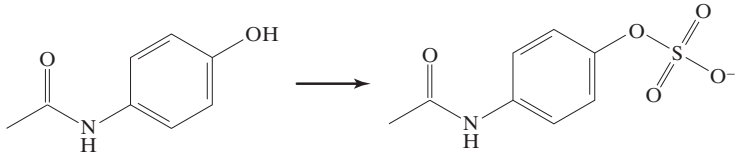
Answer:

The first step is an oxidation on the pyridine ring. In this case, a two-step process takes place, first addition of an −OH (hydroxylation) and then an oxidation (loss of hydrogen). This yields continine. The next step is a demethylation to norcotinine.

Phase 2 metabolism involves the bonding of an **endogenous** (native to the body) hydrophilic species to the drug molecule. As with metabolic transitions, there are many types of conjugations. Table 3 presents examples of two common conjugations, one with **glucuronic acid** and one with an amino acid (glutathione in this example). Formation of **glucuronides** is frequently an issue in forensic toxicology, because many drug and metabolites form these conjugates. For example, morphine can form two conjugates, 3-*O*-glucuronide and 6-*O*-glucuronide (Figure 12); many of the phenylethylamines conjugate; as do aspirin and ibuprofen. The conjugate may form with the parent drug or with a metabolite and once conjugated with the hydrophilic group, elimination of the drug or metabolite via the kidneys and urine is much more likely. The conjugates introduce a complication to analysis and require pretreatment of the sample to break the conjugation.

The process of conjugation requires an activated form of the conjugate, as shown in Figure 13. The top portion of the frame shows cyclic glucuronic acid bonded to a uridine diphosphate structure (UDP or UDPA), which activates the glucuronic acid. This is the structure that interacts with the drug molecule. The conjugate replaces a hydroxyl hydrogen and as a result, significantly increases the water solubility of the compound. In most, but not all, cases the glucuronide form of the drug or metabolite is pharmacologically inactive. Another example of conjugation is with amino acids, which can occur if the drug or metabolite has a carbonyl group. Examples include many of the NSAIDs, such as acetaminophen, shown in Table 3.

TABLE 3 Selected Phase 2 Biotransformations

Conjugate	Reaction
Glucuronic acid	
Glutathione (amino acid)	
Sulfation	

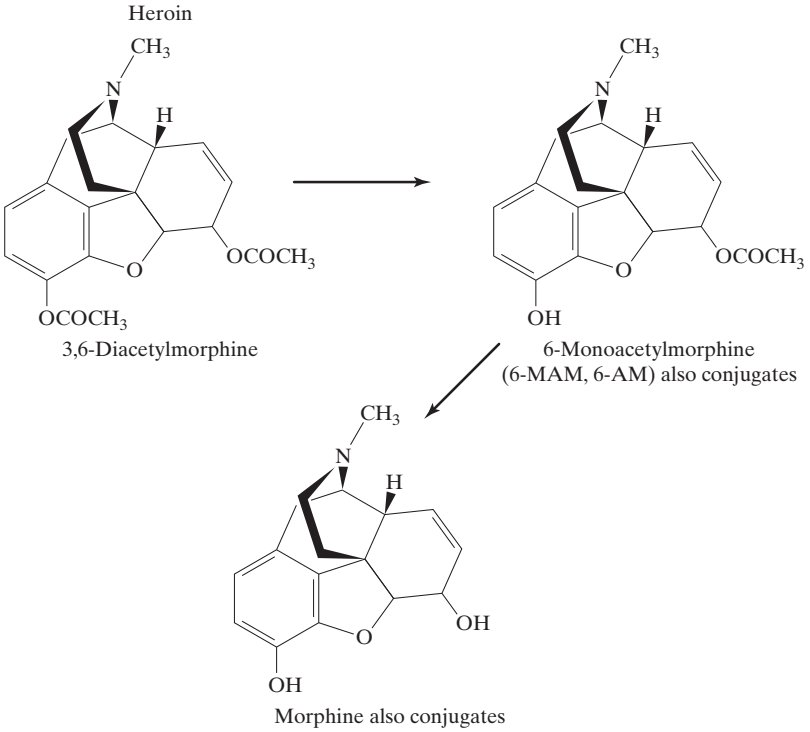


FIGURE 12
Metabolism of heroin.

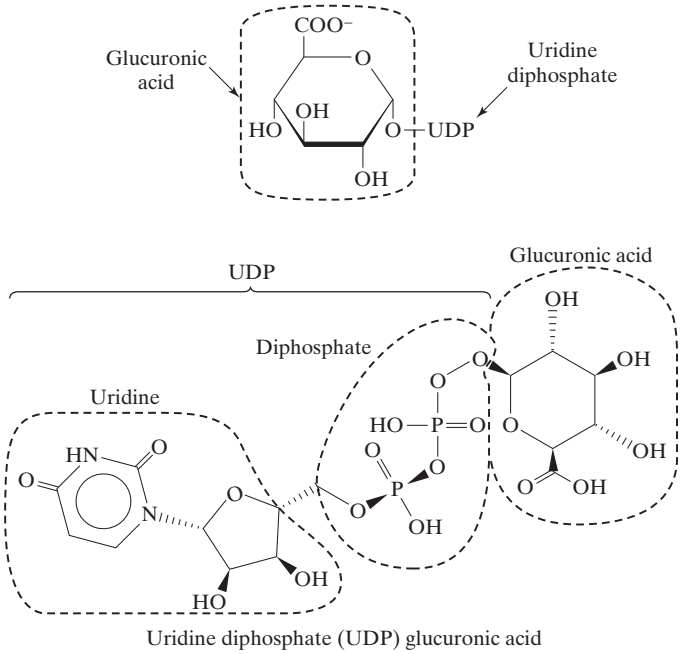
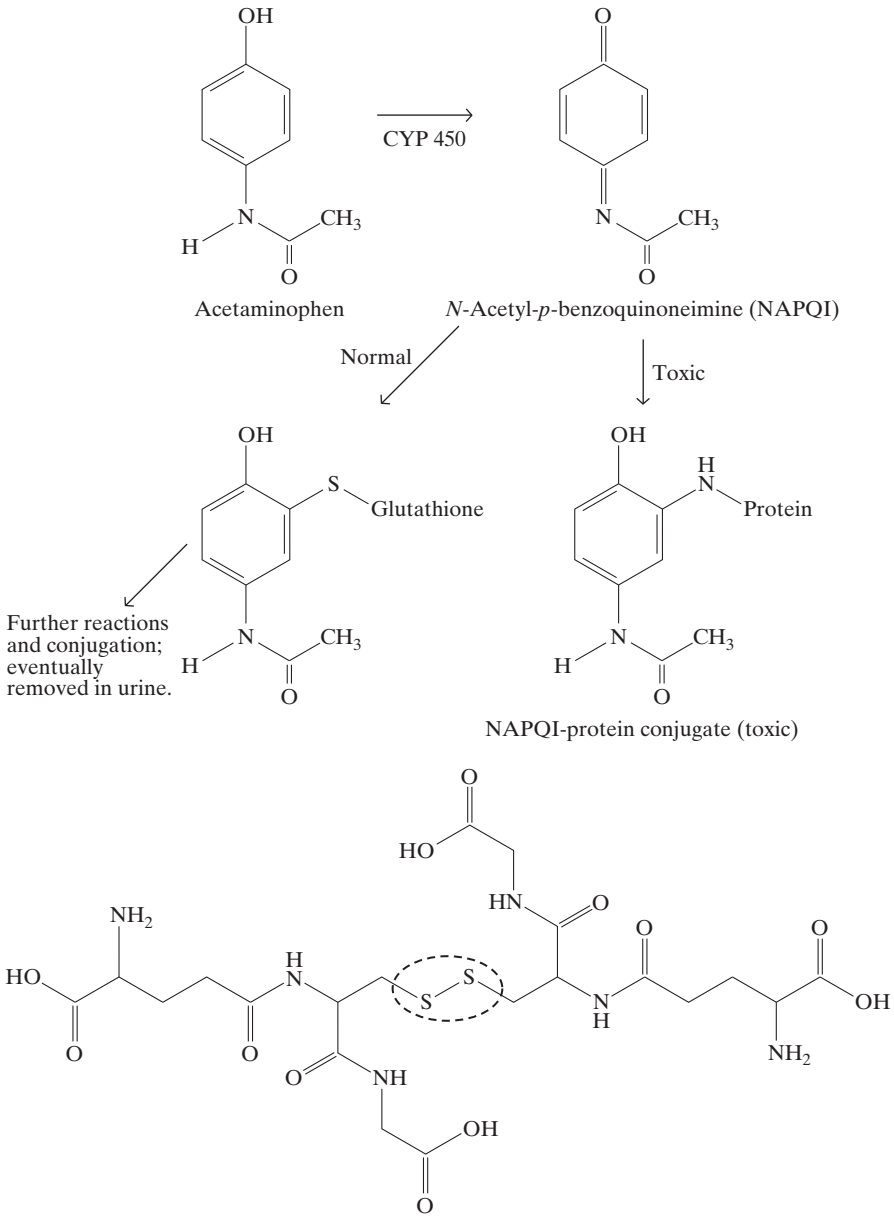


FIGURE 13 The top frame shows glucuronic acid in the activated form, bound to the UDP complex.

EXHIBIT D

Tylenol and Toxicity

Tylenol (acetaminophen or paracetamol) is one of the most widely used OTC analgesics. It is also the cause of many hospital admissions and not an uncommon means of committing suicide. The most pronounced feature of Tylenol poisoning is liver damage, which we can now understand in terms of metabolic processes. Under normal dose conditions, acetaminophen undergoes metabolism in the liver to produce primarily glucuronide and sulfate conjugates. A



small portion is oxidized (recall that a loss of hydrogen is an oxidation) to produce a compound that, through interactions with hepatic proteins, causes liver damage. At normal dose levels, much of this potentially toxic compound is eliminated via conjugation. At extremely high doses, this channel fails when glutathione is exhausted. The compound N-acetylcysteine is used to treat overdoses by providing an alternative SH pathway to eliminate the toxic compound.

Sources: Cairns, D. *Essentials of Pharmaceutical Chemistry,* 3d ed. London: Pharmaceutical Press, 2008; Moffat, A. C., M. D. Osselton, and B. Widdop, eds. Chap. 5 in "Paracetamol." *Clarke's Analysis of Drugs and Poisons,* 3d ed., Vol. 2. London: Pharmaceutical Press, 2004, p. 1392.

Phase 1 and Phase 2 biotransformations are catalyzed by enzymes. For example, the sulfation of acetaminophen is catalyzed by sulfotransferase. Note the terminology; whenever you see an *–ase* ending, the compound is an enzyme. There are several enzyme systems that come into play in drug and toxicant metabolism, the most important of which is the CYP450 family. The name is derived from *cytochrome monooxygenases* and the 450 refers to the wavelength of light where these enzymes have a strong absorption. The word **CYP** is pronounced "sip," and the collection of these enzymes is referred to as the "sips." There are many forms of CYP enzymes (collectively referred to as isoforms), which are classified into groups called families and subfamilies. The CYP450s are found primarily in the liver, bound to membranes. All CYP molecules contain a molecule of heme and a protoporphyrin. The variation among the molecules arises from an attached protein chain that is on the order of 50,000 daltons long. Table 4 presents a selection of CYPs that are involved in drug metabolism reactions of forensic interest. Of these, CYP3A4 is responsible for the largest fraction of oxidation reactions of drugs, approximately 40%. All the transformations presented in Table 4 metabolism are catalyzed by CYP450s.

Conjugation reactions are also catalyzed by enzyme families. Glucuronidation is catalyzed by the enzymes categorized as **UPD**—glucurinosyltransferases (UGTs). As with the CYPs, there are families (two) and subfamilies of these enzymes that are categorized and identified using a nomenclature similar to that used for CYPs. Although the topic exceeds the scope of this text, one of the fascinating aspects of drug metabolism is that many of these enzymes are polymorphic, meaning that the exact structure varies across the population. As a result, the efficacy of metabolism varies for some drugs. This topic is referred to as pharmacogenetics, which was mentioned in the introduction to the chapter.

TABLE 4 Selected CYP450 families

Family	Designation	Subfamilies Important in Drug Metabolism
1	CYP1	CYP1A1, CYP1A2, CYP1B1
2	CYP2	CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2D6, CYPE11
3	CYP3	CYP3A4
4	CYP4	CYP4A9, CYP4A11, CYP4B1

Sources: Cairns, D. *Essentials of Pharmaceutical Chemistry*, 3d ed. London: Pharmaceutical Press 2008; Wermuth, C. G., ed. *The Practice of Medicinal Chemistry*, 2d ed. London: Academic Press, 2003.

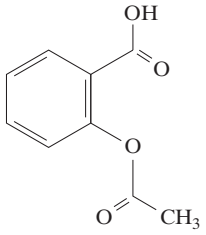
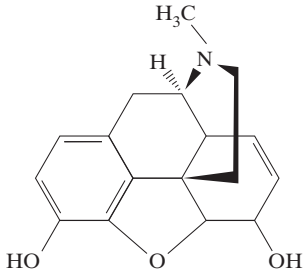
6 ELIMINATION (E)

The final step is excretion of the drug and metabolites from the body. Water-soluble materials are excreted in the urine and in sweat. If a metabolite is a gas, such as CO_2, or has appreciable vapor pressure, as does ethanol, the drug or metabolite can be eliminated by exhalation. Excretion in feces, tears, and any other body fluid is also possible.

Table 5 shows selected toxicological parameters for two sample compounds we have frequently discussed: aspirin and morphine. The pK_a values and log *P* values help predict characteristics of absorption if the drug is taken orally. Aspirin, with the more negative log *P*, is hydrophilic and lipophobic compared with the amphoteric morphine. The loss that results from oral ingestion is reflected in *F;* none is reported for aspirin, since it is delivered orally rather than by injection and is not subject to significant metabolism by the liver. Morphine can be delivered by either route, although the price paid in the oral route by first-pass metabolism is significant. The V_d and protein-binding data provide information about how the drug partitions between the central plasma compartment and other tissues, quantities that also relate to the log *P* value, which measures lipophilicity. Not surprisingly, the more lipophilic morphine has a higher V_d value and a longer half-life in plasma, likely due to its stronger association with lipophilic tissues that result in its lingering in the plasma longer than does aspirin, which is rapidly hydrolyzed to salicylic acid.

The half-life of a drug or other substance in the plasma represents the amount of time it takes for half of the original concentration to disappear. Half-life does not take into account why or how that concentration is lowered only that it is no longer present in the plasma compartment. The "missing" concentration may be attributed

TABLE 5 Example Toxicological Factors

	Aspirin	Morphine
Structure		
pK_a	3.5	8.0, 9.9
Log *P*	−1.1	−0.1
$C_{therapeutic}$	100 mg/kg	0.01–0.07 mg/L
F%	Essentially 100%	20%–30%
$t_{1/2\ plasma}$	17 min	2–3 hours
V_d	0.15 L/kg	3–5 L/kg
Protein binding	90%	20%–35%

to metabolic changes, but it may also be due to tissue uptake such that it is no longer detected in the plasma. An example of this is THC which rapidly disappears from the plasma, and then is slowly metabolized such that the metabolic products can be detected in the urine for several days after the ingestion. A more inclusive and useful parameter is the *clearance rate*, which does take into account other processes and factors, such as metabolism and distribution, along with half-life. In general, the more lipophilic a compound is, the more likely it is to be subject to various processes that in turn can increase the clearance rate relative to a less lipophilic entity.[7] A number of variables affect the actual clearance rate in an individual, and as is the case with V_d and half-life, it is often necessary to select a value from a range of possible values based on the data available.

Estimates of time since the administration of a drug are made easier when there is more than one substance present in the blood, urine, or other tissue sample. Recall that metabolism produces metabolites that are also subject to elimination. The concentration of the drug in blood or another compartment will decrease as metabolism progresses and the concentration of the metabolite increases. If the metabolite is subject to further biological transformations, it, too, will decrease as the next metabolite increases, and so on. At certain times, all three may be present, whereas at others, only the drug or the final metabolite may be detectable. Knowing the metabolic pathways and reaction rates is critical to determining the time of ingestion.

In the example shown in Figure 14 (and also in 12), injected heroin is rapidly metabolized to 6-monoacetylmorphine (6-MAM). A slower, but still quick, conversion occurs to morphine, which has a much longer plasma half-life. A blood sample drawn even an hour after the initial injection might show no heroin but a distinctive ratio of 6-MAM and morphine that could be used to estimate the size and time of the initial injection.

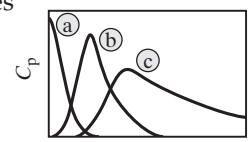


FIGURE 14 If a drug is metabolized, a drop in the concentration of the parent corresponds to an increase in the concentration of the metabolite. Here, *a* represents the plasma concentration of heroin ($t_{1/2} \sim 3$ min), *b* is the metabolite 6-monoacetylmorphine ($t_{1/2} \sim 20$ min) and *c* is morphine ($t_{1/2} \sim 2.5$ hr).

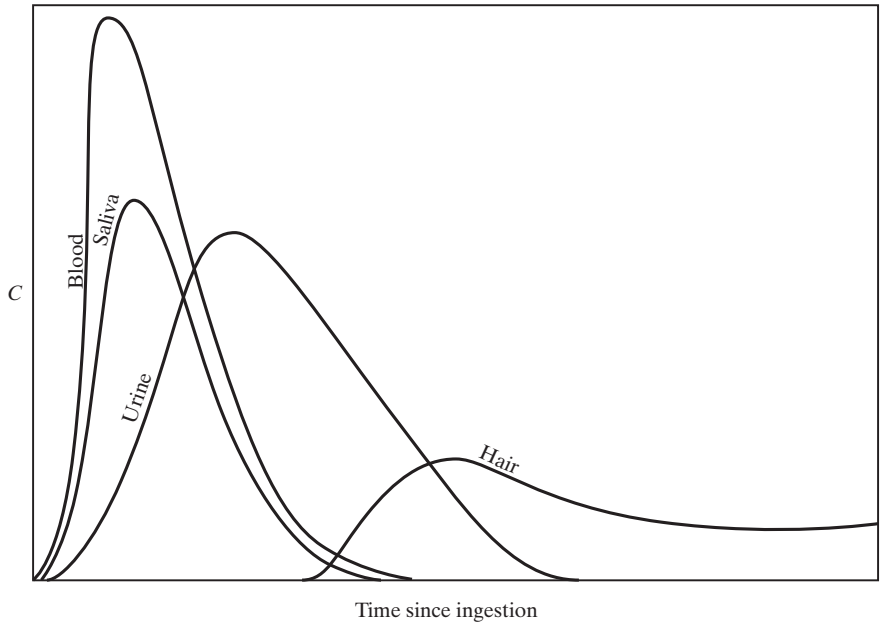


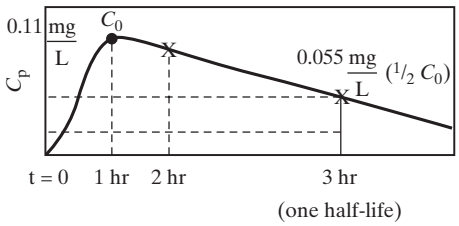
FIGURE 15 The concentration of a drug or metabolite may fall in one tissue and rise in another.

EXAMPLE PROBLEM 5

A woman weighing 135 lb takes two codeine tablets of 20 mg each as directed by her dentist to alleviate the pain associated with minor dental surgery. Assuming that the peak plasma concentration is reached in 1 hour, V_d of codeine is 3.0 L/kg, $F = 50\%$, and $t_{1/2}$ for codeine in plasma is 3.0 hours, complete the following tasks:

1. Sketch the ADME curve.
2. Calculate the peak plasma concentration.
3. Calculate the plasma concentration 2 hours after the woman took the pills. Note any assumptions.
4. Determine when the plasma concentration of codeine will become too small to be detected by a mass spectrometry method with an LOD/LOQ of 1.0 ppb. Assume that the blood sample is injected directly into the instrument.

①



②

$$V_d = \frac{D}{C_p \bullet kg} \qquad C_p = \frac{D}{V_d \bullet kg}$$

$$C_p = \frac{40\ mg}{3.0\ L \bullet 61.2\ kg} = \frac{0.22\ mg}{L}$$

but this value must be corrected for bioavailability; 50% is lost to first-pass metabolism:

$$C_p = \frac{0.11\ mg}{L}$$

③ *We will assume that the peak plasma concentration C_p is the nominal value of C_0 for the elimination calculations. Two hours after ingestion is 1 hour after peak plasma concentration is reached, as seen on the sketch.*

$$\ln C_t = -kt + \ln C_0$$

Use $t_{1/2}$ to find k:

$$t_{1/2} = \frac{0.693}{k} \qquad k = \frac{0.693}{t_{1/2}} = \frac{0.693}{3\ hr}$$

$$k = 0.23/hr$$

$$\ln C_t = (-0.23/hr)(1\ hr) + \ln(0.11)$$

$$\ln C_t = -2.44 \qquad C_t = 0.087 \frac{mg}{L}$$

④

$$LOQ = 1.0\ ppb = \frac{1.0\ \mu g}{L} = \frac{0.001\ mg}{L} \quad \text{(units must match } C_p\text{)}$$

$$\ln(0.001) = -0.23/hr\ t + \ln(0.11)$$

$$\ln(0.001) - \ln(0.11) = \frac{-0.23\ t}{hr}$$

$$-4.70 = \frac{-0.23\ t}{hr}$$

$$t = 20.4\ hr$$

Thus, the cocaine becomes undetectable after ~21 hours of ingestion.

Finally, toxicologists must consider the different tissues or fluids in which a drug or metabolite may end up. As shown in Figure 15, as a drug is processed and excretion begins, concentrations of the drug and metabolites may be detectable in more than one tissue. If a compound enters the hair, it is somewhat protected from further changes or alteration, and there is an increasing interest in hair as a sample matrix in toxicology. Figure 15 illustrates a generic model for distribution, and the picture is again complicated with the addition of metabolites that may also partition among different fluids and tissues.

7 AN INTEGRATED EXAMPLE

Consider the simplified pharmacokinetics of a familiar small water-soluble molecule shown in Figure 16. Ingestion via the oral route leads to absorption primarily in the top portion of the small intestine, as mentioned earlier. Peak ethanol blood concentration

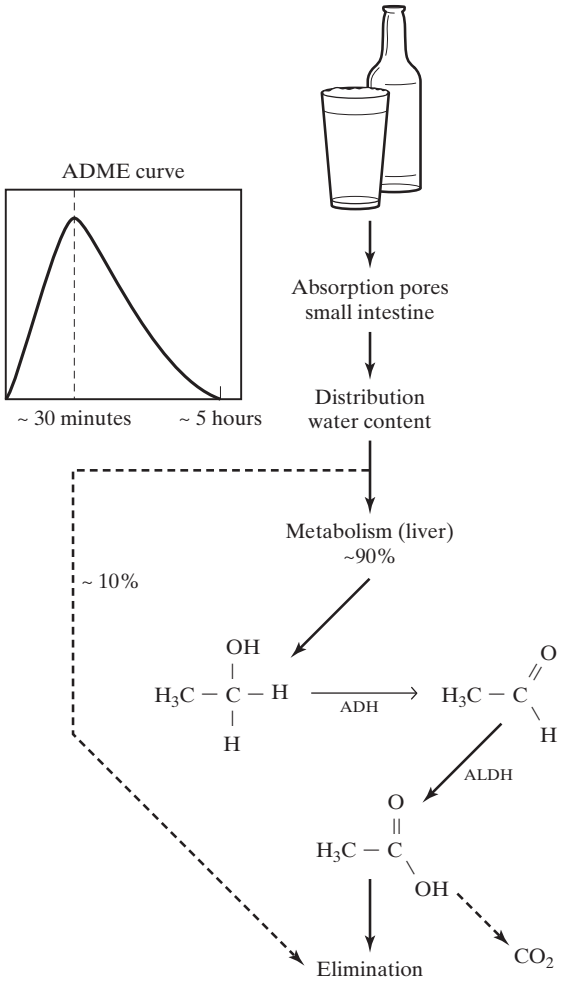


FIGURE 16 Simplified ADME pathway of ethanol. After ingestion, peak blood concentrations are reached in half an hour.

EXHIBIT E

Drug Recognition Experts (DREs)

The acronym DUI is commonly known to mean "driving under the influence," but intoxication and impaired functioning can result from the ingestion of many substances other than ethanol. Laboratory analysis is essential to determining which substance caused intoxication, but the work requires time. Rarely can it be completed while the subject is still intoxicated. To assist police officers in determining the likely source of intoxication, **drug recognition experts** (DREs) can be called. Certified DREs use a battery of tests and examinations, including interviews, physical examinations, and evaluations of vital signs, to offer an opinion as to which intoxicating agents are present. Blood and urine are also collected.

occurs roughly half an hour after ingestion, assuming that the subject stops at one dose. Ethanol distributes into tissues on the basis of their water content. The solubility of the molecule also means that a portion (~10%) of the dose will be excreted unchanged while the remaining portion is metabolized in a two-step process. Overall, the elimination of ethanol is an interesting example in kinetics because the order of the elimination depends on the ethanol concentration. At lower concentrations, on the order of 0.01 g/dL, the elimination follows first-order kinetics.[9]

To begin the elimination, alcohol dehydrogenases (ADHs) catalyze the conversion of ethanol to acetaldehyde, a compound thought to play a role in the symptoms of a hangover. This step is the rate-limiting step in the process.[9] Fortunately, the subject in this example drank responsibly and stopped at one dose, so suffering will be minimal. Aldehyde dehydrogenases (ALDHs) convert the acetaldehyde to acetate ion and acetic acid. Carbon dioxide is the final metabolic product and is eliminated via exhalation. The clearance rate of ethanol is about 0.016 g/dL per hour, leading to elimination of the initial dose in a few hours.[9]

Applying the Science 1 The More Things Change . . .

Some of the best chemists in the ancient world were brewers. The Sumerians appeared to favor beer, the Egyptians wine, but both cultures mastered brewing thousands of years ago. Making alcoholic beverages generally requires an understanding of two chemical processes: fermentation and distillation. In the investigation of archaeological samples, tartaric acid is considered a good marker for the presence of wine.

More challenging than detecting traces of ancient wine is determining whether the wine once held in a container was a red wine or a white wine. Not surprisingly, the reds are attributable to dyes. Older brewing methods were also an unintended source of lead poisoning, since lead was widely used to line pots and other distillation equipment. Lead salts are also sweet to the taste and were used frequently for this purpose given the scarcity of sugar. One early chemist offered this commonsense safety tip for distilling oils ("oyls"): *You must be very careful that the ashes and pot do not wax too hot, for if the oyl within takes fire it will break the vessels and flie up, that it can hardly be quenched, and reach the very ceiling; so that it is best to operate upon oyls in arched rooms.*

Sources: Egloff, G., and C. D. Lowry. "Distillation Methods, Ancient and Modern." *Industrial and Engineering Chemistry* 21 (1929): 920–23; Gauasch-Jane, M. R., et al. "Liquid Chromatography with Mass Spectrometry in Tandem Mode Applied for the Identification of Wine Makers in Residues from Ancient Egyptian Vessels." *Analytical Chemistry* 76 (2004): 1672–77; Wittmers, L., et al. "Archaeological Contributions of Skeletal Lead Analysis." *Accounts of Chemical Research* 35 (2002): 669–75.

8 DOSAGE CONSIDERATIONS

Unlike simple chemical reactions taking place in a beaker, biotransformation rates and effects vary with the person and the dose. Whereas the former is a function of genetics, general health, age, and many other variables, the latter is easier to quantify and describe. Drugs are taken to invoke a therapeutic physiological effect. The range of a dose, again variable depending on the person, is referred to as the effective dose (ED), and to obtain it, repeated administration is often called for. The goal in these cases is to establish a steady level of C_p.

Because there will be a range of effective doses within a population, one way to report the effective dose is as the **ED_{50}**, the dose that will lead to the desired response in 50% of the test population. In toxicology, where a death is likely involved, the same concept is used, but is reported as the **LD_{50}**, which is the dose that causes death in 50% of a test population (Figure 17). In a test population, typically laboratory animals, there will always be individuals who are sensitive and susceptible to drug effects and those who are tolerant and resistant. This variability is due to genetic factors, health, and other parameters. Even at low doses, some individuals will show a response

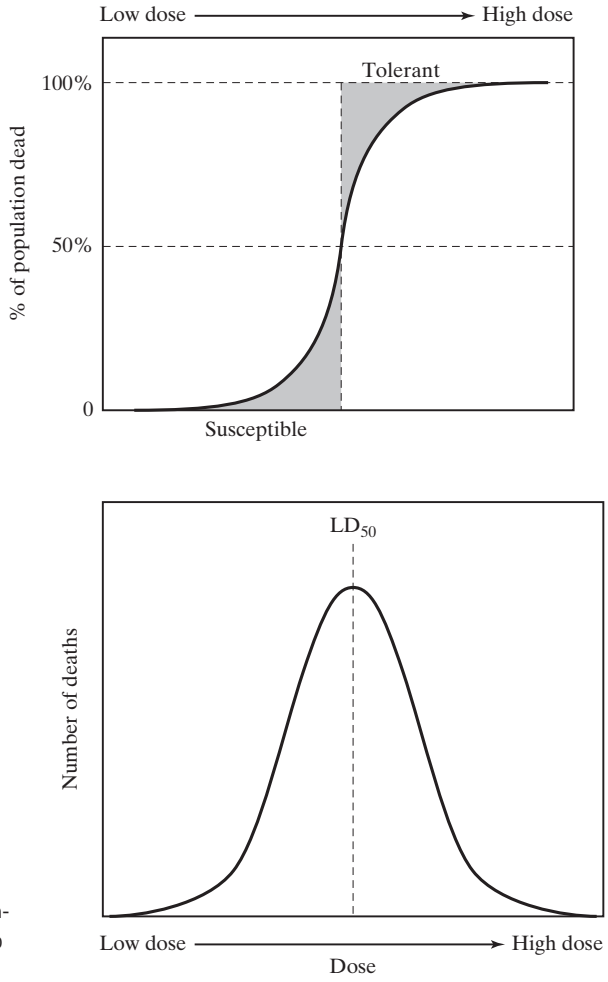


FIGURE 17 Two graphical representations of the LD_{50} concept.

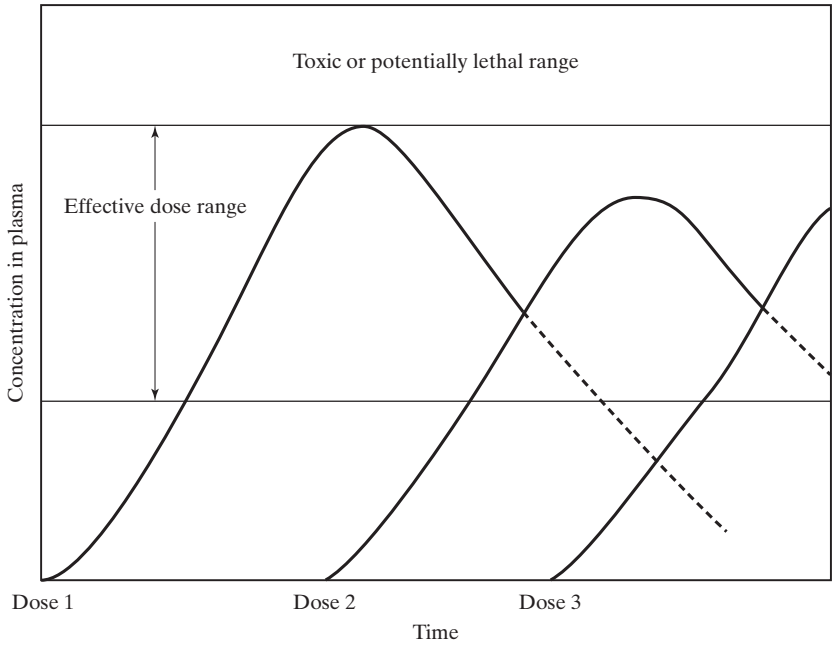


FIGURE 18 When drugs are used therapeutically, a series of doses is required to keep the plasma concentrations in the effective range.

or, in the case of a poison, will die. The values are also specific to the test population, and if that is a group of laboratory animals, there is no guarantee that the data will extrapolate to humans. The ability to extrapolate is important in the case of poisons, for which the body of research on human test populations may be small or nonexistent.

We can extend these ideas to multiple doses of a drug. The first dose behaves as we have seen several times in this chapter, with concentration rising during AD and decreasing during ME phases. To maintain the desired physiologial effect, the plasma concentration must stay within the therapeutic range. This is accomplished by repeat doses at set time intervals. Too short an interval can result in overdose to potentially lethal plasma concentrations; too long an interval can result in the loss of the therapeutic effect.

EXAMPLE PROBLEM 6

A "baby" aspirin typically has 81mg/tablet of the drug. How many tablets would a 130-lb woman have to swallow at the same time to ingest the equivalent of the LD_{50}? Assume the LD_{50} for aspirin is 225 mg/kg. Based on this result, do you think she could commit suicide this way?

Answer:
The lethal dose is the LD_{50} multiplied by the body weight in kilograms, so the first step in this type of problem is to convert the body weight from pounds to kilograms. From Table 5, we assume no loss to first-pass metabolism that has to be accounted for, so the calculation is straightforward, if lengthy. Units are the key; use your skills in dimensional analysis to check your work.

$$135 \text{ lb} \times \frac{1 \text{ kg}}{2.2 \text{ lb}} \times 225 \frac{\text{mg}}{\text{kg}} = \frac{13{,}807 \text{ mg}}{81 \frac{\text{mg}}{\text{tab}}} \sim 171 \text{ tablets}$$

Notice we are saying approximately 171 tablets. We know that the LD_{50} is not a single value but rather is the midpoint of a range, so all we can do here is provide a reasonable answer based on the data provided.

As for the second part of the question, this is similar to the type of opinions that forensic toxicologists are asked to offer in court based on the analytical data and their expertise. In this case, a reasonable response is that she might be able to swallow this many pills at once (although it would be difficult), and it is likely that she would vomit before a fatal dose reached the bloodstream. With the data provided, this is all that can be said.

9 HOW DRUGS WORK

We conclude this chapter with a brief discussion of how drugs cause a pharmacological effect. We have acquired a basic understanding of how drugs behave once ingested, but we have not talked much about how they work. We know that a drug is defined as a substance that generates a physiological change and now we want to explore why. At the core of drug action is a binding event between the drug and a receptor of some type. The binding event has the effect of altering an existing biochemical pathway and inducing a physiological change. Drugs do not create new pathways, they only can modulate existing ones. As an example, a drug binding to a receptor on a cell wall might have the effect of inhibiting production of an enzyme. Drugs that bind to a receptor in the same way as an endogenous compound and elicit the same effects are called **agonists**. In other words, these drugs mimic the behavior of compounds naturally present in the body.

An **antagonist** is a drug that binds to the site, but does not elicit the effect, and further, blocks access to the site by endogenous compounds.

Returning to morphine (and the opiates in general), we can now better explain their physiological effects. Morphine reacts with binding sites throughout the central nervous system (CNS) and in the brain. It is thought that endogenous compounds, referred to generically as *endorphins*, bind to these sites and produce an analgesic effect; thus morphine is an agonist. Rather than one receptor, there are at least three classes of opiate receptors (μ, δ, and κ) with subclasses existing within the classes. Of these three, the μ receptors appear to be the most important in producing pain relief as well as the associated side-effects such as drowsiness and respiratory depression. Several antagonist drugs are available to block the opiate receptors, including naltrexone (Figure 19).

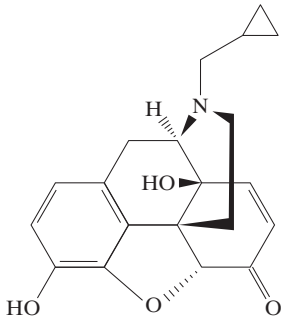


FIGURE 19 Naltrexone.

Summary

The forensic questions in forensic toxicology typically have to do with what drug was taken, when was it taken, how much was taken, and what are the likely consequences. In this chapter, we explored how drugs enter, move through, and leave the body in the form of drugs, metabolites, and conjugates. As a starting point, we can look at the ADME process as a compartment model and can apply basic kinetics to derive important information to answer the pertinent forensic question(s). With a basic understanding of drugs from the pharmacological point of view, we will next delve into the analytical aspects of forensic toxicology.

Key Terms and Concepts

Agonist
Antagonist
Apparent volume of distribution
AUC (area under the curve)
Bioavailability
Biotransformation
Clearance rate
Concentration gradient
CYP
Drug recognition expert (DRE)
ED_{50}
Endogenous
First-order process
First-pass metabolism
Glucuronic acid
Glucuronide
Half-life
Intramuscular
Intravenous
LD_{50}
Mode of ingestion
Passive diffusion
pH-partition hypothesis
Pharmacodynamics
Pharmacogenetics
Pharmacokinetics
Pharmacology
Prodrug
Subcutaneous
Toxicodynamics
Toxicokinetics
UPD
Volume of distribution
Xenobiotic

Problems

FROM THE CHAPTER

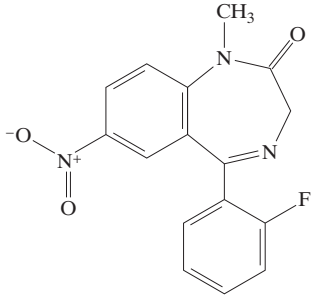
1. Based on material presented in this chapter, what would be the expected metabolic elimination pathway for isopropyl alcohol?
2. Why is it more effective to administer morphine by injection than by oral ingestion?
3. Some drugs are absorbed as ion pairs. Explain how this affects absorption would work and what drugs are most likely to follow this mechanism.
4. If you look up the apparent volume of distribution (V_d) of a few drugs, you will find that many are reported as ranges rather than as a single value. Why?
5. Fentanyl is metabolized to two compounds, norfentanyl and despropionylfentanyl. Find the structures of all three and identify the types of metabolic transformations that occur (See Example Problem 4). What is unusual about this pathway?
6. Fentanyl is a powerful synthetic opiate. Based on your findings in the previous question (*Hint:* structure), does this strike you as odd? Why?

7. Methamphetamine metabolizes to form two products, amphetamine and 4-hydroxymethamphetamine. Show these reactions and identify the type of conversion.
8. A death investigator returns from a scene of a questioned death. The victim is an older woman with a history of depression. The investigator found an empty prescription bottle for Valium (5 mg/tab) on the nightstand and a half-empty bottle of wine. The investigator suspects the woman may have committed suicide by taking the entire bottle of tablets with a glass of wine. Comment on this hypothesis, assuming the LD_{50} (oral) for diazepam is ~100 mg/kg.
9. A woman weighing 60 kg drinks the equivalent of 60 g of ethanol. Her peak plasma concentration is found to be 1.91 g/L.
   a. What is the value of V_d for ethanol in this example?
   b. Assume that the woman's weight is 55% water. How do the weight of water in her body and the value of V_d compare? What does this result mean in terms of the distribution of ethanol?
10. When administered orally, the predator drug Rohypnol® (flunitrazepam) has a bioavailability *F* of 70%. A woman arrives unconscious in the emergency room with signs that a sexual assault may have occurred within the last hour. She weighs 120 lb, and a blood analysis reveals a C_p of flunitrazepam of 0.05 mg/L. Estimate the size of the initial dose in milligrams, assuming that the concentration found is the peak concentration. Is it conceivable that a dose of this size could be administered surreptitiously in an alcoholic beverage?
11. Consider the following data regarding aspirin:

pK_a	$t_{1/2\ plasma}$	Principal Metabolic Reaction	Conjugates with	~LD_{50}
3.5	17 min	Hydrolysis	glucuronic acid	225 mg/kg

Source: Galichet, L. Y., et al., eds. "Aspirin," in *Clarke's Analysis of Drugs and Poisons*. London: Pharmaceutical Press, 2004, p. 652.

Answer the following questions:
   a. What is the reported lethal dose for an adult female (130 lb), in number of tablets of aspirin, each of which typically contains 325 mg of aspirin?
   b. Can the plasma half-life be used to determine how long aspirin will remain in the tissues? Explain.
   c. Assuming a first-order process and rapid absorption, what would be the concentration of aspirin in the woman an hour after she took two 325-mg tablets?
   d. Show the hydrolysis of aspirin and the expected product.
   e. At what stage (ADME) does conjugation play a role?
12. The following data apply to the drug Rohypnol (flunitrazepam), FW 313.39/mol:

log *P*	pK_a	Structure	$t_{1/2\ plasma}$	$t_{1/2\ elimination}$	Elimination
2.1	1.8		3hr	16–35 hr	1% unchanged urine; 10% feces and the rest metabolites in urine

Source: Galichet, L. Y., et al., eds. *Clarke's Analysis of Drugs and Poisons*, Vol. 2. London: Pharmaceutical Press, 2004.

Answer the following questions:
   a. Assuming a typical dose of 1 mg, what would be the expected concentration of unchanged drug in a urine sample of 25 ml?
   b. If the LOD/LOQ for this drug in a mass spectrometer is 2.5 μg/L, how long after the dose will it be possible to detect the unmetabolized drug in urine?
   c. Propose a sample extraction scheme for flunitrazepam from blood.
   d. The four major peaks in the mass spectrum of this drug are 312, 285, 266, and 238. Propose fragment losses to explain this distribution of peaks.
   e. Based on the structure of the drug, what distinctive IR spectral features might be expected?

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Forensic Toxicology

1 Sample Types
2 Types of Forensic Toxicology
3 Analytical Methods in Forensic Toxicology

OVERVIEW AND ORIENTATION

We will look at how this knowledge is applied in the field of forensic toxicology. Forensic toxicology encompasses everything from **blood alcohol content (BAC)** to postmortem toxicology. The single drug that would be evidence to the seized drug chemist morphs into many compounds as a result of ingestion, creating a new universe of analytes and matrices such as blood and tissue. Although the fundamentals of analytical chemistry are applied as usual, quantitative analysis becomes central in forensic toxicology. For example, it is not enough to know that ethanol is present in blood; the forensic question asks How much? To determine whether the ingestion of a drug, drugs, or other exogenous substance played a role in causing a death, the toxicologist needs to know what parent drugs and metabolites are present, in what tissues, and at what concentration. This is the information needed to reconstruct the dose event or events through knowledge of clearance rates, half-lives, plasma protein binding, and conjugation. In other words, this chapter describes the application of the principles introduced in the previous one.

We will approach our investigation of forensic toxicology using the model shown in Figure 1. The process followed by toxicologists can be divided into three phases. The first involves qualitative analysis and tentative identification of the drugs, metabolites, and other analytes of interest. As with seized drug analysis, these are determined by screening tests, but with fewer options than are available

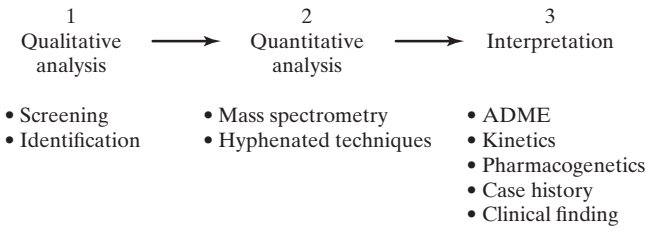


FIGURE 1 Three stages of forensic toxicology.

to the seized drug chemist. This limitation arises from two factors. First and more important are the sample matrices. It is neither feasible nor appropriate to apply a color or microcrystal test to liquid blood or urine, for example. Second, the drugs and analytes are typically present at low levels (mg/L or less) in most samples and therefore are not amenable to typical screening tests even if the matrix does allow for it. All is not lost however; toxicologists utilize a battery of immunoassay techniques, which will be introduced in Section 3.2.1. An increasing number of laboratories are using HPLC-MS^n methods for screening as well as quantitation.

The second phase of toxicology is quantitation. Perhaps in no other area of forensic science is quantitation required so frequently and routinely as in forensic toxicology. Seized drug analysts occasionally quantitate drug exhibits such as methamphetamine, cocaine, and heroin to determine the purity of the sample as a weight percent. In toxicology, if a drug or metabolite of forensic interest is identified, it is usually quantitated. Because many cases involve more than one drug and several metabolites, this can be a fairly daunting task. Not surprisingly, principles of method validation and QA/QC are integral to the practice of forensic toxicology.

The third phase shown in Figure 1 is the interpretation phase, in which all the analytical data are interpreted in light of all pertinent factors. Central to interpretation is a knowledge of the pharmacokinetics and pharmacodynamics of the drugs present. Other information vital to the toxicologist includes case history, any clinical findings if the person was being medically treated, any disease conditions, and similar information. This phase is what makes forensic toxicology unique as well as challenging. Good forensic toxicologists are masters not only of the analytical sciences but also of the relevant medical, pharmacological, and biological sciences.

1 SAMPLE TYPES

Forensic toxicologists work with biological samples. Most frequently analyzed are blood (plasma) and urine, but others may be analyzed depending on the case and the forensic questions at hand. Postmortem toxicology typically uses the largest variety of samples. Some atypical matrices used on occasion include fingernails, saliva, hair, and sweat. We will discuss each type briefly.

1.1 Blood and Plasma

Blood is an extracellular fluid that is a complex mixture of organic fats and proteins, cells, and inorganic salts. Analytically, it is a challenging matrix to work with. The characteristic color of blood comes from the complex formed between hemoglobin in **red blood cells (RBCs)** and oxygen. Spinning a blood sample in a centrifuge separates the blood into a cellular component (approximately 45 percent by volume) and a noncellular component called *plasma*. Plasma can further be subdivided into serum and fibrinogen, the material that forms clots. Serum is a clear straw yellow and contains ions and proteins, albumins, and globulins. As we will see later in the chapter, these proteins can present a challenge in sample preparation. In forensic toxicology, the concentration of compounds found in the bloodstream is designated as the whole blood, plasma, or serum concentration. We utilized this convention in the last chapter when we referred to the peak plasma concentration, C_p. The pH of blood and plasma is typically 7.4, maintained by a carbonate buffer system. This value is also referred to as **physiological pH**.

The cellular portion of blood can be divided into three types of cells: red blood cells (RBCs, also called **erythrocytes**); white blood cells (**WBCs**, **leukocytes**); and platelets (**thrombocytes**). RBCs, which transport oxygen and bicarbonate, are the most numerous and are unique in that they lose their nucleus before entering into the circulatory system. WBCs (several types exist) are the next most numerous of the cell types and are

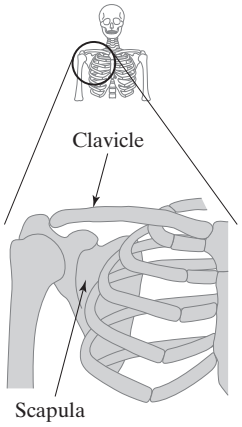


FIGURE 2 Location of the clavicle.

active in fighting diseases. Platelets are needed for clot formation. Unlike red blood cells, the white blood cells have a nucleus.

Blood that is drawn from a living or very recently deceased person will have the cellular component intact, and the plasma can be isolated from the cellular component using centrifugation. However, shortly after death, cells start to lyse (burst) and the contents mix with the plasma. At this point, the term "blood concentration" is appropriate. Clotting can also occur, further complicating sample preparation issues. Also, because blood ceases to flow when the heart stops beating, postmortem blood samples may be drawn from more than one source (if possible) including from the heart, the femoral vein (**femoral blood**), below one of the clavicles (Figure 2) (**subclavian blood**), and vessels away from the body core such as the femoral vein (**peripheral blood**). Key features of the circulatory system as well as other relevant organs are shown in Figures 3 and 4.

1.2 Urine

Urine is chemically less complex than blood and generally easier to work with from the sample preparation point of view. It is a concentrated filtrate of blood that contains the water-soluble parent molecules, metabolites, and conjugates that result from a drug ingestion.

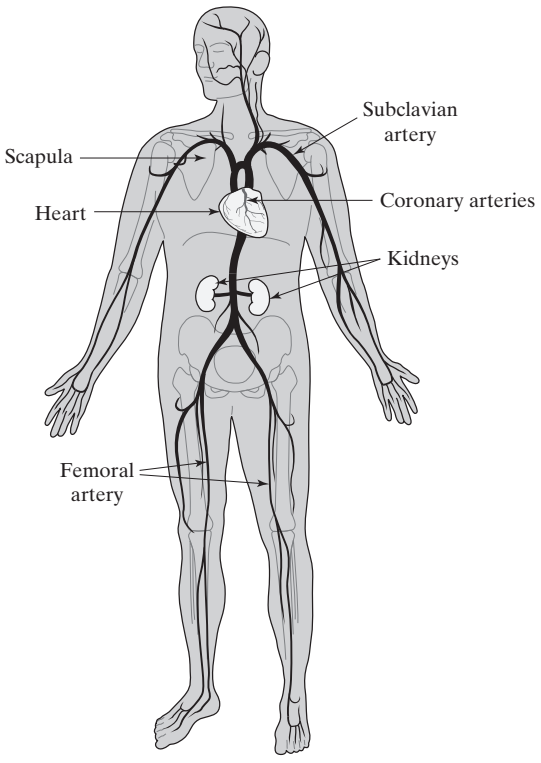


FIGURE 3 Location of key arteries and organs.

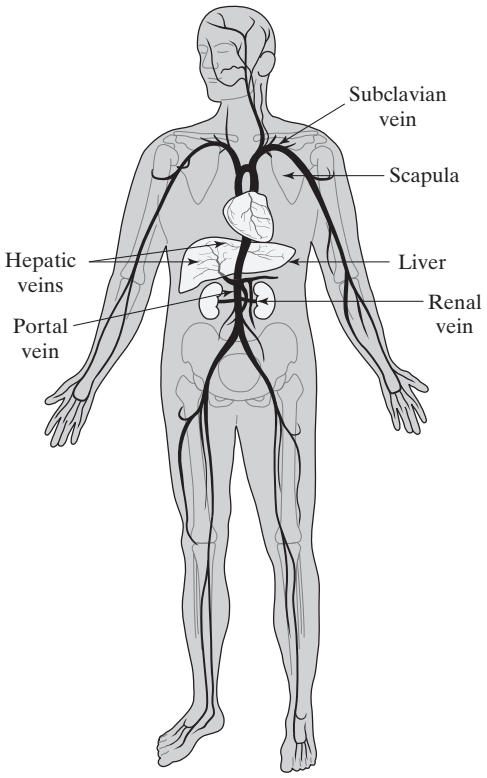


FIGURE 4 Location of key veins and organs.

Because it does not directly reflect the current plasma concentration of a drug, urine results can be difficult to interpret and cannot be used to gauge level of intoxication, for example. However, drugs and metabolites are often found in higher concentrations in urine than in blood and will be detectable in the urine longer than in blood. The kidneys are capable of filtering the entire plasma volume of blood more than 50 times in a day. The characteristic straw-yellow color of normal urine is attributed to **urochrome**, which is a by-product of the breakdown of hemoglobin. The characteristic smell is due to urea and ammonia, a breakdown product of urea. One of the older presumptive tests used for urine in forensic science was to heat a suspected stain to see if an ammonia odor resulted.

Urine is about 95% water and 5% solutes by volume. Of these solutes, urea is present in the largest concentration and is a product of the breakdown of amino acids. The ionic solutes include sodium, potassium, phosphate, sulfate, creatinine, and uric acid. The high solute load in urine results in its having a specific gravity greater than 1.000. This value can be of clinical importance in diagnosing illnesses or problems. The pH of normal urine is slightly acidic (around 6) but varies based on a number of factors including diet and health conditions.

1.3 Vitreous Fluid

The **vitreous fluid**, found inside the eyeball, can be valuable in postmortem cases, particularly when other postmortem samples have been compromised. It resides in what is called *anatomical isolation* (at least relatively isolated) and thus will resist changes associated with decomposition longer than blood, urine, and other tissues. Because there are no metabolic enzymes present, relative concentration ratios of parent compounds to metabolites are preserved. The limitation is volume; typically, 4 mL is about the most than can be recovered from both eyes at autopsy. Generally speaking, the vitreous fluid reflects plasma concentrations of drugs and metabolites present in the hours prior to death. Vitreous fluid is useful for estimating alcohol levels at the time of death; outside the vitreous, changes associated with decomposition can alter the blood alcohol content.

1.4 Other Tissues and Fluids

Samples of liver can be useful in postmortem cases, since it is the primary site of metabolism in the body. Sample preparation of liver usually involves homogenization coupled with aggressive extraction. Liver samples are important in cases where advanced decomposition is noted. In a living person, the liver produces **bile**, which collects in the gallbladder. Because of their origin in the liver, many drugs and metabolites are detectable in the bile. Other materials that may be collected include kidney, brain, and stomach contents (**gastric contents**).

1.5 Hair

Over the past 20 years, hair has become an important sample matrix in forensic toxicology.[1–7] The advantage of hair is that it can provide a chronological record of exposure based on known growth rates (about half an inch per month). Because it can be collected **non-invasively** (as opposed to a blood draw), it has become widely used in workplace drug monitoring. One of the challenges in hair testing is to ensure that whatever is detected originates inside the hair, deposited by physiological processes, as opposed to material present as surface contamination. The latter could indicate passive or innocent exposure to substances such as cocaine, whereas the former is evidence of ingestion of cocaine. Hair is also a difficult matrix to work with given the high protein content and usually a fairly aggressive digestion step using strong base, enzymes, or

methods such as microwave digestion.[8] To preserve the chronological record of exposure, hair is collected at the scalp and sectioned accordingly; the farther the section from the scalp, the longer the elapsed time from the dose. Hair has proven useful in drug-facilitated sexual assault cases, particularly with GHB, which clears out of the system so rapidly that victims may not seek treatment until after it could have been detected in traditional matrices.[9–12]

1.6 Other Matrices

Several other matrices have been used in forensic toxicology. One of the most promising is **oral fluid** (saliva), which, like hair, can be collected noninvasively. Saliva is generated by the secretions of several glands, so its composition can vary significantly. Saliva is about 98% water and has a lower protein load than plasma. Amylase, an enzyme that assists in the initial digestion of carbohydrates, is present in large quantities and is the basis of forensic presumptive tests for saliva. The pH of oral fluid is usually slightly acidic (around 6.8) but variable; the typical rate of production of saliva is a surprising liter or more per day. Saliva for analysis is usually collected using a swab or a gauze that is chewed for some period to maximize the amount of fluid collected. Sweat,[13–18] nails[13, 14, 19, 20] and tears[19] can also be collected non-invasively and have been used to a limited extent in forensic investigations. Drugs enter oral fluid primarily by passive diffusion driven by a concentration gradient.

Recently, **meconium** has become an important matrix for monitoring prenatal drug ingestion by expectant mothers. Meconium is the first solid waste (stool) produced by an infant and is a blackish green in color. As the baby grows in the uterus it consumes amniotic fluid, which contains a variety of materials including bile, shed skin, and urine. Working with this material is as pleasant as the description indicates, but like hair, it is a matrix that captures a record of past ingestion of drugs by the mother. In some states, such ingestion has legal consequences, so there is increasing interest in its analysis.[14, 16, 19, 21–23]

2 TYPES OF FORENSIC TOXICOLOGY

There are many ways to categorize and subdivide the discipline of forensic toxicology. What is presented here is typical but by no means exclusive. All forensic toxicologists do not work in all areas, and some may end up specializing in one area (postmortem toxicology, for example). The categories are listed alphabetically, not in order of importance.

2.1 Alcohol

Alcohol plays a role in forensic toxicology in obvious as well as less obvious ways. Ethanol intoxication and driving while intoxicated (DUI) are best known, but there are other alcohols of interest and other ways in which ethanol concentration is important, notably in postmortem toxicology. We will discuss those alcohols and considerations in that section.

When ingested orally, ethanol is absorbed to some extent in the stomach but predominantly in the top of the small intestine. Factors that play a role in the rate of absorption include what if any food is ingested at the same time (or is already present), type of beverage (carbonation can facilitate increased absorption), alcohol content of the beverage (maximum absorption occurs around 35% full) and the presence of fatty foods, which tend to decrease the absorption rate. Once absorbed, ethanol spreads to tissues as a function of their water content, given the water solubility of ethanol.

This factor has some interesting consequences; for example a woman will tend to have higher relative blood ethanol concentrations than a man for the same amount of ethanol ingested owing to the smaller amount of water present in her body compared to a man's of similar weight.[24] Ethanol is classified as a central nervous system depressant even though symptoms at lower ethanol concentrations seem contradictory (i.e., talkativeness, loss of inhibitions), owing to the functions depressed. In general, at lower ethanol concentrations, higher brain functions are more affected, and at higher ethanol levels, lower-level brain functions are depressed. Blood alcohol concentrations are reported as g/100 mL or g/dL, and death is possible at levels of around 0.40 or above. Because of the depressant effect of ethanol, the cause of death in ethanol poisoning is usually respiratory arrest. It is important to keep in mind that apparent tolerance can be developed to ethanol by the induction of the enzyme responsible for its breakdown. This allows an individual to consume more ethanol than might otherwise be considered normal.

The metabolic transformation and elimination of ethanol is summarized in Figure 5. The majority is metabolized to acetaldehyde (catalyzed by alcohol dehydrogenase, ADH). Most of the acetaldehyde is oxidized to acetic acid, catalyzed by aldehyde dehydrogenase. A small portion, < 0.1% typically, will form conjugates with glucuronic acid and sulfate, and the remainder is excreted unchanged. A fraction of the acetate is further metabolized to CO_2 and exhaled in the breath. One of the most interesting features of ethanol metabolism is the mixed kinetic control of the elimination, shown in Figure 6. At blood alcohol concentrations above ~ 0.02 g/dL, the rate of elimination follows zero-order kinetics. As the concentrations fall below this level, first-order kinetics (as discussed in the last chapter) are followed.

Recall that for first-order reactions, the rate of elimination depends on the concentration of the material present. For a zero-order reaction, the rate of elimination of ethanol is independent of the concentration of ethanol in the blood. Now that we understand a bit about metabolism, this puzzling observation can be explained.

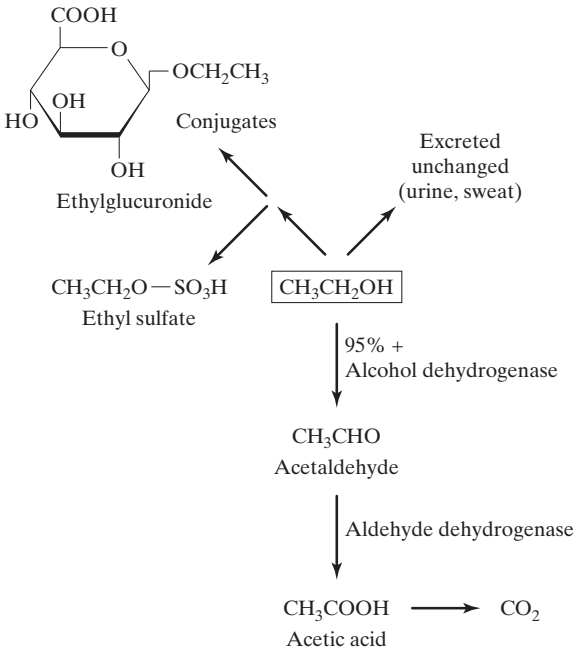


FIGURE 5 Metabolism of ethanol.

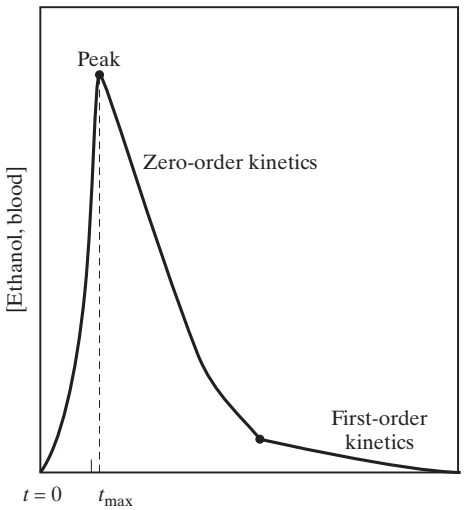


FIGURE 6 A simplified depiction of mixed kinetic control of ethanol elimination.

The ADH enzymes required for metabolism of ethanol to acetaldehyde become saturated at high ethanol concentrations. During this phase of elimination, zero-order kinetics are followed. As the ethanol concentration decreases and sites on ADH enzymes become available, the kinetics become first order.[25] As a rule of thumb, an elimination rate of 0.02%/hour is used although 0.015%/hour is also seen.[26]

EXAMPLE PROBLEM 1

A man ingests several shots of whiskey in rapid succession on an empty stomach, resulting in a peak plasma concentration of 0.120 g/dL 1 hour after the shots are consumed. If he does not drink any more alcohol, how long will his blood alcohol concentration be at or above 0.08?

Answer:

The difference in BAC's is $0.04\frac{g}{dL}$

To determine the time needed for the BAC to drop by this amount, divide by the estimated rate:

$$\frac{.04\frac{g}{dL}}{.02\frac{g}{dL}\text{ per hour}} = 2\text{ hours.}$$

Although ethanol is the biggest forensic concern, other alcohols also have forensic relevance. Methanol (CH_3OH, referred to as wood alcohol) is sometimes consumed by those seeking ethanol effects. Methanol is present in many products and is found in illicitly produced liquor and beverages. Methanol follows a similar metabolic path as ethanol (Figure 5), with potentially disastrous consequences. Formaldehyde, a toxin, is formed instead of acetaldehyde and goes on to form formic rather than acetic acid. Formic acid is eliminated slowly, allowing damage to accumulate. Blindness can result owing to damage to the optic nerve, although the mechanism of this process is debated. The lingering presence of the acid can lead to a condition called *metabolic acidosis.* This condition leads to rapid breathing due to disruption of blood pH (which is related to the concentration of CO_2 and thus breathing rate) and symptoms associated with this and other metabolic issues. Other alcohols encountered forensically include isopropanol and ethylene glycol. However, the primary concern remains ethanol and ethanol intoxication.

Currently, the federal government sets the legal limit for DUI at 0.08%, and all states follow this requirement. The limit varies internationally, ranging from a "zero tolerance" level of 0.0 to 0.02 in Sweden, 0.03 in Finland, 0.05 in Germany, to 0.08 in the United States, Canada, and the UK. There is little debate over the dangers of DUI; according to the National Highway Traffic Safety Administration (NHTSA, www.nhtsa.gov), there were 37,261 fatalities in automobile accidents in 2008 (latest data available), of which 32% involved alcohol. State by state, the percentage ranged from a high of 46% (North Dakota) to a low of 16% in Vermont (http://www-nrd.nhtsa.dot.gov/Pubs/811172.pdf, last accessed November 2011). Chronic alcoholism is a contributing factor to accidental death and disease conditions. There is increasing concern over binge drinking, defined as having more than five drinks at a single sitting. Statistics provided by the Centers for Disease Control and Prevention (CDC) show that in 2010, 23% of adults over 18 years of age drank five or more drinks in one day at least once during the year

before the question was asked. For women, the percentage peaked in the 18–24-year-old age group (27.7%) and for men, the percentage peaked for the 25–44 age group, at over 40% (http://www.cdc.gov/features/ds5drinks1day/, last accessed November 2011). Even if one ignores the issues of alcohol intoxication, there are many peripheral but closely related issues. One of those is **drug-facilitated sexual assault (DFSA)**.

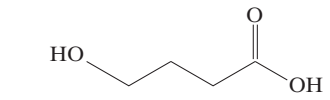
2.2 Drug-Facilitated Sexual Assault (DFSA)

DFSA is defined as a sexual assault (a rape) that is perpetrated on the victim (usually a woman, but not always) who is incapacitated by drugs and/or alcohol. The consumption of the incapacitating agents may have been voluntary (e.g. having several drinks at a bar) or involuntary when administered secretly by the perpetrator(s). Thus, in a common scenario a woman arrives at a bar with friends, has a few drinks, and then a man surreptitiously spikes another drink with GHB. He then takes her to another location and perpetrates the assault. How the victim ingests the incapacitating drugs does not matter; if the sexual contact was involuntary, a DFSA has occurred.

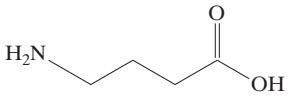
DFSA is a crime that brings together a number of forensic practitioners. If the victim awakes the next day in unfamiliar surroundings and has reason to believe that an assault may have occurred, she could call police and would be taken to a hospital for an examination. In many locations, her primary medical contact would be a **Sexual Assault Nurse Examiner (SANE)**, specially trained to deal with sexual assault cases and in gathering the pertinent physical evidence as well as biological samples for toxicological analysis. As you might imagine, DFSA is much more than a toxicological problem.

The number of sexual assaults that occur each year in the United States is unknown because the rate of reporting is unknown. Estimates are that about 35% of the assaults that take place are reported. In 2009 (latest data available), the FBI Uniform Crime Report showed more than 88,000 forcible rapes (http://www2.fbi.gov/ucr/cius2009/data/table_01.html, last accessed November 2011) or about 29 incidents per 100,000 people. The highest reported rate was in 1992, at about 43 per 100,000. These statistics do not address how many of these rapes qualified as DFSA, the rate of which is equally difficult to pin down. In a recent report in *Forensic Science International,* the authors reported toxicological analysis in sexual assault and found alcohol present in 62% of the cases, benzodiazepines alone in 3.8% of the cases, and ethanol plus benzodiazepines in 4.0%.[27] There were no GHB positives in this study, and the frequency of use of GHB as a date rape drug is unknown. This lack of data could in part be attributed to its rapid clearance rate and relatively short window of detection.

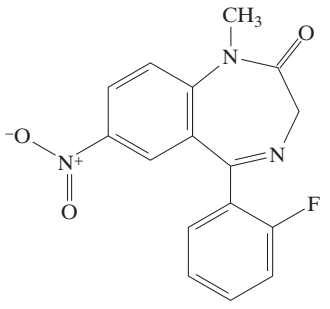
We will focus our discussions on GHB and flunitrazepam, or Rohypnol, which is a benzodiazepine that is significantly more potent than Valium (diazepam). Both drugs interact with the receptors for the neurotransmitter **GABA**, which has an inhibitory action on the central nervous system (Figure 7). Both drugs are rapidly absorbed after oral ingestion. Flunitrazepam is



Gamma-hydroxybutyric acid (GHB)

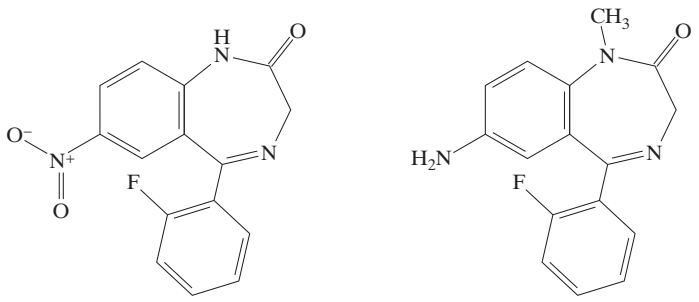


Gamma-aminobutryic acid (GABA)



Flunitrazepam

FIGURE 7 Date rape drugs and the neurotransmitter GABA.



Desmethylflunitrazepam (norflunitrazepam) 7-Aminoflunitrazepam

FIGURE 8 Metabolites of flunitrazepam.

metabolized principally to desmethylflunitrazepam and 7-aminoflunitrazepam with 3-hydroxy-7-acetamidoflunitrazepam and 7-amino-1-desmethylflunitrazepam possible (Figure 8). The plasma half-life of flunitrazepam is between 15 and 35 hours.[28]

GHB is an endogenous compound (present naturally), with background levels varying widely but typically in the low ppm range. After about 12 hours post-administration, it is difficult to detect with a half-life of between 0.3 and 1 hour.[28]

For these reasons GHB presents the most difficult analytical challenge. GC-MS is an excellent tool for the job but requires derivatization given the poor chromatographic performance of GHB. LC works well for GHB, but interestingly, LC-MS^n methods are not particularly useful. The issue is not the instrument per se, but the detection limits; GHB analysis does not require ppb detection and as discussed above, this is counterproductive. Accordingly, recent research has centered on establishing threshold levels for endogenous and exogenous concentrations[9, 11, 29–31] in various matrices and on examination of alternative matrices such as hair[11, 32] and saliva.[33]

2.3 Postmortem Toxicology

Most people associate forensic toxicology with death investigation and postmortem toxicology, although as we have seen already, this is one aspect of a broad field. The role of postmortem toxicology is to assist in the determination of the cause of death (**COD**), so these toxicologists work closely with death investigators, medical examiners, and coroners. Death investigators are tasked with identifying the **cause of death** as well as the manner and mechanism of death. The **manner of death** is categorized as natural, accidental, suicide, or homicide (frequently abbreviated **NASH**) and undetermined. The cause of death is where the forensic toxicologist may play a role; toxicological findings, coupled with the case and scene history and autopsy results are all used to arrive at the cause of death; for example, blunt force trauma, electrocution, or acute fentanyl toxicity. In cases such as automobile fatalities, levels of potentially intoxicating drugs are measured as part of the investigation, even though the intoxicating agents did not cause the death directly. Thus, drugs and alcohol levels play a role in postmortem toxicology.

Postmortem work typically involves the widest variety of sample matrices (discussed in detail below) including blood, urine, vitreous fluid, and tissues. The toxicologist must pull together data on drugs and metabolites found in the different sample types to understand the antemortem dosing event or events. Usually, more than one substance is found (polypharmacy) along with alcohol, and factors such as drug interactions, enzyme activation, enzyme inhibition, and others have to be considered in drawing conclusions.

One of the most interesting and challenging aspects of postmortem toxicology is the lack of complete understanding of what happens to drugs and metabolites after death. Even death itself is difficult to define in forensic terms. We are familiar with medical death as defined by the cessation of a heartbeat and respiration, and the more updated brain death criteria. However, this is death on a macro scale and does not capture the complexity of the process on the micro and biochemical level. Death is a process, not a discrete event and an understanding is important to interpretation of postmortem toxicological results[34–37] because of the effects of potential phenomena such as **postmortem redistribution (PMR)**.

Once breathing and circulation stop, little or no fresh oxygen reaches the tissues, and processes that were aerobic either cease or follow an anaerobic pathway. The ATP/ADP cycle under anoxic conditions leads to the production of lactic acid and a decrease in pH in the blood and other compartments. Transport mechanisms break down, and enzymes once confined to the cells may move out into the general environment. Other enzymes will continue to function for some period postmortem. Cell death results in rupturing of the cell (**autolysis**), which releases cell contents into the mix. Bacteria that are native to the GI tract can enter the system, as can bacteria from external sources. Many of these bacteria can function in anoxic or aerobic environments and can further change drug and metabolite concentrations. Gastric contents can migrate into the bloodstream as well. Clearly, in all these situations, time since death is a critical factor in these considerations.

After death, drugs that had been in different compartments or tissues are subject to change, driven by concentration gradients and solubility considerations as well as other processes. For example, tissues, such as the liver, with high drug concentrations, can release drugs into the bloodstream. The lungs are also a source of release. Additionally, conjugation may still occur, further complicating the picture. As an example, we know that heroin is metabolized to 6MAM and morphine, which in turn forms a glucuronide conjugate. Postmortem changes can generate glucuronides of both 3- and 6MAM.[34]

We noted in Section 2.1 that ethanol and other alcohols may play a significant role in postmortem toxicology. Both ethanol and methanol are endogenous, but typically at negligible concentrations; the situation is nothing like that discussed for GHB. However, alcohols can be produced after death; recall that methanol and ethanol are fermentation products that can be produced postmortem. Microbes can alter ethanol concentrations as well. Fortunately, vitreous fluid (discussed shortly) provides a valuable matrix for ethanol determinations as long as samples are obtained in a reasonably short time after death.

There is no shortage of famous cases that hinge on postmortem toxicological findings. These range from the death of Marilyn Monroe in 1962 from a barbiturate overdose to more recent cases such as those of Anna Nicole Smith, Heath Ledger, and Michael Jackson. In all these cases, the postmortem toxicology told the tale and led to the determination of the cause of death. As prescription drug use and multiple drug use continues, the number of such cases (famous and otherwise) will continue to grow.

2.4 Sports Toxicology

Forensic toxicology has achieved a high profile in performance toxicology, both in humans and in animals such as horses and greyhounds. Although controlled substances are part of sports toxicology, they are not the primary concern, whereas substances that are not controlled (such as caffeine) can be an issue. Athletes use and abuse substances in an attempt to enhance athletic performance, and so the target lists are often referred to as *performance-enhancing substances*. The **World Anti-Doping Agency** (WADA, www.wada.org) oversees internal standards, maintains lists of banned substances (published as the Prohibited List), funds research, and accredits laboratories involved in sports doping analysis. WADA breaks down the banned substances list using the following categories:

S0 Non-approved substances
S1 Anabolic agents
S2 Peptide hormones, growth factors, and related substances
S3 Beta-2 agonists
S4 Hormone agonists and modulators
S5 Diuretics and masking agents
M1 Enhancement of oxygen transport
M2 Chemical and physical manipulation
M3 Gene doping

The last three entries are prohibited performance enhancing methods that do not involve ingestion of a particular substance in the traditional sense. For example, gene doping involves insertion of a gene into the body to generate a performance-enhancing substance, and oxygen transport can be enhanced by blood doping. One practice is to collect blood from the athlete during a training phase, centrifuge and separate the cellular component, and replace the plasma component. The cells, including the red blood cells that transport oxygen, are frozen and preserved and given to the athlete immediately prior to an event.

Diuretics and masking agents (S5) may be taken in an attempt to foil analytical testing. For example, a high level of a banned substance or metabolite in urine could be hidden by dilution of the urine to a point at which the concentration of the performance enhancing substance drops below the LOD of the analytical method used to detect it. A diuretic, which increases the rate of water can be used for this purpose. Plasma expanders can be used to accomplish the same goal in the bloodstream. Beta-2-agonists are a class of drugs widely used to treat asthma and are allowed at low levels if needed for such purposes. Higher doses have been used for performance enhancement purposes. As the name suggests, hormone agonists are substances that compete for receptor sites and thus can modulate and mimic hormone effects. Peptide hormones and growth factors, such as human growth hormone, have also been used to enhance performance.

Currently, the largest number of positive results for doping analysis comes from the class anabolic agents, or **anabolic agent steroids (AAS)**. Anabolic agents are those related to or derived from testosterone and that promote the development of male sexual characteristics such as increased muscle mass. Male sex hormones are called androgens collectively, and the term **androgenic hormones** is also seen. These substances can be divided into endogenous hormones such as testosterone and exogenous hormones such as nandrolone (nortestosterone) and boldenone. Commercial preparation of nandrolone include “Deca QV” and “Deca Durabolin,” and the slang term “deca” is used

EXHIBIT A

Toxicological Samples from Athletes

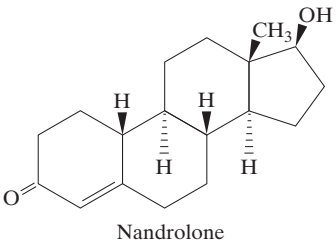
Sample analysis for banned substances in athletes is similar to forensic analysis for controlled substances. Urine is the primary matrix, but blood may also be collected. The National Collegiate Athletic Association (NCAA) stipulates how urine is to be collected and maintains a list of banned substances for college athletes. Not all these substances are illegal.

Andy Crawford/Dorling Kindersley Media Library

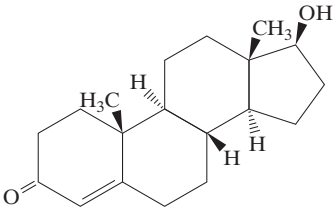
The procedure for collecting urine starts when the athlete arrives at a collection station, where he or she selects a beaker. The athlete must deliver 85 mL of urine while being watched by a crew chief; until that volume is obtained, the athlete stays at the collection station. If a single urination event fails to deliver the needed volume, the athlete can be given approved beverages only from sealed containers. The urine collected must have a pH between 4.5 and 7.5 and a specific gravity of above 1.005; a lower value suggests the possibility of dilution. Once the urine sample meets these requirements, it is divided into two containers, with approximately 60 mL in "A" and the balance in "B." Both the crew chief and the athlete witness all steps, including the creation of the chain-of-custody forms.

to refer to these preparations. They are perhaps best known as the steroids thought to have been widely used in major league baseball in the 1990s and early 2000s. Structures are shown in Figure 9.

Because steroids such as testosterone are endogenous, the difficulty in detecting and interpreting the results is similar to that with GHB. The goal is to detect the hormone and identify it as endogenous or exogenous. The first stage in steroids analysis is what is called a **steroid profile**, in which the sample (urine or blood) is characterized for the presence of a target group of steroids and metabolites. In addition to detection, ratios are determined for several of these and used to flag a sample as suspicious.[38–42] Once this is accomplished, attention often turns to determination of endogenous to exogenous ratios. The tool that is used for this purpose is **isotope ratio**



Nandrolone



Testosterone

FIGURE 9 Steroids common in sports doping.

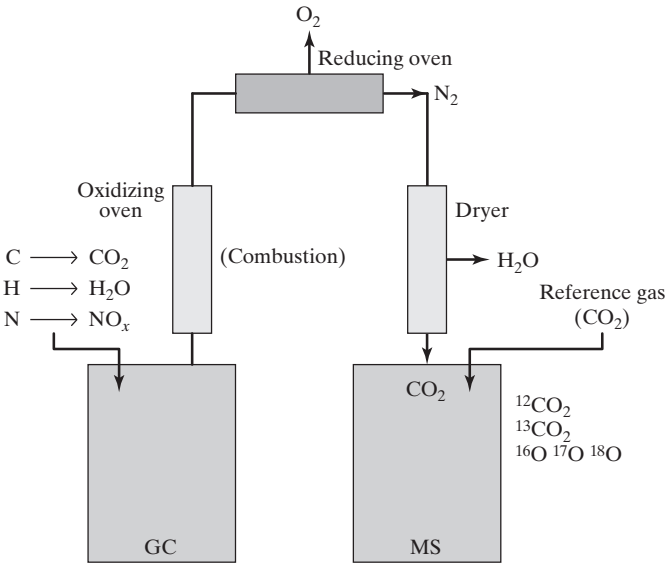


FIGURE 10 Isotope ratio GC-MS.

mass spectrometry (IRMS) coupled to GC.[43] A schematic of the instrument is shown in Figure 10. Eluent from the GC enters an oxidizing oven, where carbon is converted to CO_2, hydrogen to water, and nitrogen to the NO_x forms. In the reducing oven, the NO_x gases are reduced to N_2, and water is removed in the drying tube. The remaining CO_2 is directed into the mass analyzer (high resolution) for detection and isotopic characterization. The stable isotopes of carbon are ^{12}C and ^{13}C, and those of oxygen are ^{16}O, ^{17}O, and ^{18}O. The CO_2 detected can have a mass of 44 (12 + 32) amu up to 49 (13 + 36) with 44, 45, and 46 being of primary interest.[43] Software tools are required to sort out the relative contributions and isotopic abundances of interest. IRMS was first used in Olympic competition in 1996 at the summer games in Atlanta and has seen increasing use ever since.[43]

The basis of distinguishing between endogenous and exogenous steroidal compounds is the $^{13}C/^{12}C$ ratio, which is compared with accepted endogenous reference compounds (ERCs).[44] Compounds will have either more ^{13}C than the ERC ("enriched") or less ("depleted"), and the basis of the determination is the value determined by

$$^{13}C = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample} - \left(\frac{^{13}C}{^{12}C}\right)_{standard}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} \quad \textbf{(1)}$$

The units for this value are parts per million, symbolized as ‰. The standard for carbon is a material called **Vienna Pee Dee Belemnite (VPDB)** to which all other ratios are compared. Note that both endogenous and exogenous compounds will deviate from the VPDB standard values but in differing amounts. The key, as with GHB, is determination of the range of typical endogenous values.

3 ANALYTICAL METHODS IN FORENSIC TOXICOLOGY

Once the sample is prepared, the analytical instrumentation utilized in forensic toxicology is similar to that used in seized drug analyses. With the exception of blood alcohol measurements, confirmation of identification and quantitation (step 2 in Figure 1) is accomplished using a hyphenated instrument with mass spectrometry as the detector. However, getting to the instrument stage is more complicated than in seized drug analysis and makes use of a battery of tools we have discussed, with an emphasis on liquid–liquid extraction (LLE) and solid-phase extraction (SPE). Tandem MS methods, because of their sensitivity, sometimes allow for a simpler preparation, but this is not always possible.

Applying the Science 1 Stable Isotope Ratios and Wine Identification

Wine is derived from grapes and can be classified by stable isotope ratio analysis. In a 2004 report, researchers employed ^{13}C isotope enrichment values for glycerol and ethanol to categorize wines. Glycerol forms naturally during fermentation by the degradation of sugars from grapes. Fermentation also produces ethanol and CO_2. Four countries, two vintage years, and two wines (rosé and red) were characterized, and the δ‰ for ethanol was plotted against that of glycerol. Differences among countries were evident in the slopes and axis values of the plots. PDB was used as the reference standard.

Source: Calderone, G., et al. "Characterization of European Wine Glygerol: Stable Carbon Isotope Approach." *Journal of Agricultural and Food Chemistry,* 2004, Web Release August 28, 2004.

3.1 Sample Preparation

Everything we discussed in the context of preparing seized drug samples for analysis applies here, including ionization state (HA/A^- or B/BH^+), pH considerations, and solubility considerations. One sample preparation issue pertinent to forensic toxicology is the need to remove proteins from matrices such as blood and liver, typically using a protein precipitation technique. The goal is to denature the protein (destroy its three-dimensional configuration) so that it will form a solid that can be removed by centrifugation. The most commonly used reagents are organic solvents such as acetonitrile, perchloric acid, or trichloroacetic acid (TCA). The supernatant may be analyzed directly, or it may be dried and reconstituted if needed to avoid the dilution effect that arises from the addition of the precipitating reagent. For some tandem MS methods, the sample can be diluted with a small volume (μL range) of acetonitrile and centrifuged, followed by a direct injection of the supernatant into the instrument. This process is referred to informally as **crash and shoot**, and it works best with relatively simple matrices such as urine or saliva. More complex matrices such as plasma and tissue typically require additional treatment, particularly if a variety of drugs and metabolites are being targeted.

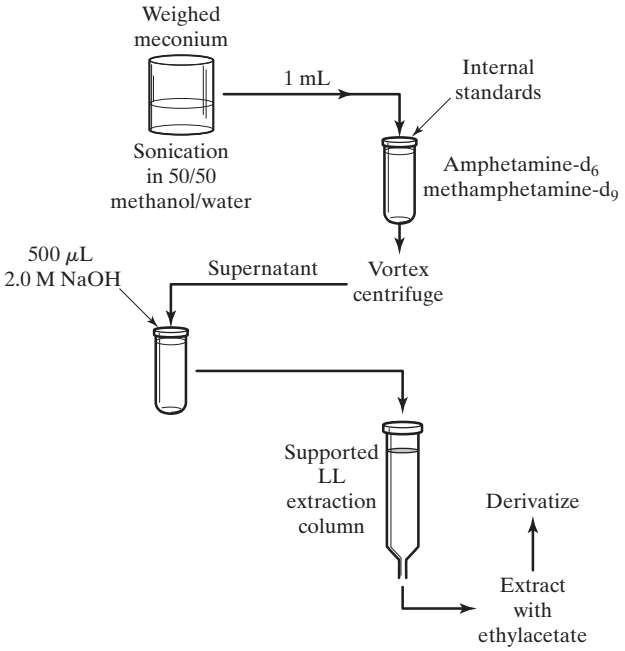
LLE can be performed using a separatory funnel, but increasingly, microextraction methods are being employed in which very small volumes of liquid matrices are used. For example, if a plasma sample is to be screened for the presence of basic drugs and metabolites, a small amount of plasma (~ 50–500 μL) can be placed in a small test tube along with an internal standard (in a water-soluble solvent) and a basic buffer. If the sample is to be analyzed using LC, a precipitating reagent can be added at this point. After vortexing, an extraction solvent such as ethyl acetate, 1-chlorobutane, or butyl acetate is added in a volume of ~100 μL. The sample is again vortexed and then centrifuged to force a clean separation of the layers. The organic layer containing the basic drugs is drawn off for further analysis. This may involve direct injection of 1–2 μL

into the instrument or drying of the extract and reconstitution in a small volume of the appropriate solvent/buffer mix, depending on the instrument used. If a GC method is to be used, no buffer is added.

For blood, plasma, and solid samples such as liver, SPE is essential. In general, two types of SPE columns are widely used in forensic toxicology—those that contain ion-exchange capabilities and those that do not. The ion-exchange phases are capable of retaining the charged form of a drug (A^- or BH^+), depending on the composition of the solid-phase resin. First, the column is usually conditioned with methanol or similar solvents. Once the sample is loaded onto the column, a series of washing steps remove soluble matrix components, and the packing acts as a physical filter to trap proteins and other solids. After the washing stages (and often a drying stage), the analyte of interest is eluted using the appropriate solvent. For example, to elute basic drugs from a nonpolar stationary phase, they must be forced into the ionized BH^+ form. Thus, a solution such as 95% methanol/5% dilute NH_4OH is a reasonable choice. Once eluted, the extracts are dried under a stream of nitrogen or air. Care must be exercised with volatile drugs such as methamphetamine at this stage. Often, an aliquot of dilute HCl is added prior to the drying stage to convert the basic drug back to the nonvolatile salt form. The sample is reconstituted as appropriate for the instrument. Typically, an internal standard is added prior to the SPE process. There are methods and columns for basic, acidic, neutral, and combined extractions.

Applying the Science 2 Messy Meconium

Meconium is a difficult matrix to work with, but one that can provide important information regarding maternal ingestion of controlled substances during the prenatal period. A recent article in the *Journal of Analytical Toxicology* reported on a method for sample preparation and GC-MS analysis of methamphetamine and amphetamine in meconium. The authors used a technique called supported liquid extraction, which combines elements of LLE and SPE. A supported liquid extraction column consists of solid support beads coated with a layer of solvent that acts as the extraction medium, just as it would in LLE. In supported LE, an aqueous buffer system is supported on a surface such as diatomaceous earth. After sample loading, a few minutes are allotted to allow for absorption into the buffer that is on the support. Now the solution is dispersed such that there is a large surface area available for contact rather than the single interface that would be present in a separatory funnel. Once absorption is complete, the analytes are eluted using an organic solvent appropriate for the task. The advantages of SLE include a reduced number of steps compared with SPE. The authors utilized selected ion monitoring (SIM) for the detection. LODs were reported as 10 ng/mL and the lower limit of quantitation as 25 ng/mL. A comparable SPE procedure would have required several cleaning and drying steps to achieve similar results.



Flow of preparation.

Source: Gunn, J. A., B. Sweeney, T. Dahn, S. Bell, R. Newhouse, and A. R. Terrell. "Simultaneous Quantification of Amphetamine and Metham-phetamine in Meconium Using ISOLUTE® HM-N-Supported Liquid Extraction Columns and GC-MS." *Journal of Analytical Toxicology* 32, no. 7 (2008): 485–90.

Another extraction consideration relevant here but not in seized drug analysis is the issue of conjugates produced by Phase II metabolism, which is particularly important in the analysis of urine. Breaking up the conjugations requires treatment with acids (acid hydrolysis) or treatment with enzymes such as β-glucuronidase. Care must be exercised to avoid unwanted hydrolysis reactions such as the hydrolysis of cocaine.

EXAMPLE PROBLEM 2

Design an SPE preparation for opiates in urine.

Answer:

Opiates form a number of conjugates and must be addressed. The opiates are also basic, so the pH needed is known. A 5-mL sample of urine is first treated with enzymes to break up the conjugates but not risk acid hydrolysis. The urine is then diluted with a basic buffer (pH 9) and loaded onto a normal-phase SPE column. The un-ionized drug is held on the column while aqueous and methanolic rinses are used to remove unwanted components. The column is dried to remove water, and then the compounds are eluted with methanol/dilute ammonium hydroxide.

3.2 Screening Methods

3.2.1 IMMUNOASSAY Because of the variety and complexity of the sample matrices encountered in forensic toxicology, the battery of presumptive tests is limited compared with those used in seized drug analysis. Color tests are used occasionally, as is thin-layer chromatography, but the primary screening tool is immunoassay.

Not quite instrumental, but beyond sample preparation, immunoassay detects analytes on the basis of an **immunological reaction**. The technique was developed in the 1970s and has been refined to the point that automated immunoassay systems are a common sight in toxicology laboratories.

As with extraction and partitioning, equilibrium is involved:

$$\mathrm{Ab} + \mathrm{Ag} \longleftrightarrow \mathrm{Ab} - \mathrm{Ag} \tag{2}$$

$$K = \frac{[\mathrm{Ab} - \mathrm{Ag}]}{[\mathrm{Ag}][\mathrm{Ab}]} \tag{3}$$

In these equations, Ab is an **antibody**, Ag is an **antigen**, and K reflects what is called the **binding strength** of the antigen–antibody complex. In toxicology, the antigen is the drug or metabolite (or any number of other types of compounds), and the antibody is manufactured specifically against the drug or metabolite. This sounds simple enough, but drug molecules are typically too small to have any significant antigenic properties. This property of drugs is a blessing, since a drug that elicited an immune response would be quickly attacked and inactivated. As a result, for purposes of immunoassays, drugs are usually bound to a larger molecule such as a protein that will elicit an immunological response that is reasonably specific to the drug or drug class of interest. The challenge is to find an antibody that reacts to the drug part of the drug-protein conjugate and therefore with the free drug as well.

To produce antibodies, the antigen is introduced into an experimental animal previously sensitized. To induce an immunological response, the drug is combined with a protein. The drug molecule is sometimes referred to as the **hapten** portion of the complex. The drug molecule and the protein molecule are together called the **immunogen**. The protein portion is always larger than the drug molecule, often by a factor of a thousand or more. After the introduction of the immunogen into the animal and an

incubation period, the animal is bled, and the antibodies in the blood are isolated and purified to yield an antiserum. The stronger the reaction of the antiserum to the antigen (the drug immunogen), the stronger is the serum's **titer**. Under the conditions of a standardized immunological assay, an antiserum with a titer of 1:1000 will be active up to a dilution of 1 in 1000.

An antiserum produced this way is not pure but, rather, is a mixture of different antibodies that respond to different areas of the large immunogen molecule. Such antisera have ranges of specificity, which means much the same in this context as it does in analytical chemistry. A specific reagent acts on or responds to one and only one substance. Similarly, an ideal specific antiserum will react with one and only one immunogen. However, because the immunogen molecules are large and spatially complex, such specificity is difficult to achieve with an in vivo technique. These antisera are called **polyclonal**, and they can vary from batch to batch even when obtained from the same animal. However, this problem has been greatly reduced with the use of **monoclonal** techniques, illustrated in Figure 11.

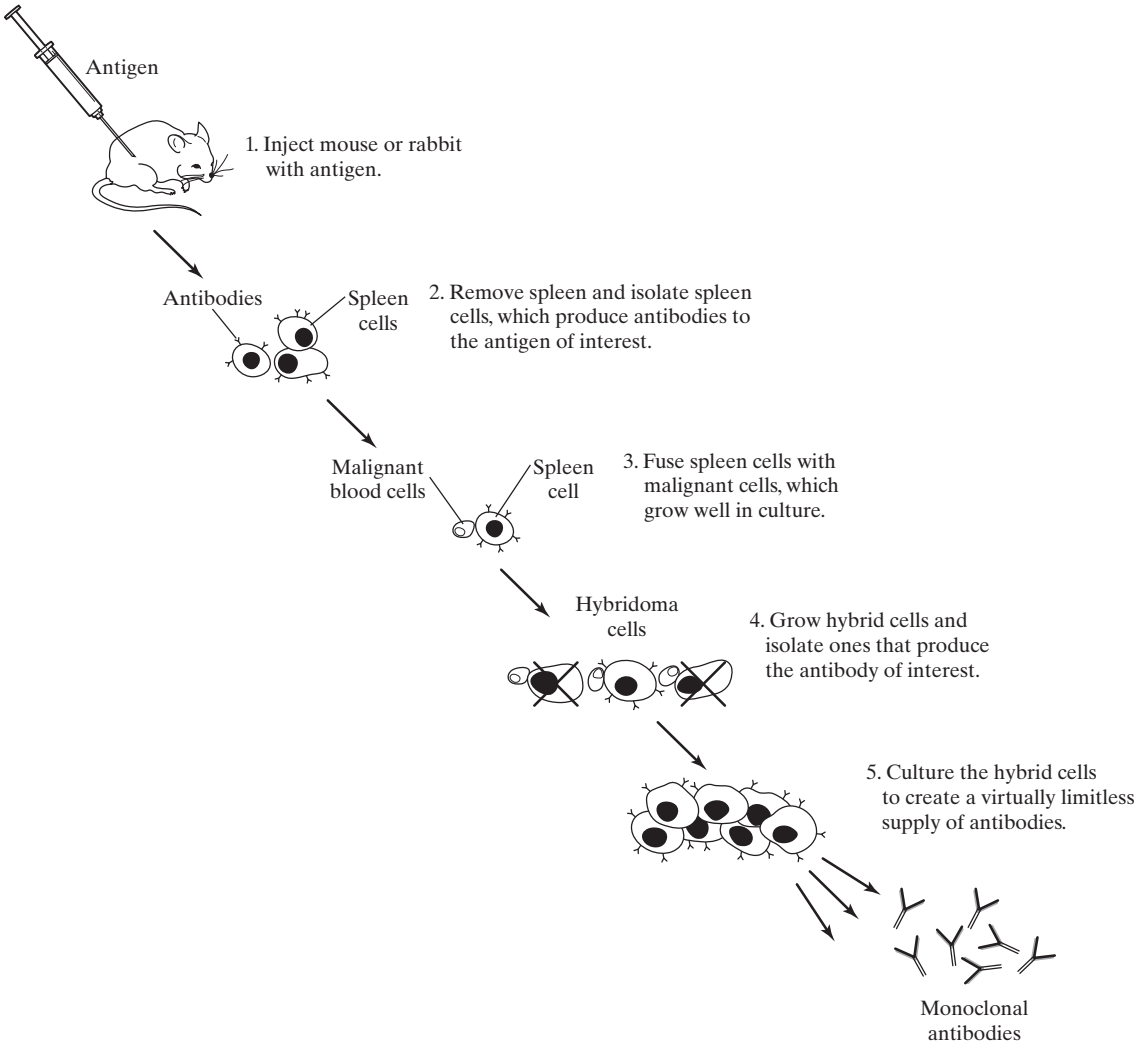


FIGURE 11 An overview of the production of monoclonal antibodies.

Like the process of producing an ordinary antibody, the process of producing a monoclonal antibody starts with the injection of an antigen into an animal. Rather than being bled, the animal is sacrificed, and cells from the spleen are collected. These cells, which will produce the antibody, are then isolated and fused (hybridized) with cells from tumors, creating **hybridoma** cells. The next step is another isolation, in which the most productive hybridoma cells are kept and cultured (cloned), resulting in steady yields of high-specificity, high-titer antisera. However, even such highly purified antibodies can still react to some extent with antigenic molecules that have similar structures. This overlap of reactivity is similar to that seen with ion-selective electrodes such as used in pH meters. A pH electrode is designed to respond only to H^+ ions, but high concentrations of Na^+ will also generate a response.

The same concept applies to antisera and is referred to as **cross-reactivity**. For example, the noncontrolled substances pseudoephedrine, ephedrine, and phenylpropanolamine react with antisera used in amphetamine–methamphetamine assays. The result of cross-reactivity can be false positives, and it is this issue (among others) that leads to the classification of the immunoassay as a screening technique. However, with knowledge of potential cross-reactivity taken into account, immunoassay can be at least semiquantitative, something difficult to achieve with thin-layer chromatography. A typical immunoassay is used to determine whether the analyte of interest is likely present above a set threshold, a value referred to as the *cutoff*. Although there is wide variation in cutoff concentration, the detection limits of current assays are in the range of micrograms to nanograms per milliliter of sample, and many techniques allow for simultaneous screening of numerous drugs and metabolites.

EXHIBIT B

Magic Brownies?

Cross-reactivity is a recognized limitation of immunoassays and is generally well characterized and well understood in forensic applications. However, new findings are always possible. In one case argued not long ago, a defendant maintained that a recent intake of a large amount of chocolate led to the in vivo production of cannabinoids that cross-reacted with the antibodies designed for marijuana immunoassays. An immunological experiment followed and debunked the idea, relegating the legal strategy to the "nice try" category.

Source: Tytgat, J., et al. "Cannabinoid Mimics in Chocolate Utilized as an Argument in Court." *International Journal of Legal Medicine* 113 (2000): 137–39.

Although the discussion that follows uses drugs as examples, immunoassay is not restricted to drug and metabolite analysis. If an antibody can be made to a molecule, an immunological technique can be developed to detect that molecule. Immunoassays have long been used as field tests for polychlorinated biphenyls (PCBs), and there are immunoassays for explosives such as trinitrotoluene (TNT). Unlike thin-layer chromatography, immunoassays are amenable to automation. This is a boon to forensic toxicology laboratories that may have to screen tens or hundreds of samples in a variety of matrices on a daily basis.

3.2.2 TYPES OF ASSAYS Immunoassay can be broadly categorized as **competitive** or **non-competitive**. With noncompetitive methods, also called *immunometric* methods, the antibody is usually present in excess. In the competitive mode (Figure 12), antigens compete for a limited number of antibody binding sites. Initially, the antibody is bound to labeled antigen. The label is used in the detection scheme. The labels can be radioisotopes, species capable of fluorescence, or enzymes, to name the most common.

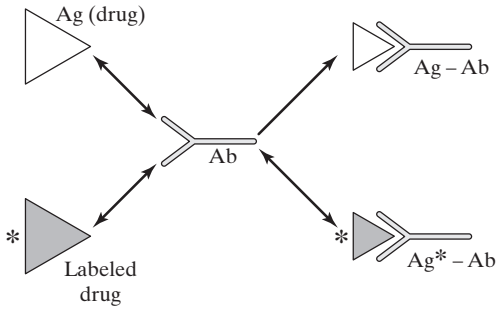


FIGURE 12 A simplified depiction of competitive binding in immunological reactions. Drug from a sample (the antigen Ag) will compete with the labeled drug for the limited available antibody (Ab) binding sites. The labeled sites are indicated by an asterisk (*).

Early assays used radioactive iodine (^{131}I; later, ^{125}I) as labels, and detection employed scintillation counting of emitted gamma (γ) radiation. Beta (β) particles are also used in certain techniques.

The bound, labeled drug–antibody complex is coated on the inside of a tube and thus is immobilized. Suppose that the label is a radioactive material. Prior to the addition of sample, the equilibrium

$$\text{Ab} - \text{drug*(immobile/bound)} \longleftrightarrow \text{Ab(immobile)} + \text{drug*(free, unbound)} \quad \textbf{(4)}$$

is established. A sample such as urine or blood is then added to the tube, and the system is allowed to incubate. If there is a high concentration of drug in the sample, it will eventually displace the bound and labeled drug. When the tube is rinsed, there will be little or no radioactivity associated with the bound or immobile antibody phase left behind. Concentration can be related to degree of displacement. The weaker the radioactive signal in the bound phase, the more complete the displacement is and the more concentrated the drug was in the sample. Calibration curves can be established to make the assay quantitative. The method described in this example, in which the label is a radioactive material, is referred to as **radioimmunoassay** (RIA). RIA is a **heterogeneous assay**, meaning that the bound phase must be separated from the unbound phase before the detection method is employed. This process is shown in Figure 13.

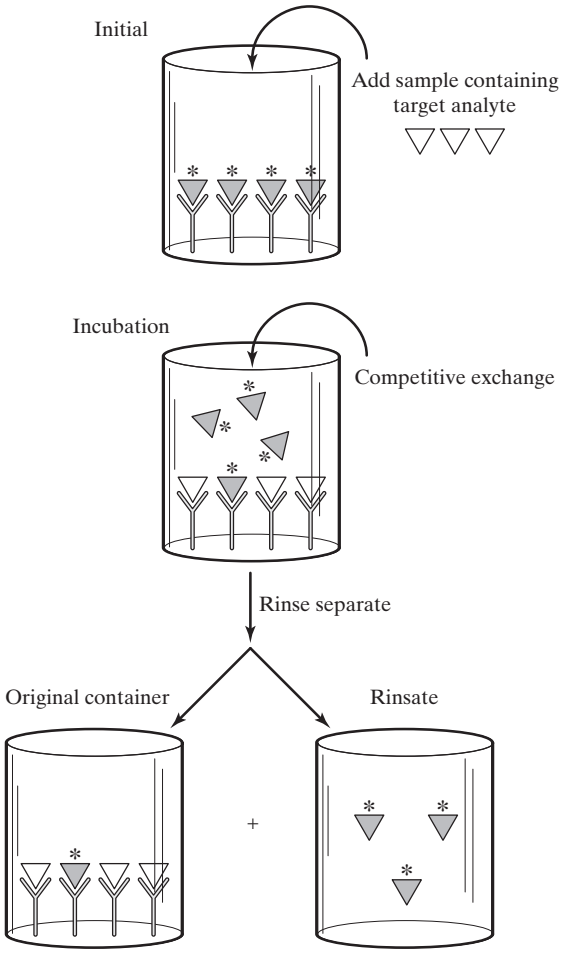


FIGURE 13 A simplified depiction of a competitive binding heterogeneous assay using a labeled antigen.

HOMOGENEOUS ASSAYS These elegant techniques do not require separation of the bound and unbound fractions prior to measurement of response. Such assays

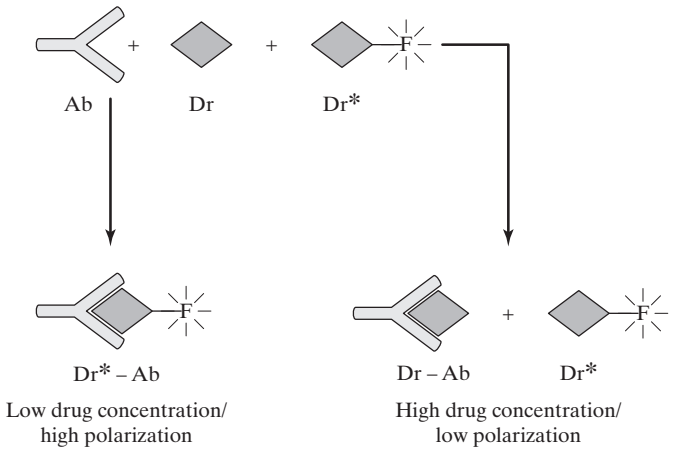


FIGURE 14 Fluorescent polarization immunoassay (FPIA), a homogeneous assay. The F indicates a fluorescing label.

offer advantages by simplifying and eliminating a step but are more prone to matrix effects. An example of a homogeneous assay is **fluorescence polarization immunoassay** (FPIA, Figure 14).

Because there is no separation step, the sample and tube have the same total fluorescent signal before and after sample is added. However, when the labeled drug is bound to the antibody and immobilized, its movement is constrained, and as a result, the light transmitted through it will be strongly polarized. Light coming from unbound labeled molecules will not be polarized, because their motion is not constrained and will be random over the collection. A high concentration of the drug in the sample will displace more labeled drug and decrease the polarization of the emitted light. Two other techniques used in forensic laboratories are enzyme-multiplied immunoassay and enzyme-linked immunosorbent assay.

ENZYME-MULTIPLIED IMMUNOASSAY TECHNIQUE (EMIT, FIGURE 15) This colorimetric technique is widely used to screen for drugs and metabolites. In EMIT, a complex between an enzyme and the target drug is created so that the enzyme retains its catalytic activity in a conversion reaction, such as NAD (colorless) ⟶ NADH (colored). The active enzyme complex can

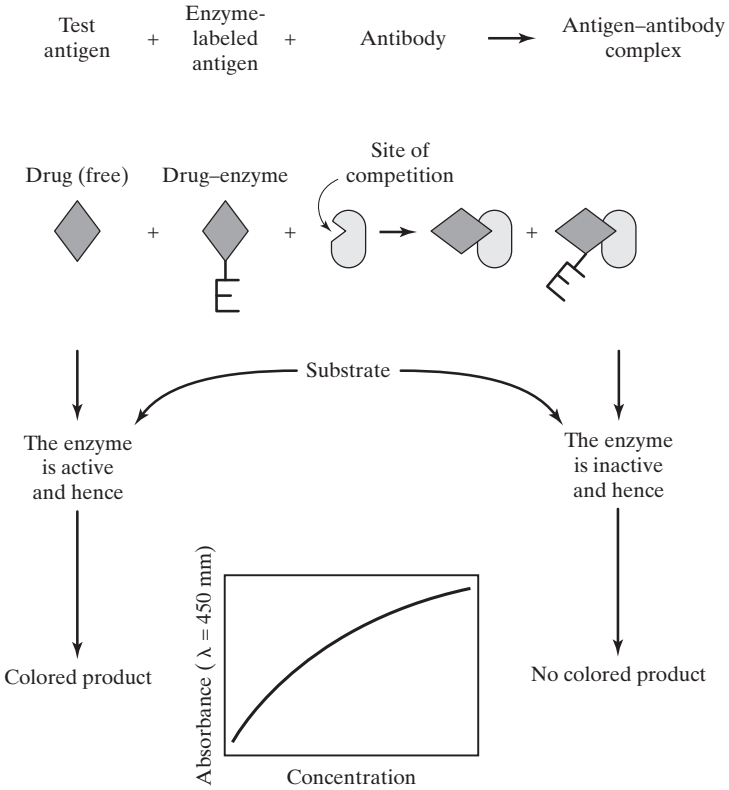


FIGURE 15 EMIT.

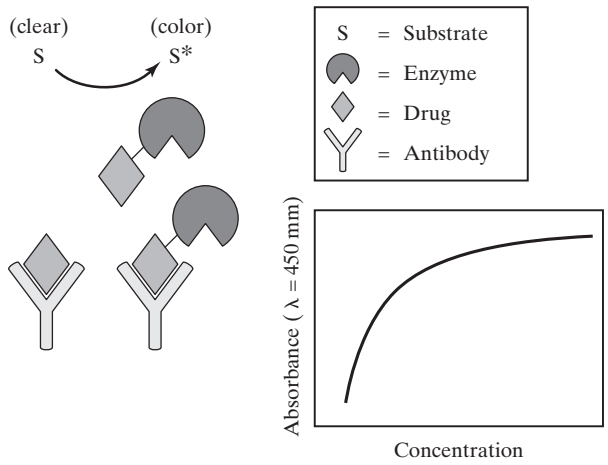


FIGURE 16 ELISA.

be inhibited by binding to an antibody molecule added to the system. This antibody can bind either with the drug or with the drug–enzyme complex.

When there is excess drug antigen available, it will tend to displace the enzyme–drug complex from the antibody, and as a result, active enzyme will be available to catalyze the NAD ⟶ NADH reaction. In turn, this will increase absorption at 340 nm, λ_{max} of NADH. EMIT is a homogeneous assay.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA, FIGURE 16) This is another colorimetric procedure that exploits unbound enzyme to catalyze a reaction that produces a color change. The label is the enzyme, and when labeled drug is displaced, the color-change reaction is catalyzed. The intensity of the color is proportional to the concentration of the drug. A darker color correlates with high displacement and high drug concentration in the sample, whereas faint or no color means that little of the enzyme has been liberated and thus little drug is available in the sample to displace it. ELISA is classified as a heterogeneous assay but otherwise shares many characteristics with EMIT. An advantage of ELISA is that it can be used on sample matrices that are opaque and not amenable to other methods described above. The antibody is bound to the reaction vessel, usually a 96-well styrene plate. Following the application of the sample and labelled enzyme complex, the solutions can be rinsed off prior to the addition of substrate and color development reagents. For this reason, ELISA is favored in many labs performing post-mortem toxicology.

3.2.3 BREATH ALCOHOL One of the most widely used and legally contested presumptive tests in forensic toxicology is the breath alcohol (**BrAC**) test. Breath testing can be based on selective partitioning of alcohol into the gas phase above a liquid based on Henry's law. Breath alcohol testing is the field application of these principles. The blood in the lungs is separated from air by only a thin layer of epithelial cells, so ethanol (and other volatiles) can partition at a predictable rate into exhaled breath. This fact, combined with the value of K_H at the temperature of exhaled air, allows for the correlation of breath alcohol content to blood alcohol content. At 34°C, the value of K_H is now generally taken as 2300 but still occasionally quoted as 2100,[24] which means the concentration in the breath is diluted 2300 times relative to the concentration in the blood. Another way to view this is that it would take 2300 mL of air to contain the same amount of ethanol as is found in 1 mL of blood. Breath alcohol detectors must be designed to detect and quantitate this ethanol vapor.

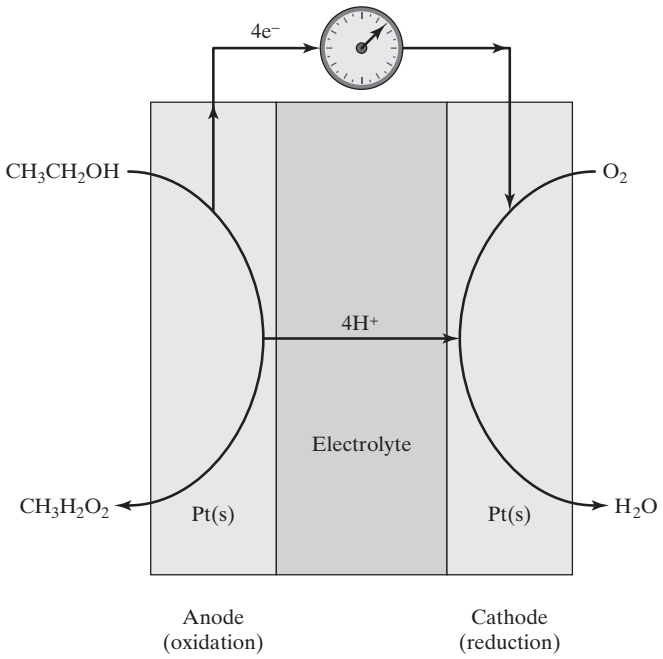


FIGURE 17 Fuel cell schematic.

Breath testing instruments can be based on electrochemical and spectrophotometric detection. The first instrument, called a Breathalyzer, was developed in 1954 by Robert Borkenstein of the Indiana State Police. In honor of his contributions to the field, the University of Indiana offers courses referred to as the Borkenstein classes in drug and alcohol testing and toxicology (www.borkensteincourse.org/index.html, last accessed November 2011). The original Breathalyzer was based on a color test that resulted from a redox reaction (Eq. 5); later models are based on IR spectrophotometry, fuel cells, and solid semiconductor detection systems.

$$\underset{\text{(red-orange)}}{2K_2Cr_2O_7} + 3CH_3CH_2OH + 8H_2SO_4 \rightarrow \underset{\text{(green)}}{2Cr_2(SO_4)_3} + 2K_2SO_4 + 3CH_3COOH \quad \textbf{(5)}$$

A common commercial design for a fuel-cell-based breath alcohol detector is shown in Figure 17. Breath is directed over the anode, which contains platinum, as is the cathode. The platinum catalyzes the oxidation of ethanol to acetic acid, which is esterified to ethyl acetate, releasing four protons and four electrons. The movement of the electrons to the cathode generates a current that is proportional to the concentration of ethanol present.

Because of the importance of breath alcohol testing, it is frequently challenged in court. Clearly, there are many variables that must be controlled, such as the calibration of the instrument and operating temperature. Significant variation arises from how the person exhales into the device, and different jurisdictions have different protocols to address these issues.[45, 46, 47]

3.3 Hyphenated Instrumental Techniques

With the exception of the blood alcohol assays, drugs and metabolites in biological matrices are almost universally identified and quantitated using a chromatographic inlet and a mass spectrometer. Until recently, GC-MS was the dominant tool, but now,

LC-MS methods have taken center stage and continue to find ever-expanding applications in forensic toxicology.

Blood alcohol samples are to forensic toxicology what marijuana is to seized drugs—the source of the largest number of cases and samples. Analytically, blood alcohol determination may seem a bit anachronistic, but the methodology employed is fast and cost-effective. The basis of detection of blood alcohol is detection of ethanol in the headspace above the blood. Instrumentally, this is relatively simple to implement using automated headspace analysis systems. A small amount of blood (typically 5 mL) is placed in a glass vial and capped. The cap consists of a metal rim that supports a septum centered over the vial and loaded into the headspace unit. When it is time for the vial to be analyzed, it is moved to a heated chamber long enough for thermal equilibrium to be established. A needle or tube punctures the septum and draws off a set amount of the headspace vapor and delivers it to a gas chromatograph equipped with a flame ionization detector.

Because the FID is not a specific detector, some form of confirmation of identification by retention time is required. This is usually accomplished by using two different chromatographic columns. Often, the injection port of the GC is connected to a splitter, allowing the sample to be analyzed simultaneously on two columns within a single analytical run. Alternatively, another aliquot of the sample can be tested using a second instrument equipped with the confirmation column. Ethanol has a different retention time on the two different columns, and these must match to constitute a positive identification. Quantitation is straightforward and utilizes an external standard curve.

GC-MS is still used for routine screening and quantitative analysis in forensic toxicology.[42, 48–53] As we saw in sports toxicology, IRMS coupled with GC is a newer application of GC-MS to toxicology, albeit with a new flavor of MS detection. As a screening tool, GC has the advantage of being able to detect a large number of compounds in a single run; the disadvantages relate to the usual issues of volatility, polarity, and thermal liability. Increasingly, GC-MS screening methods are utilizing the selected ion monitoring capability (SIM) of the detector to improve detection limits. An example is shown in Figure 18. A total of 16 analytes were targeted with three ions.[48] The urine samples were prepared by the addition of an internal standard (a deuterated compound, THC-COOH-d_3) followed by incubation with β-glucuronidase for half an hour in a buffered solution at pH 7.4. After the conjugates were broken, the pH of the sample was adjusted to 9, and extraction was performed using a mixture of chloroform and isopropanol. The organic layer was centrifuged, solids were removed, and the sample was evaporated to dryness. The sample was derivatized using MSTFA and TMCS, and 1 μL was injected. For example, the ions selected for morphine (414, 429, and 401) correspond to the most abundant and diagnostic peaks of the derivatized molecule. LODs ranged from 50 to 3 ng/mL.

One of the most important developments in instrument technology for forensic toxicology is tandem MS, which has had a significant impact on the practice of forensic toxicology in the past 10 years,[5, 14, 21, 54–59] and as the cost of these instruments continues to decrease, they will likely become ubiquitous. In addition to the capability to achieve low LODs and LOQs, the variety of tandem designs increases the utility of the systems. Given that the front-end inlet is HPLC or increasingly UPLC, the number of analytes that can be targeted in single runs is as good as or better than with many GC-MS methods. The number of compounds that can be practically detected in a given run is sometimes referred to as **peak capacity**. For example, there are increasing reports of using an LC-QToF for screening samples and then an LC triple quadrupole for confirmation and quantitation. The time-of-flight instrument provides exact mass data along with a retention time, leaving only a few potential isobaric (same mass)

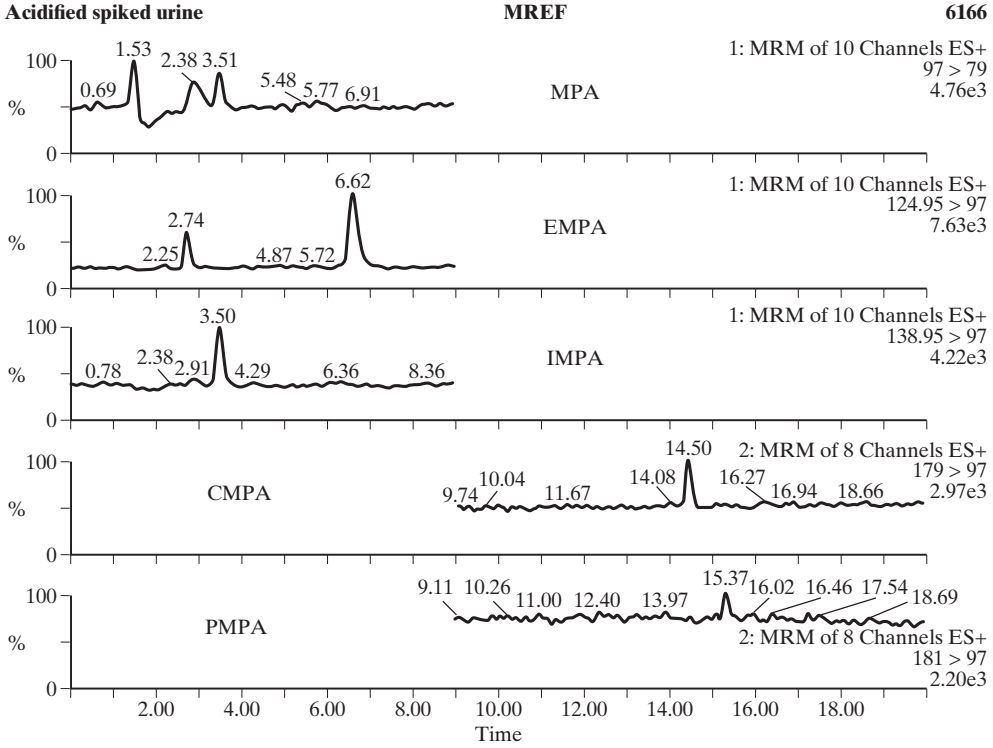


FIGURE 18 Extracted ion chromatograms for selected phosphonic acids in urine. These acids are associated with chemical warfare agents. MREF is the background reference; MPA is methylphosphonic acid; EMPA is ethyl methylphosphonic acid; IMPA is the isopropyl analog: CMPA is the cyclohexyl analog; and PMPA is the pinacolyl analog. From "Feasibility of Direct Analysis of Saliva and Urine for Phosphonic Acids and Thiodiglycol-Related Species Associated with Exposure to Chemical Warfare Agents Using LC-MS/MS" by T. L. Hayes, D.V. Kenny, and L. Hernon-Kenny in JOURNAL OF MEDICAL CHEMICAL, BIOLOGICAL, AND RADIOLOGICAL DEFENSE, Volume 2, August 9, 2004, http://www.jmedcbr.org/Issue_0201/Kenny_0804.html. Used by permission.

interferences. Quantitation on the triple quadrupole provides additional confirmation, particularly if a different column is used.

An example of a recent application of LC-tandem MS is shown in Figures 19–20. An emerging concern in seized drugs and forensic toxicology is synthetic **cannabimimetics** designed to mimic the physiological response to marijuana and THC.[60–65] These compounds are referred to as "legal marijuana," "K2," and "spice" and are

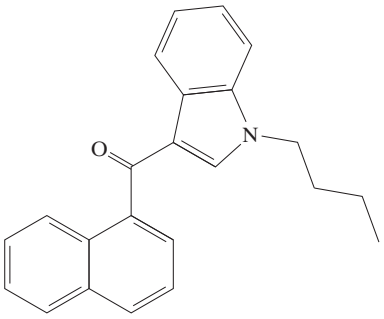


FIGURE 19 JWH-073

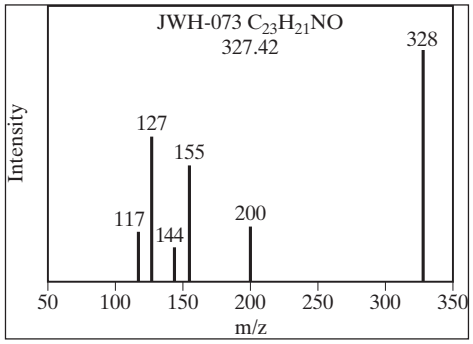


FIGURE 20 Reconstructed mass spectrum

available over the counter in many locations, sold as incense or similar products. The products can contain one or more of a variety of synthetic cannabinoids. These cannabinoid agonists bind with the cannabinoid receptors to varying degrees and cause similar physiological effects.[63] These compounds are often identified as one of the **JWH series**, named after chemist John W. Huffman who synthesized them while studying the cannabinoid receptor system. Thus, the family of mimics includes compounds referred to as JWH-073 and JWH-012.

Figure 19 shows one of these compounds, JWH-073 and its metabolites. Figure 20 shows the ESI-MS spectrum (single quadrupole) for the corresponding structures in Figure 19. This is fairly typical of an ESI spectrum; recall that ESI is a soft ionization technique, and depending on instrument conditions, fragmentation is limited, certainly as compared with EI mass spectrometry used in GC-MS.

Summary

This concludes our discussion of forensic toxicology and forensic drug analysis. We have learned about drugs of abuse and how they are addressed when presented as physical evidence (seized drug analysis) and as biological evidence (forensic toxicology). Although postmortem and alcohol toxicology are best known, sports testing and workplace drug monitoring are also areas in which forensic toxicologists may play a significant role. The matrices are as varied as the analytes, and case history, scene information, pharmacological knowledge, and analytical skill are all needed to address the forensic questions typically presented. In the next section, we will switch gears and begin our exploration of combustion chemistry.

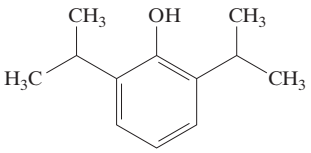
Key Terms and Concepts

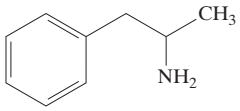
Anabolic agent steroids (AAS)
Androgenic hormones
Antibody
Antigen
Autolysis
Bile
Binding strength
Blood alcohol content (BAC)
BrAC
Cannabimimetics
Cause of death (COD)
Competitive immunoassay
Crash and shoot
Cross-reactivity
Drug-facilitated sexual assault (DFSA)
Erythrocytes
Femoral blood
Fluorescence polarization immunoassay (FPIA)
GABA
Gastric contents
Hapten
Heterogeneous assay
Hybridoma
Immunogen
Immunological reaction
Isotope ratio mass spectrometry (IRMS)
JWH series
Leukocytes
Manner of death
Meconium
Monoclonal
NASH
Non-invasive sampling
Noncompetitive immunoassay
Oral fluid
Peak capacity
Peripheral blood
Physiological pH
Polyclonal
Postmortem redistribution (PMR)
Radioimmunoassay (RIA)
RBC
Sexual Assault Nurse Examiner (SANE)
Steroid profile
Subclavian blood
Thrombocytes
Titer
Urochrome
Vienna Pee Dee Belemnite (VPDB)
Vitreous fluid
WBC
World Anti-Doping Agency

Problems

FROM THE CHAPTER

1. Why is the term "blood concentration" not preferred for describing concentrations of drugs and metabolites in the bloodstream of living persons?
2. In normal urine will the following substances be protonated or not?





Propofol, pK_a 11 Amphetamine, pK_a 10.13

3. Show the net cell reaction for the fuel cell shown in Figure 17.
4. If a fuel cell such as shown in Figure 17 is used to detect breath alcohol, what other compounds might cause a false positive?
5. Design a micro-LLE extraction for whole blood designed to detect acidic drugs.
6. An analyst prepares a homogenized liver sample by placing a weighed amount in a test tube, adding methanol, and sonicating. She places the entire sample on an SPE column, extracts it, and dries it. She then reconstitutes the extract in a known volume of internal standard solution. What is the problem with this approach?
7. An analyst loads a blood alcohol sample onto an instrument equipped with a single column and an FID detector. After the run is completed, the analyst recaps the vial and places it on a second headspace unit connected to a GC-FID containing the confirmation column. What is the problem with this approach?
8. A woman consumes ten beers over 2 hours resulting in a BAC peak concentration of 0.16. How long must she wait before her BAC level falls below the legally intoxicated level of 0.08 g/dL?
9. How would you classify the transformation of flunitrazepam to desmethylflunitrazepam? What pattern do you detect in naming?
10. What are the potential isotopic compositions of the 45 and 46 amu fragments discussed in the context of IRMS?

INTEGRATIVE

1. What is unusual about the formation of 7-aminoflunitrazepam based?
2. Unknown to her, a woman is dosed with the typical anesthesia-inducing dose of 50 mg/kg of GHB. She weighs 130 lb. If the half-life is taken as 0.7 hour, when will the concentration in her blood approach a threshold value of 10 ug/mL? You will need to look up some values.

FOOD FOR THOUGHT

1. Could IRMS be used to help solve the endogenous/exogenous problem with GHB determinations? Research what, if anything, has been reported in this regard and comment.

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The Chemistry of Combustion and Arson

OVERVIEW AND ORIENTATION

The next few chapters will tackle the second most important work area (behind drug analysis and toxicology) in forensic chemistry. We will begin with an exploration of the fundamentals of combustion, a specialized type of oxidation–reduction that spans candle flames to powerful and destructive explosions. The important differences between a fire and a bomb are the speed at which the combustion occurs and the degree to which it is confined. As we will see, combustion can be thought of as a continuum. Forensically, combustion is the process at the heart of arson, bombing, and, perhaps less obviously, gunshot residue. In this chapter, we will cover the fundamental principles that underlie these three types of forensic cases and evidence and discuss the physical evidence associated with fires.

1 THE COMBUSTION CONTINUUM

Combustion is an oxidative decomposition in which oxygen (the oxidant) oxidizes a fuel. The different manifestations of combustion (Figure 1), ranging from a gentle candle flame to a violent military explosive, are part of a continuum that includes the **propellants** used in firearms. The terms we use to describe this decomposition depends on characteristics such as the speed of the reaction. For example, in the **detonation** of an explosive, the reaction front moves faster than the speed of sound (~ 741 mph, or 331 m/s). If this same reaction occurs at less than the speed of sound, it is called **deflagration**. This term can also be applied to what we mean by the term "burning," in which the flame speed is less than the speed of sound. As the flame front's speed increases, oxygen from the atmosphere is incapable of sustaining it, and additional oxygen must come from another source such as potassium nitrate (KNO_3, or **saltpeter**). The fuel must also change as the energy derived from it increases.

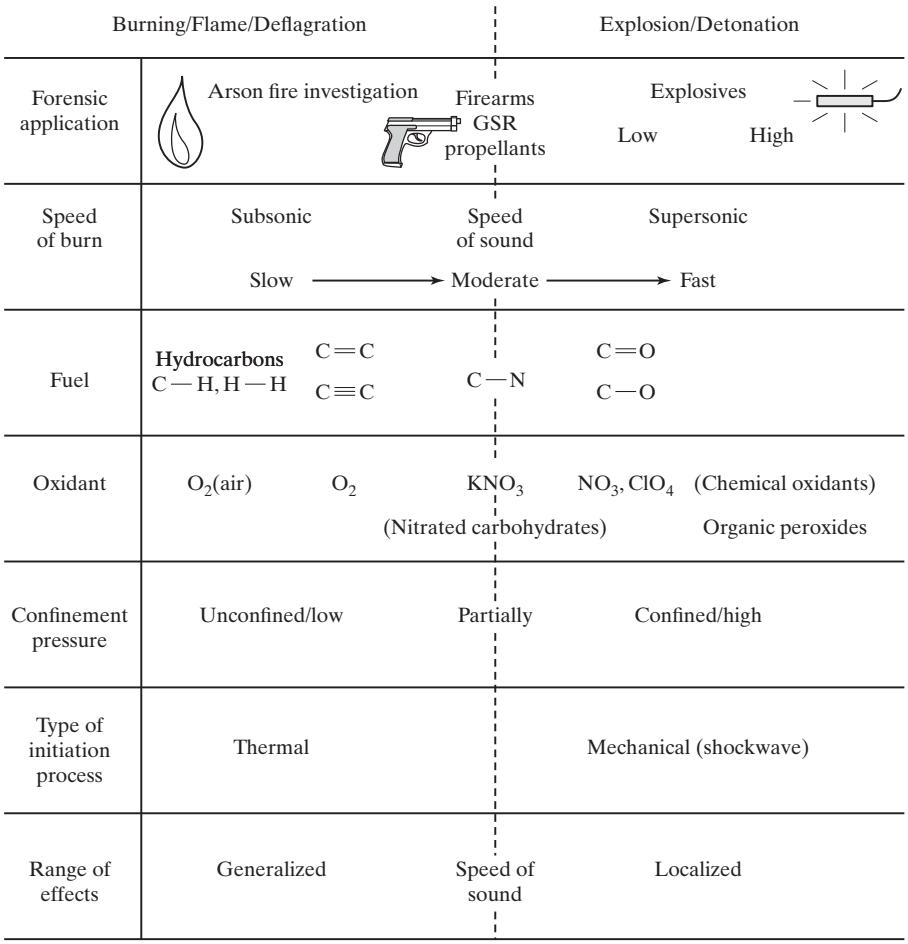


FIGURE 1 The continuum of combustion. The dividing line between burning, deflagration, and detonation is the speed at which the reaction front propagates.

Combustion is an exothermic reaction in which reactants are converted to principally gaseous products. Because the reaction is exothermic, the product gases heat up and expand, and in turn can be harnessed to do work. In a fire, such as arson, this expansion generates plumes with predictable behaviors that leave distinctive markings at the crime scene. In contrast, the expansion of hot gases is utilized in propellants to drive a projectile forward (pressure–volume, or *PV* work), whereas in explosives the expanding gases are confined as long as possible to generate a destructive shock wave. Because of this confinement, a detonation is a mechanical process, whereas deflagration is a thermal one. This critical distinction will be discussed in detail in the next section.

EXHIBIT A

Salt, Peter?

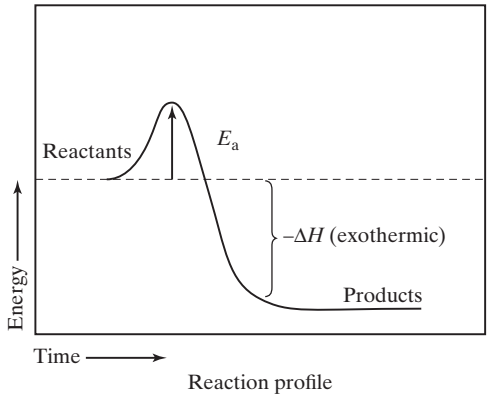
An older name for the salt KNO_3 (potassium nitrate) is saltpeter (also spelled salt Peter and salt peter), which is used as a chemical oxidant in gunpowder and explosives. Saltpeter is a mineral that forms when organic material such as waste, decaying plants, and animal manure is placed in contact with soil high in alkali content (such as limestone). Saltpeter is found on the earth's

surface as well as in caves and is easily collected and mined. The first recorded mention of saltpeter was by a Taoist alchemist writing and working in the ninth century. Although the mineral was once used for medicinal purposes, demand "skyrocketed" with the invention of gunpowder and explosives. Lammont du Pont, of the famous du Pont family of chemists, was able to patent a method for making blasting powder without saltpeter, a much more economical approach. Unfortunately, he died in 1884 while experimenting with dynamite and sulfuric acid. Du Pont and several of his assistants were literally blown apart in the accident.

Sources: Harmon, M. B. "Gunpowder, Ingenuity, Madness, and Murder: The Saga of the du Ponts." *Biography,* November 2002, 92; Morrison, P., and P. Morrison. "Nitrogen: The Dark Side." *Scientific American,* 281 (October 1999): 125–26.

Combustion requires reactants and enough energy to exceed the energy of activation (E_a) required to initiate the reaction. The reaction profile shown in Figure 2 illustrates the exothermic nature of a combustion reaction, as well as the need for enough energy to initiate it. Once initiated, enough energy is produced to supply the necessary E_a to sustain the reaction until one of the reactants is exhausted. With a simple flame, the fuel is exhausted first, since the oxidant is atmospheric oxygen. When chemical oxidants are employed, either fuel or oxidant may be the limiting reagent.

The energy released in a combustion reaction results from the increased stability (lower potential energy) of the products relative to that of the reactants. The energy (ΔH) released can be estimated with the two methods shown in Figure 3. The first method is based on table values of thermodynamic quantities under standard conditions of temperature and pressure (STP, 25°C and 1 atm). The second method calculates how much energy is required to break the chemical bonds in the reactants and form the bonds of the products. As shown in the figure, this result should be relatively close to that determined from the table values; however, the quantities calculated are used only as starting points, because the combustion reactions encountered in forensic chemistry are complex and rarely occur close to standard conditions. Even if the reaction starts at near standard conditions, the reaction itself quickly pushes the system well beyond standard conditions.



Oxidized

$$CH_4(g) + 2O_2(g) \longrightarrow CO_2(g) + 2H_2O(g)$$

Oxidizing agent

FIGURE 2 A generic reaction profile for combustion, along with a simple example of a combustion reaction. Combustion is a specialized type of redox reaction.

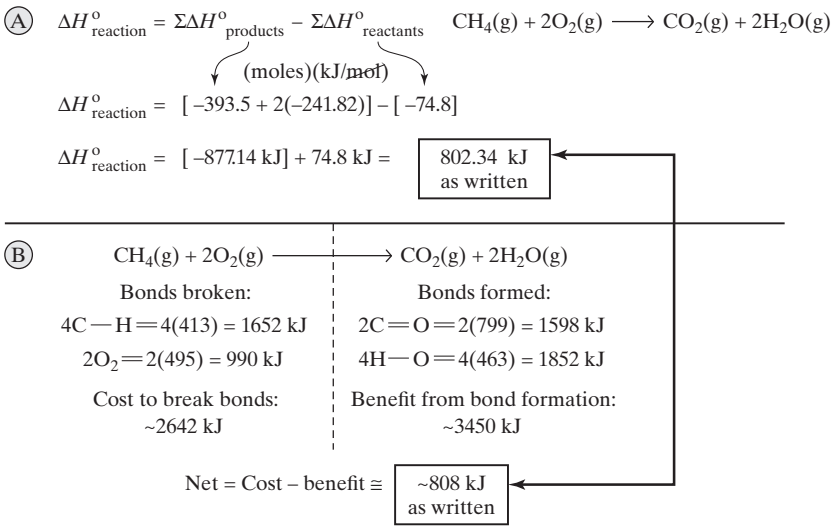


FIGURE 3 Two methods of estimating how much energy is released by a combustion reaction. In the top frame (A), ΔH° is calculated from table values, whereas in (B), the energy balance between bonds broken and bonds formed is used.

Some readers may be familiar with the fire triangle, which is one way of summarizing the requirements for a combustion reaction. Such a triangle is divided into three regions, identified as fuel, oxidant, and heat (the last of which supplies E_a). Building on the concept of the triangle, we will consider the requirements for combustion to be

1. fuel and oxidant in appropriate quantities and concentrations,
2. a source of E_a, and
3. sufficient contact time for the energy source to initiate the reaction.

The absence of any one factor prevents combustion. We will examine the particulars of each factor next.

The first requirement, fuel and oxidant in proper proportions, illustrates key points and unmasks common misconceptions. Wood does not burn; rather, what burns are the vapors emanating from heated wood. Gasoline in a can will not explode because the proper fuel–air mixture does not exist. A cigarette tossed into a pool of gasoline usually smothers before it has a chance to ignite the vapor above it. Similarly, the Hollywood staple of exploding gas tanks in cars is more fiction than fact. Rapid burning can occur, but only when the gas tank is ruptured, the contents leak and vaporize, and the proper air–fuel vapor mixture is created at the same time and place as a source of ignition that stays in contact long enough to spark the reaction.

2 ASPECTS OF COMBUSTION

Consider a simple model of combustion: burning wood (Figure 4). Once the fire is burning, heat must be transferred efficiently to the wood to vaporize reactants. Heat transfer is also needed to ensure that E_a is overcome and the reaction is self-sustaining. Specifically, the activation energy must be sufficient to form free radicals, the heart of combustion reaction mechanisms. Oxygen must move into the reaction zone via mass transfer, and the rate of this transfer directly affects the reaction. Think of blowing across a smoldering fire, an action that increases the efficiency of mass transport of oxygen and speeds the reaction by supplying more oxidizing agent. The kinetics

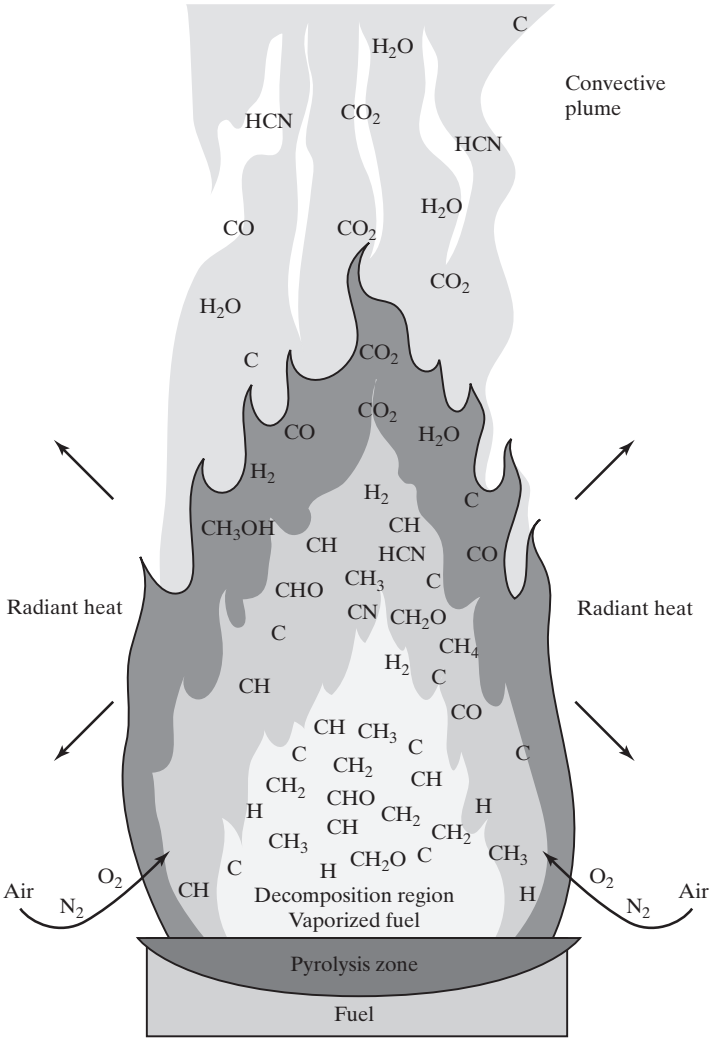


FIGURE 4 The combustion of wood (fuel), illustrating aspects of combustion addressed in this chapter. Note the complexity of what would at first seem to be a simple chemical reaction.

(speed) of combustion depends on the rate of formation of free radicals in the flame flickering above the wood. The reaction speed also depends on multiple rate constants and reactant concentrations in multiple connected chain-reaction pathways. The heat evolved, favored pathways, and the balance of products will all depend on thermodynamic considerations, including stoichiometric ratios and equilibria. Thus, although we will address each of these topics individually, all interact to control and define the complex process of combustion.

2.1 Thermodynamics

Thermodynamics relates concepts of energy flow, enthalpy (H), entropy (S), free energy (G), and equilibrium.[†] The first law of thermodynamics states roughly that energy is neither created nor destroyed but only changes form. In combustion and in explosions, potential energy in chemical bonds (chemical energy) is converted to heat and

[†] Equilibrium is defined as the point at which $\Delta G = 0$.

work. *Energy* is defined as the ability to do work and can be categorized by the type of work done. For example, there is chemical energy, potential energy, mechanical energy, and kinetic energy. The second law of thermodynamics relates to entropy and (again, roughly) states that in any spontaneous process, the disorder of the universe increases. Entropy increases during combustion because gaseous products are formed and heat is released by the exothermic reaction. Molecules move faster at higher temperatures relative to molecules at lower temperatures, resulting in increased disorder.

The free-energy change of any reaction, including combustion, is defined in terms of enthalpy and entropy:

$$\Delta G = \Delta H - T\Delta S \quad \textbf{(1)}$$

where S is entropy and G is the Gibbs free energy. In a flame, the combination of an exothermic reaction with increasing disorder leads to a large negative value for the change in free energy (ΔG). ΔG also is a measure of how much work can be done by a system in a spontaneous reaction. Work can be divided into two components: actual work (w) and heat (q). Both aspects come into play across the combustion continuum. In a fire, such as an intentionally set arson fire, heat and gases are produced but are not exploited to do work. In propellants and explosives, heat and work play critical roles and the work done is the central issue.

In chemical applications, the most common type of work is *PV* work. Examples are shown in Figure 5. The top frame illustrates how the evolution of a gas (H_2) in a spontaneous process is used to do *PV* work in a system in which heat is a minor contributor. The lower frame depicts the relationship between *PV*, work, and force. To move the piston, work—specifically, *PV* work—must be done, and the force acting inside the cylinder must exceed the force exerted by atmospheric pressure. If the piston was removed from the system, the gasoline would still ignite, burn, and generate hot expanding gases; however, this expansion is not purposely directed into doing *PV* work. This is the situation we see in arson fires.

Forensic examples involving energy and illustrating how work is done are shown in Figures 6 and 7. In Figure 6, a bullet is propelled out of a gun by *PV* work done by expanding gases produced by the burning propellant. Here, the bullet is analogous to the piston (Figure 5), which is moved as a result of the production of gas. In the gun, primer ignites when struck by the hammer, provides the initial E_a spark, and initiates combustion, which produces heat and hot expanding gases. The bullet is held in the cartridge by compression and friction, but the joint is designed to give way once sufficient pressure builds up. The result is movement of the bullet down the barrel, just like movement of the piston shown in Figure 5, except that the force must overcome the compression and friction forces holding the bullet in place. As gas expansion continues, much of the energy is transferred to the bullet as kinetic energy. The energy trace of a gun firing can be summarized as a mechanical energy (hammer striking primer) $\longrightarrow$ chemical energy $\longrightarrow$ heat and work (heat and mechanical) $\longrightarrow$ kinetic energy. This progression can be simplified to ME $\longrightarrow$ CE $\longrightarrow$ ME (and heat) $\longrightarrow$ KE.

For a crude pipe bomb (Figure 7) made of galvanized steel pipe and gunpowder, the energy pathway is the same. What differs is that no joint is designed to fail, as was the case with the gun firing. A pipe bomb is a mechanically stronger containment device that allows pressure to build until it exceeds the strength of the container at its weakest point. Pressure and confinement are critical factors in explosions and detonations. The pipe shatters and ejects shrapnel in all directions. In contrast, a gun is designed to focus and direct the gas expansion and work in order to impart the most kinetic energy to a single projectile traveling in a controlled trajectory in one direction.

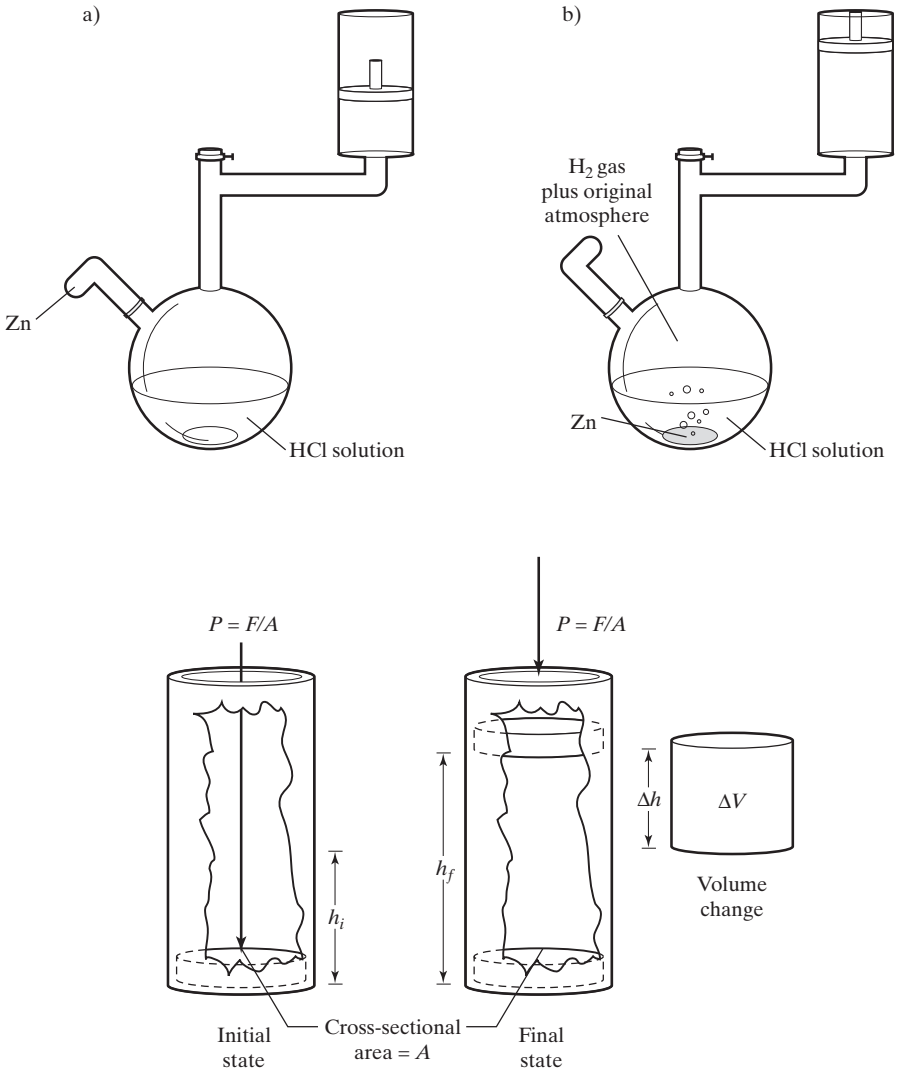


FIGURE 5 Two illustrations of *PV* work. In the top frame, zinc metal is dropped into acid, and pressure exerted by the evolving gas raises the piston. In the lower frame, pressure is defined as force per unit area (*F*/*A*), but the concept is the same and the $P\Delta V$ term can be calculated geometrically by using the volume of a cylinder. For the piston to move, work of quantity $-P\Delta V$ must be done by the system. An example of this mechanism is gasoline burning in the cylinder of an internal combustion engine.

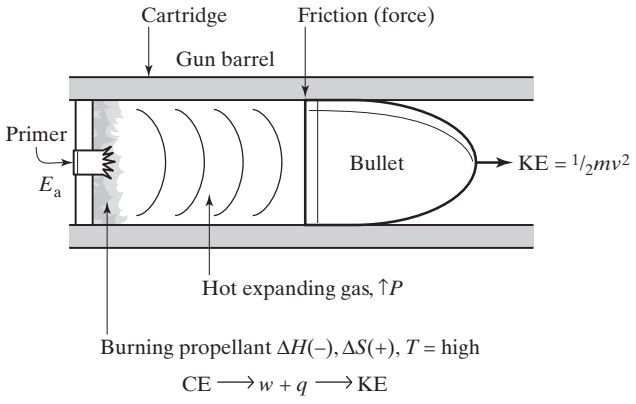


FIGURE 6 How a combustion reaction is used to create sufficient energy to do the work of moving a bullet out of a gun barrel.

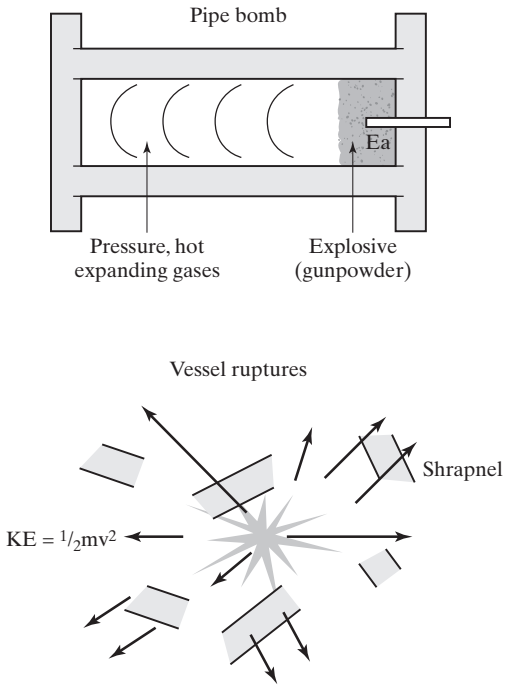


FIGURE 7 A pipe bomb is designed to rupture and not to fail at a specific joint. The result is catastrophic destruction of the container and the ejection of sharp shrapnel moving at high speed.

Before going further, we should introduce terminology to eliminate the negative–positive sign confusion often associated with the heat of reaction and the release of heat. All of the combustion reactions we will deal with in this chapter are exothermic, all release heat, and all have a negative value for $\Delta H^{\circ}_{\text{reaction}}$. The heat released by the system is absorbed by the surroundings and is always positive. In the examples just discussed, the system is the burning propellant or the detonating explosive. These reactions release heat to the surroundings, such as the gun barrel. For this discussion, heat released will be referred to as Q, in keeping with traditional thermochemical notation. In combustion, Q is always positive and numerically the opposite of $\Delta H^{\circ}_{\text{reaction}}$:

$$Q = -1\,\Delta H^{\circ}_{\text{reaction}}) \tag{2}$$

We will also assume that combustion is **adiabatic combustion**—that is, that the heat released is used only to heat the products and that there is no heat exchange to the surroundings. Again, using the gun example, if the firing process is adiabatic, it means that all the heat evolved is consumed by heating of the products created by the combustion reaction. Of course, this is only an approximation, but a useful one.

Heating the products also increases their kinetic energy. This heating causes pressure to increase, according to the ideal gas law:

$$PV = nRT \tag{3}$$

This law shows that temperature is directly proportional to pressure and volume. In the case of an explosive, the higher the pressure generated, the more powerful is the explosive. The power of an explosive can be defined as VQ, where V is the volume of gaseous products and Q is the heat evolved.[1] Thus, the most powerful explosives (per gram) are those that create the largest number of moles (n) of gas at the highest temperatures. A common way to express this relationship is via a modification of the ideal-gas law[1]:

$$F = nRT_{\text{e}} \tag{4}$$

where F is force of the explosive, n is number of moles of gas produced, and T_e is the temperature of the resulting product mix.

The value of Q (used to heat the products to T_e) is central to describing and estimating explosive power. Figure 3 illustrated two methods of calculation of the heat of reaction, but of more interest here is gauging the relative heat generated per gram of fuel. Also, the calculations shown in Figure 3 assume that the fuel and oxidant are in **stoichiometric equivalence** (which we abbreviate as STE), meaning that the molar amounts of fuel and oxidant are in exactly the correct proportions according to the balanced equation for the reaction to go to completion. In the case of methane combustion (Figure 2), if 1.0 mol of methane was present when the reaction started, then at STE there would be exactly 2.0 mol of O_2 present. This situation, however, is rarely encountered, and as a result, both the products of the reaction and the heat evolved will be affected.

EXAMPLE PROBLEM 1

Assuming that conditions are such that methane (CH_4) and methanol (CH_3OH) combust via an explosion, which is the more powerful per gram detonated?

Answer:

To answer this question, the value of heat released (Q or ΔH) for each compound is needed, as well as the number of moles of gas produced. Use thermodynamic values to determine Q per mole combusted. Some assumptions are necessary, but for a rough comparison, this approach is reasonable. Here, we will treat all species in the gas phase.

$$CH_4 + 2O_2 \longrightarrow CO_2(g) + 2H_2O(g)$$

$\frac{\Delta Hg}{kJ/mol}$	−74.8	−393.5	−241.82	
moles	1.0	1.0	2.0	(3.0 total)
grams	1.0			
moles/gram	0.063	0.063	0.125	(0.19 total moles per gram)

$$0.19 \times \frac{22.4\ L}{mol} \cong 4.3\ L/g$$

$$\Delta H^o_{reaction} = [-393.5 + 2(-241.82)] - (-74.8)$$

$$= \frac{-802.3\ kJ}{mol\ CH_4} = \frac{-50.1\ kJ}{g\ CH_4(g)};\ Q \cong \frac{50\ kJ}{g\ CH_4}$$

$$2CH_3OH(g) + 3O_2 \longrightarrow 2CO_2(g) + 4H_2O(g)$$

$$2\ mol \longrightarrow 6\ mol\ gas$$

$$moles/gram\ 0.0313 \longrightarrow 0.0313\ mol + 0.0625\ mol\ or,$$

calculated as above, $\frac{2.1\ L}{g\ CH_3OH}$

$$\Delta H^o_{reaction} = [2(-393.5) + 2(-241.82)] - (-201.2)$$

$$\Delta H^o_{reaction} = \frac{-1069\ kJ}{2\ mol\ CH_3OH} = \frac{-535\ kJ}{mol\ CH_3OH} \cong \frac{16\ kJ}{g\ CH_3OH}$$

$$QV_{CH_4} \cong 50 \times 4.3 = 215 \qquad QV_{CH_3OH} = (16)(2.1) \approx 36$$

The difference is ~ a factor of 6 for 1 g of each substance.

Notice that, in balancing the equations, clearing the fractions was not necessary, since the oxygen does not contribute to Q. The combustion of a gram of methanol produces about half the gas volume of the combustion of a gram of methane. Thus, ethanol is a better fuel, at least using this one metric.

Using the ratio of fuel to air at STE as a reference point, we can describe situations in which we do not have STE. When the amount of fuel relative to the amount of oxidant decreases, the mixture becomes a **lean mixture**. In chemical terms, the system is overoxidized, and if the fuel concentration drops too low relative to the concentration of oxidant, combustion cannot take place. When there is more fuel relative to the oxidant, the mixture is **rich**, and the system is underoxidized. At the extreme of richness, combustion is impossible. An underoxidized system favors production of the less oxidized product and releases less heat.

The calculation of the **fuel/air ratio** (F/A) is an important one and can assist in determining such properties as potential flammability of mixtures. Returning to the combustion of methane as our example, the molar ratio of methane to oxygen in a balanced equation at STE is 1:2, respectively. Because the types of combustion of interest here take place in air, additional corrections are required. Assuming that the atmosphere is 21% oxygen, the number of moles of air supplied must be adjusted upward. A mole of air contains 0.21 mol of O_2; to obtain 1 mol of O_2, we need to multiply this value by 4.76($0.21 \times 4.76 = 1.0$). One last adjustment is to multiply this result by 2, since the complete combustion of 1 mol of methane requires 2 mol of oxygen. This value is easily converted to a mass ratio using the formula weight of methane (16.0 g/mol) and the weighted average mass of air, which is generally taken to be 28.85 g/mol:

$$\text{F/A} = \frac{(16.0\ \text{g/mol})(1\ \text{mol})}{(2.0\ \text{mol O}_2)\left(\dfrac{4.76\ \text{mol air}}{1\ \text{mol O}_2}\right)\left(\dfrac{28.85\ \text{g}}{1\ \text{mol air}}\right)} = 0.0583 \qquad \textbf{(5)}$$

Thus, at stoichiometric equivalence, the mass ratio F/A is 0.0583. It is important to note that here we are assuming a closed system, in that the fuel and air are being held at a fixed volume at 1.0 atm. We will see many instances in which we have to make some basic assumptions about conditions, and in many cases, our calculations are reasonable estimates, not hard values. However, these types of calculations are the foundations of complex models used to describe combustion, deflagration, and detonation.

Now, consider an example in which 5.0 g of methane is released into a container of air with a volume of 20.0 L at a temperature of 25°C. To determine whether the resulting mixture is combustible, rich, or lean, the mass ratios of fuel to air are calculated and compared with the ratio at STE just calculated. The first step is to obtain mole ratios via partial pressures and the ideal gas law:

$$P_{\text{CH}_4} = \frac{nRT}{V} = \frac{\left(\dfrac{5.0\ \text{g}}{16.0\ \text{g/mol}}\right)\left(0.0821\dfrac{\text{L}\cdot\text{atm}}{\text{mol}\cdot\text{K}}\right)(298\ \text{K})}{20.0\ \text{L}} = 0.38 \qquad \textbf{(6)}$$

therefore, $P_{\text{air}} = 0.62$

We convert these mole fractions to mass using formula weights:

$$M_{\text{CH}_4} = M_{\text{F}} = (0.38\ \text{mol})(16.0\ \text{g/mol}) = 6.1\ \text{g}$$
$$M_{\text{air}} = (0.62\ \text{mol})(28.85\ \text{g/mol}) = 18.0\ \text{g} \qquad \textbf{(7)}$$

The last step is to determine the F/A ratio relative to that at STE:

$$\frac{(M_{\text{F}}/M_{\text{A}})_{\text{SYS}}}{0.0583} = \frac{6.1\ \text{g}/18.0\ \text{g}}{0.0583} = 5.8 \qquad \textbf{(8)}$$

The mixture in the container is rich relative to the STE ratio. As seen in Figure 8, deviations from STE affect the temperature of the flame; we will see why momentarily.

Staying with our methane example, Figure 8 illustrates how rich and lean mixtures alter the heat evolved. The heat released at stoichiometric equivalence was previously calculated (Figure 3); note that the heats of formation for water (in gas or liquid form) and methane are constants in this combustion, and the contribution of elemental O_2 is zero. As a result, the expression for calculating ΔH_i to compare the three reactions depicted in the figure can be simplified as follows:

$$\Delta H^\circ_{\text{reaction}} = X_{CO}\,\Delta H^\circ_{CO(g)} + X_{CO_2}\,\Delta H^\circ_{CO_2(g)} \quad \textbf{(9)}$$

$$\Delta H^\circ_{\text{reaction}} = X_{CO}(-110.5\ \text{kJ/mol}) + X_{CO_2}(-393.5\ \text{kJ/mol}) \quad \textbf{(10)}$$

where X_i represents the mole fraction of each species produced. The most negative value (the largest Q) occurs when only CO_2 is produced. Any carbon monoxide pro-

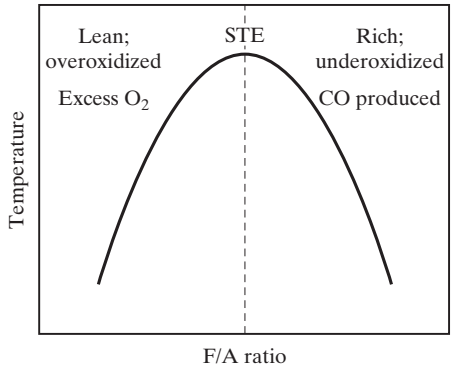


FIGURE 8 Temperature of a flame as a function of the fuel/air ratio.

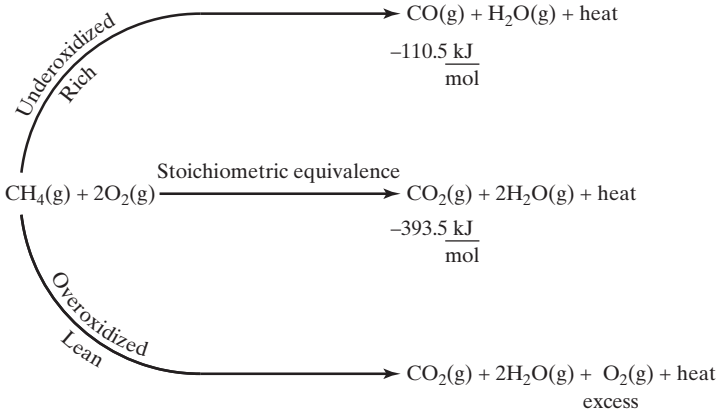


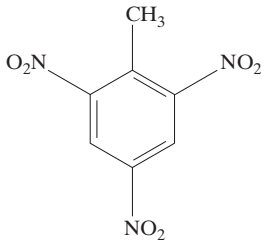
FIGURE 9 The effect of underoxidation and overoxidation on heat released.

$$\Delta H^\circ_{\text{reaction}} = \Sigma\Delta H^\circ_{\text{products}} - \Sigma\Delta H^\circ_{\text{reactants}} \rightarrow \Delta H^\circ_{\text{reaction}}$$

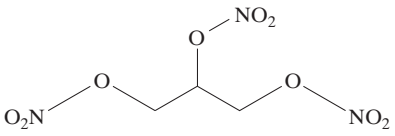
$O_2 = 0.0$; H_2O = constant; [–74.8] constant

Controlled by CO/CO_2

duced decreases the heat released, since the overall heat of the reaction becomes more positive ($\Delta H^{\circ}_{\text{reaction}}$ becomes less negative). Thus, underoxidized systems, which favor CO production, release less heat than do systems at stoichiometric equivalence. However, if there is excess oxidant, some of the heat evolved is diverted to heat that oxidant, rather than just the products, as we assumed in the adiabatic combustion model. As a result of heat diversion, Q decreases from the maximum produced at stoichiometric equivalence, at which Q heats only the products.



Trinitrotoluene (2,4,6 TNT) $C_7H_5N_3O_6$



Nitroglycerin $C_3H_5N_3O_9$

FIGURE 10 Two common explosives.

The discussion becomes more interesting when we examine reactions in which the oxidant is not atmospheric oxygen or in which the source of oxygen is chemical, as in the case of explosives such as TNT or nitroglycerin (Figure 10). With these explosives, part of the oxidant is supplied by the molecule, part is supplied by the atmosphere, and the ratio is expressed as the **oxygen balance**. As demonstrated in Example Problem 3, nitroglycerin has a positive oxygen balance, meaning that when the explosive decomposes to gaseous products, the explosive molecule itself can supply all the needed oxygen, with some to spare. The reverse is true for explosives such as TNT, which require oxygen from the atmosphere or another chemical source. When the oxygen balance is negative and relatively large, CO will form in preference to CO_2. In other words, the system is underoxidized and lean. The oxygen balance can also be expressed as a weight-percentage-like quantity derived from the ratio

$$\frac{\text{Mass O released or consumed}}{\text{Mass of explosive}} \qquad \textbf{(11)}$$

EXAMPLE PROBLEM 2

For combustion of a hydrocarbon such as methane or ethane, is the oxygen balance positive or negative?

Answer:

The oxygen balance is always negative. There is no oxygen in the molecule.

Example Problem 3 illustrates how oxygen balance is calculated, and Table 1 gives the oxygen balance of some representative explosives.[1]

The concept of oxygen balance is analogous to the definition of a rich or lean mixture of fuel versus oxidant. The difference is that the oxygen balance is internal

TABLE 1 Oxygen Balance of Representative Explosives

Explosive	Oxygen Balance
Ammonium nitrate	+20%
Nitroglycerin	+4
Picric acid	−45
TNT	−74

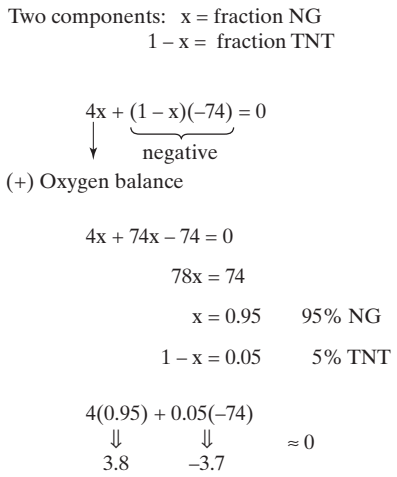


FIGURE 11 Oxygen balance calculations for a mix of explosives.

to the fuel molecule; in other words, the molecule supplies both fuel and at least part of the oxidant. Because the oxygen ratio is related to stoichiometric ratios, it also relates to the heat release *Q*. When a given explosive is the only material combusted, the more positive the oxygen balance, the greater is the heat released. By itself, TNT does not generate as much heat as a compound such as nitroglycerin. However, explosives are often formulated such that their combined oxygen balance approaches zero, which corresponds to stoichiometric equivalence (STE). For example, suppose an explosive mixture consists of TNT and nitroglycerin. To maximize *Q*, the combined oxygen balance should be as close to zero as possible. TNT has a large negative oxygen balance, whereas nitroglycerin has a small positive balance. Clearly, then, the mixture should contain a little TNT and lots of nitroglycerin. The calculation is shown in Figure 11.

Applying the Science 1

It is not unheard of for fires to be set with the intent of destroying evidence of other crimes such as murder. A recent study addressed how fires affect blood evidence – both detection of bloodstain patterns and the ability to obtain typable DNA samples from blood after a fire. In this project, fires were set under tightly controlled conditions after bloodstains had been purposely created in several rooms in the structure. Postfire, analysts studied the performance of reagents used for presumptive testing of blood including phenolphthalein, luminol and fluorescein on the stains, most of which remained visible even after burning. Results were generally poor, although some positives were obtained. The authors correlated maximum temperatures with the ability to obtain full and partial DNA profiles and found that in areas where the temperature exceeded 923°C, they obtained full profiles from about a third of the samples, partial profiles from about 12%, and no profile from more than half of the samples. In contrast, 85% of the samples from a room where the temperature was 307°C yielded full profiles, 6% partial profiles, and fewer than 10% yielded no profile. An important finding of this work is that at fire scenes, the lack of a positive presumptive test for blood does not preclude the ability to obtain viable DNA evidence.

Source: Tontarski, K. L., K. A. Hoskins, T. G. Watkins, L. Brun-Conti, and A. L. Michaud. "Chemical Enhancement Techniques of Bloodstain Patterns and DNA Recovery after Fire Exposure." *Journal of Forensic Sciences* 54, no. 1 (2009): 37–48.

EXAMPLE PROBLEM 3

Calculate the oxygen balance for the following materials: aluminum metal and nitroglycerin (NG, $C_3H_5N_3O_9$).

Answer:

The first step is to balance the combustion equation, with products going to their completely oxidized state. Any nitrogen is assumed to go to N_2. The relative excess or deficit of oxygen is calculated as a weight percent.

For Al:

$$Al + O_2 \longrightarrow Al_2O_3$$

Balanced:

$$4Al + 3O_2 \longrightarrow 2Al_2O_3$$

One way to approach this problem is to use a table, as follows. Note that we do not need to consider the products once the equation is balanced.

	4Al	$3O_2$
Moles	4	3
Formula weight (g/mol)	27.0	32.0
Grams	108	96

Oxygen balance:

$$\frac{96 \text{ g } O_2}{108 \text{ g Al}} \times 100 = -89\%$$

The balance is negative because additional oxygen is required for the reaction to proceed.

For NG:

This part of the problem is more complicated because oxygen is present in the molecule. We need to determine whether the amount is sufficient by itself to support the reaction, so we first balance the equation:

$$C_3H_5N_3O_9 \longrightarrow 3CO_2 + 5/2H_2O + 3/2N_2$$
$$2C_3H_5N_3O_9 \longrightarrow 6CO_2 + 5H_2O + 3N_2$$
$$18O \longrightarrow 12 + 5 = 17O; \text{ balance for excess}$$
$$4C_3H_5N_3O_9 \longrightarrow 12CO_2 + 10H_2O + 6N_2 + O_2$$

Here, oxygen is in excess, so the oxygen balance will be positive.

	$4C_3H_5N_3O_9$	O_2
Moles	4	1
Formula weight (g/mol)	227.0	32.0
Grams	908	32

Oxygen balance:

$$\frac{32 \text{ g } O_2}{908 \text{ g NG}} \times 100 = +3.5\%$$

2.2 Kinetics of Combustion

Kinetics is the study of the speed of reactions and their mechanisms. Combustion is a complex free-radical process in which many reactions can occur and in which a complex mixture of products forms. The pathways favored and resulting products depend on which reactions are favored under the given conditions.

Combustion does not involve simple single-step collisions between a fuel molecule and an oxidant. Rather, the reactions that occur during combustion are based on free radicals. In a free-radical[2] mechanism, three generic steps take place:

- *initiation*, in which the first free radicals are formed
- *propagation*, in which reactions among radicals produce more radicals
- *termination*, which results from the combination of two free radicals to form a neutral species

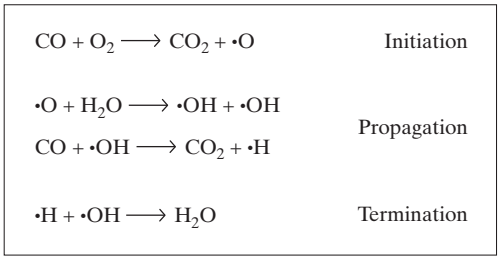


FIGURE 12 Sample free-radical reactions that occur in a flame.

Because radicals react with neutrals to create new radicals, a chain reaction results. Each step in a generic free-radical reaction has an associated rate constant. One of these steps will be the slowest and is termed the *rate-limiting step.* Just as the slowest member of a relay team limits its performance, the rate-limiting step limits the speed of the chemical reaction. The rate-limiting step is generally the step with the highest energy of activation. Partial and simplified steps for the combustion–oxidation of hydrogen are shown in Figure 12.

EXHIBIT B

It's a Radical Difference

Radicals form when a bond such as H—H in H_2 is cleaved homolytically: $H_2 \longrightarrow 2\,H\bullet$. A heterolytic cleavage would send two electrons to one atom and none to the other: $H:H \longrightarrow H^+ + H:^-$. That which causes the formation of the free radical is called the initiator, which may be a chemical species or energy such as UV light. In forensic situations, peroxides (ROOR) are frequently encountered as free-radical initiators: $ROOR \longrightarrow 2\,RO\bullet$. The relative stability of radicals is comparable to that of carbocations, with tertiary free radicals being the most stable and primary the least.

The combustion of even a simple hydrocarbon such as methane is extraordinarily complex. A recent count revealed 277 known elementary reactions for this well-studied combustion involving 49 different chemical species.[3] Compare this with the combustion of an accelerant such as gasoline, itself a complex mixture of hydrocarbons, and the task of accurately modeling the combustion becomes impossible. Fortunately, in forensic chemical applications, the most important point is that complex and competitive free-radical pathways lead to a mixture of products.

2.3 Heat Transfer

Heat transfer and heat flow in combustion begins with the premise that all the heat Q evolved in the process goes into heating the products and raising their temperature.[†] In other words, we assume an adiabatic flame. Excess oxidant (a lean mix) reduces temperature, because Q must be distributed to excess reactant as well as to the products. There are other places heat can and must flow to sustain combustion, some

[†]Our discussion here will focus on heat transfer via convection (matter transport), but heating by conduction and radiation also take place.

of which were seen in Figure 4 and which are also shown in simplified form in Figure 13. Heated air is less dense than cooler air, and as a result, much of the radiant heat produced in a simple combustion like a burning candle is carried away in rising air and gases. This kind of combustion is also called a **buoyant flame**. Heat is also required for phase transitions, as shown in Figure 14. In the candle, the wax must first be melted to liquid and vaporized before combustion occurs. For some heavier hydrocarbons, the melting point and heat needed to vaporize them can be quite high. If Q dwindles, so will the supply of vaporized fuel.

Heat transfer to the substrate has interesting effects on fire behavior and arson investigation. As shown in Figure 15 (top frame), heat may reach deep into a substrate such as wood even when oxygen cannot. The result is **pyrolysis** ("fire cutting") or decomposition in a reducing environment. The products of pyrolysis are different from those of oxidation and can be identified as a layer in burned wood. Typically, the pyrolysis zone is some distance below the burned surface. The layer is defined by the availability of oxygen and by the depth to which heat can penetrate.

The bottom frame of Figure 15 illustrates a situation seen in many arson cases: a liquid accelerant such as gasoline is poured over a surface and the vapors are ignited. If the pool is deep enough, it insulates the substrate below and limits the temperature increase. Just as the temperature of liquid water (not steam) cannot exceed 100°C, the temperature of the liquid accelerant cannot exceed its boiling point. Consequently, the pattern of burning and scorching at the edge of the pool and away from it will be different from the patterns directly beneath.

Heat transfer is directly related to the concept of **mass transfer**. Q is used to heat products of the reaction, which, on a molecular level, means that the greater Q is, the more kinetic energy is transferred to the product molecules. This kinetic energy can be transferred to other molecules via collision. However, for that energy to be transferred to key molecules such as those found in the vaporized candle wax, the energy has to be delivered, via fast-moving molecules, to the right place. That movement of mass is called mass transfer.

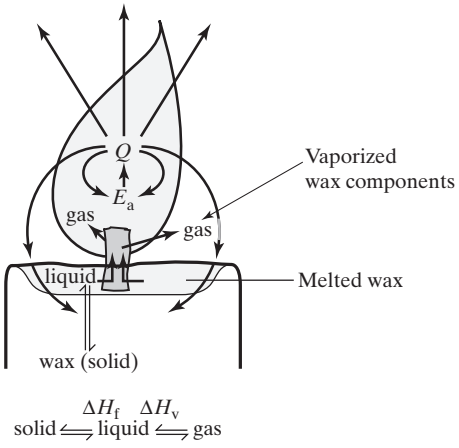


FIGURE 13 Simplified heat flow paths in a candle flame.

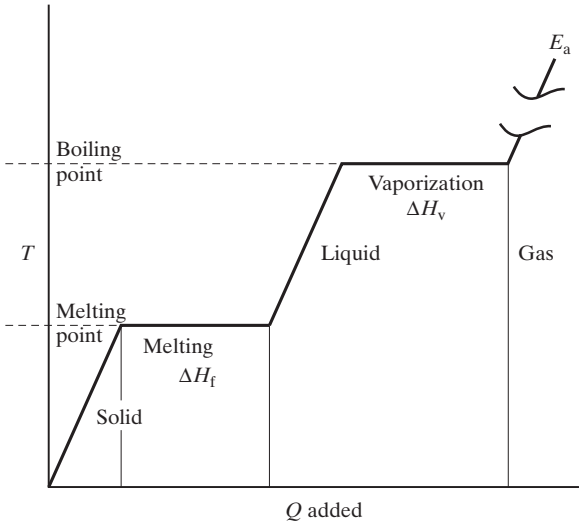


FIGURE 14 Some of the heat generated by the combustion is consumed in the necessary phase transitions of the fuel.

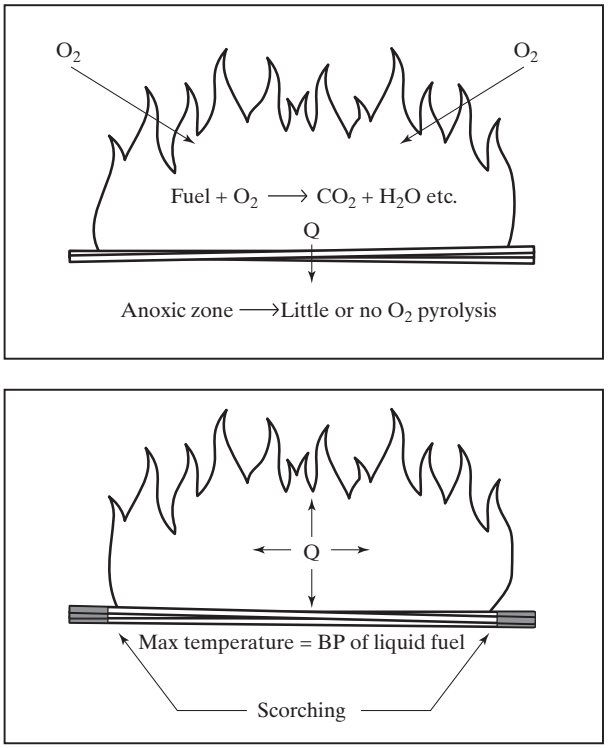


FIGURE 15 Burn patterns reflect heat flow as well as the presence or absence of oxygen.

2.4 Mass Transfer

Figure 4 shows one aspect of mass transfer: the movement of atmospheric O_2 and N_2 to the combustion zone. This diffusion is driven by a concentration gradient, a process aided by rising heated gases. As the heated products rise, surrounding air moves in to fill the void. As these molecules approach the reaction zone, they collide with molecules heated by the combustion, which increases the velocity of those heated molecules. This is a simple view of how heat is conducted in the combustion zone.

Mass transfer and diffusion are also important considerations in evaluating how well the fuel and oxidant mix. In a quiescent (quiet and not mixing) solution of water, a drop of food coloring added will diffuse over time until it is equally distributed throughout the liquid. This is another example of diffusion driven by a concentration gradient. The same will happen in the gas phase. However, in combustion, the process is more complex and dynamic. Consider the simple systems shown in Figure 16. Heat transferred to the fuel imparts the energy needed to vaporize the fuel or its pyrolysis products. These vapors diffuse away from the surface, a zone of high concentration. In the combustion zone, the fuel is consumed, as is oxygen, which diffuses inward toward the combustion zone. Figure 17 illustrates two simple combustions and the gradients created. The combustion zone approximates the concentration ratios shown in Figure 16. The right frame shows a fire burning on a vertical surface such as a wall, and the lower frame shows a "pool fire," which would occur in an arson in which gasoline was used as an accelerant.

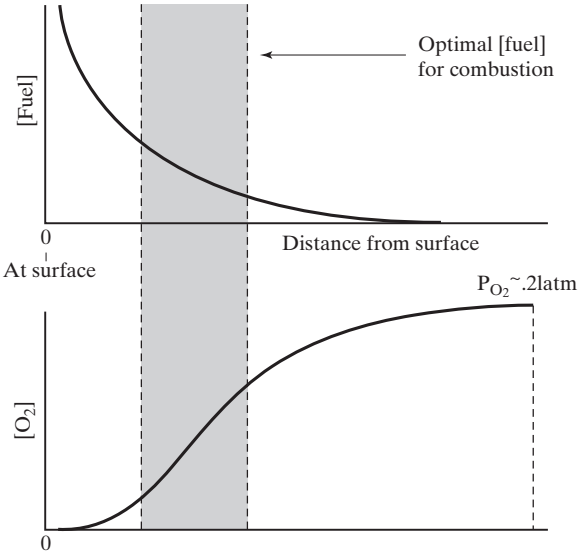


FIGURE 16 Concentration gradients of fuel and oxidant as a function of distance. The [ ] notation refers to concentration.

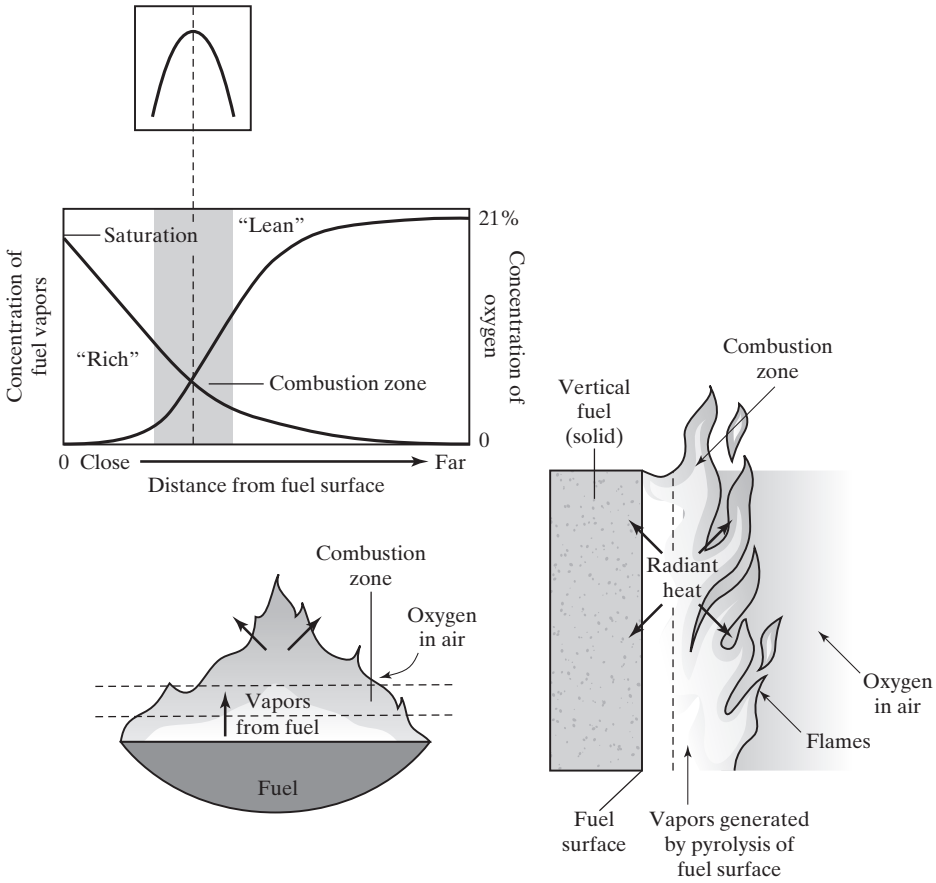


FIGURE 17 Different views of the concentration gradients and the zones at which the concentrations of the fuel and the oxidant support combustion. Figure 8 is superimposed for reference.

Finally, in cases involving a poured liquid **accelerant**, mass transport of the fuel occurs in a lateral direction, controlled by the characteristics of the surface. Gasoline on a nonporous surface like concrete will diffuse easily, whereas gasoline on a porous surface like wood or carpet will tend to be absorbed. As a result, porous and semiporous surfaces should be sampled in depth, since the chances of finding residual accelerants is increased in such cases.

Applying the Science 2 Spontaneous Combustion? Human Candles

One of the more persistent urban myths of forensic science is the belief that the human body can somehow erupt into flame and rapidly burn down to ashes with no discernible ignition source and nearly no peripheral burning. The material discussed in this chapter concerning heat and mass transfer, fuel–air ratios, flammability limits, and the conditions that must be met for successful ignition are sufficient to debunk any ideas of spontaneous human combustion, but the myth persists. To combat misperceptions, a series of experiments was conducted and the results recently reported in the *Journal of Forensic Sciences*. The author noted that in most cases of spontaneous human combustion, a source of ignition can be located, with cigarettes being one common culprit. The mystery centers on how a body can burn with such ferocity (a large Q value) and become incinerated while nearly all the furniture—even furniture the victim is sitting on—is barely damaged. One theory is the "wick effect," in which the fat in a body supplies a wick consisting of clothing to facilitate a long burn of a victim such that most of the body's mass is consumed as fuel, leaving little but small amounts of bone behind. The author of the article cited here conducted experiments and monitored the heat of combustion of biological materials, including fat, tissue, bone, and an amputated leg. The results supported the theory of the wick effect of human combustion, but not spontaneous human combustion.

3 FIRES AND FLAMES

3.1 Flames

A common type of fire or flame is a **laminar flame**, meaning that the flame has definable layers or regions. Within the laminar category, there are two familiar examples: a candle and a Bunsen burner, shown in Figure 18. In a Bunsen burner, gaseous methane is mixed with air and injected into the combustion zone. Hot gaseous products diffuse away while atmospheric oxygen diffuses inward, creating a second region in which the fuel–air mixture is no longer in the same proportions as in the premix zone. In a candle, fuel is vaporized and diffuses upward as oxygen diffuses inward. The regions in the candle flame are defined by temperature. Pyrolyzed waxes form particulates (soot) that grow and rise. Partial oxidation occurs, and any residual soot diffuses upward and outward as smoke. In both cases, the flame is self-sustaining once ignited and burns until the fuel is exhausted.

A visible combustion wave is created if the fuel and oxidant gases are stationary, as shown in Figure 19. A flame front is visible when, for example, a large puddle of gasoline is ignited at one end and the flames propagate across the surface much as waves on a pond propagate from the point where a stone is dropped into the water. Typical flame velocities for a mixture of methane and air are in the range of approximately 15 to 45 cm/sec, depending on factors such as the fuel–air ratio.[4] In a candle or Bunsen burner, the wave front is stationary because the gases are moving into and out of the combustion zone. The combustion wave remains stationary because the unburned gas molecules are moving away at a rate equal to the burn rate.[4] In simple combustion (deflagration), the combustion wave never exceeds the speed of sound.

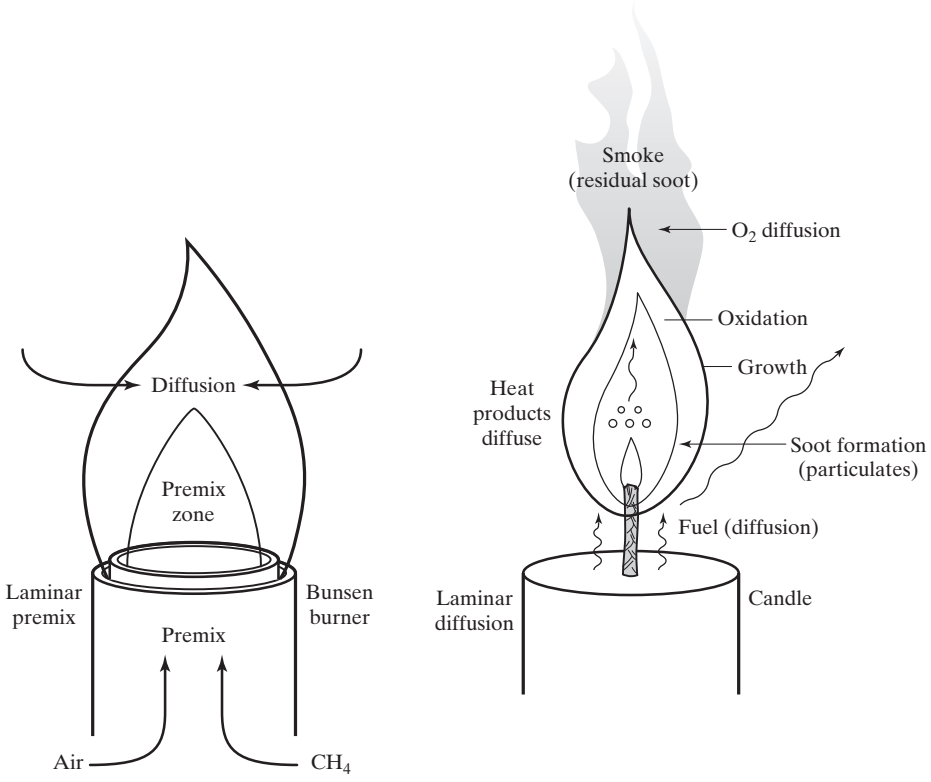


FIGURE 18 A Bunsen burner and a candle are examples of laminar flames.

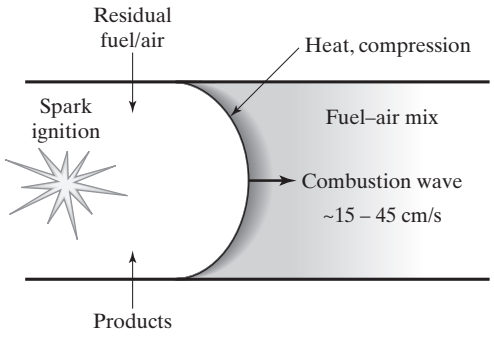


FIGURE 19 A visible combustion wave generated in an enclosed tube containing fuel and oxidant.

As long as the fuel–air mixture remains in the combustible range, the flame will be self-sustaining. The events that bracket the flame "event" are initiation (ignition) and quenching or suppression. The range of combustibility is referred to as the *flammable range* and is defined as the fuel–oxidant ratios that permit steady propagation of the flame. The lower end of the scale is called the **lower flammability limit** (LFL) or the lean limit, whereas the upper range is the **upper flammability limit** (UFL) or rich limit. The terms "lower explosive limit" and "upper explosive limit" (LEL and UEL, respectively) are also used. Some examples are presented in Table 2.

TABLE 2 Example flammability limits (in air), % by volume

Fuel	LFL (lean)	UFL (rich)
Methane CH_4	4.5	15
Propane C_3H_8	2.1	9.5
Butane C_4H_{10}	1.9	8.5
Acetylene C_2H_2	2.5	81
Hydrogen H_2	4	75

Source: Hazardous Substances Database (HSDB, http://toxnet.nlm.nih.gov/).

You will note that in Table 2, the flammability limits are presented in volume %. How could you use this data to assist in a fire investigation? This information is essential to deciding whether a combustible mixture could have existed in the right place and at the right time to support combustion. For example, assume you are investigating a fire that occurred in a small storage shed with dimensions of 4 ft × 4 ft × 6 ft. Inside the destroyed shed, you find what is left of a propane bottle, labeled as containing 1 lb of propane. Is it possible that the F/A ratio could have supported combustion? Having the dimensions allows you to calculate the total volume of the shed:

$$4\text{ ft} \times 4\text{ ft} \times 6\text{ ft} = 96\text{ ft}^3$$

$$1\text{ ft}^3 = 28.32\text{ L}$$

$$96\text{ ft}^3 \times \frac{28.32\text{ L}}{1\text{ ft}^3} = 2718\text{ L}$$

Table 2 indicates that the flammability range of propane is 2.2%–9.6% by volume. We can use this information and the ideal gas law to estimate what these limits correspond to in liters of propane. Initially, we will assume a temperature of 25°C and a pressure of 1.0 atm:

$$0.022 \times 2718\text{ L} = 60\text{ L}$$

$$0.096 \times 2718\text{ L} = 261\text{ L}$$

These values provide an estimate of the volume of fuel that would have to be present. Using the ideal gas law, we can estimate how many moles of propane these volumes correspond to:

$$n = \frac{PV}{RT} = \frac{1.0\text{ atm} \times 60\text{ L}}{0.0821\dfrac{\text{L}\cdot\text{atm}}{\text{mol}\cdot\text{K}}\,298\text{ K}} = 2.4\text{ mol at LFL}$$

$$n = \frac{PV}{RT} = \frac{1.0\text{ atm} \times 261\text{ L}}{0.0821\dfrac{\text{L}\cdot\text{atm}}{\text{mol}\cdot\text{K}}\,298\text{ K}} = 10.7\text{ mol at UFL}$$

The molar mass of propane (C_3H_8) is 44.09 g/mol, and 1.0 lb = 453.59 g, so we can convert the moles to pounds. Of course, you could convert the propane can weight to grams as well; both approaches are valid.

$$2.4\text{ mol} \times \frac{44.01\text{ g}}{\text{mol}} \times \frac{1.0\text{ lb}}{453.59\text{ g}} = 0.2\text{ lb}$$

$$10.7\text{ mol} \times \frac{44.01\text{ g}}{\text{mol}} \times \frac{1.0\text{ lb}}{453.59\text{ g}} = 1.0\text{ lb}$$

The empty bottle was labeled as containing 1.0 lb of propane, indicating that the contents released into the shed could have supported combustion. You will notice we are being a bit cavalier about significant figures; there is a reason for this. This approach is by its nature an estimate and does not consider many factors that would be important in an arson investigation. For example, we made the implicit assumption that the shed represented a closed system, all the propane left the bottle, and none of the contents escaped the shed. We also estimated temperature and pressure. In this type of situation, all we are after is a reasonable and defensible answer to an investigative forensic question: Could the propane have supported combustion? In this case, there was sufficient propane to sustain combustion. However, this would represent a starting point for further investigation, not the end. Alone, this calculation would not prove or disprove arson, but it would provide investigators with important information.

As demonstrated and discussed already, many other variables will alter the fuel/air ratio that exists in a given place at a given time. Because the fuel and oxidant are gases, pressure and temperature are among the most important variables to be considered in a fire investigation. For example, the higher the temperature, the wider is the flammable range. In arson cases, homogeneous mixtures rarely exist, and the relative densities of materials become critical factors, as shown in Figure 20.

The weighted-average formula weight of air is taken to be approximately 29 g/mol. The relative weight of hydrogen (H_2; formula weight FW $\sim$ 2) is therefore 2/29, or about 0.07. If hydrogen is released into the air, it rapidly dissipates upward and away from the release point. Conversely, gasoline vapors tend to sink. If gasoline is crudely represented by *n*-octane (C_8H_{18}, FW $\sim$ 114), the weight ratio relative to air is 114/29, or $\sim$3.9. Therefore, in a quiescent or nearly quiescent environment, fuel vapors disperse according to their weight and density. This phenomenon is illustrated in Figure 20.

The presence of vapors within the explosive limits is a necessary condition for combustion to occur, but it is not a sufficient one. The initial E_a barrier must be overcome, and the energy to do so is supplied by an ignition source that must have sufficient energy available and that must remain in contact with the flammable mixture long enough to ignite it. In arson investigations, the ignition source is called an **incendiary device**, such as a match, a cigarette, or a more sophisticated apparatus. As demonstrated in Figure 20, this device must also be in the right place at the right time. In that example, gasoline has been poured down the basement stairs and enough time has passed that a stratification has developed in this closed, quiescent system. The gasoline vapors, which are heavier than air, move lower in the room and create a rich layer near the floor. Someone wanting to ignite the mixture would have to place the incendiary device in the flammable region. A match tossed to the floor would not work because the mixture is too rich, and a spark at the top of the stairs would fail because there the mixture is too lean. In addition, the incendiary device would have to produce sufficient energy to initiate the

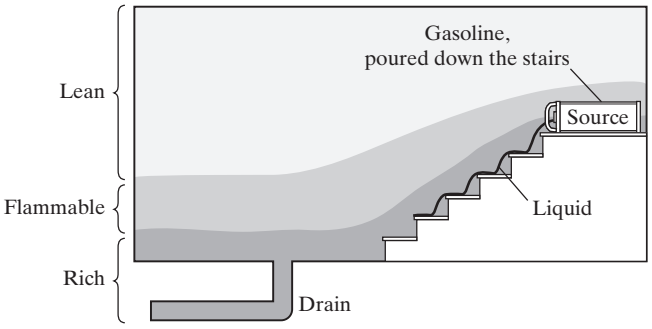


FIGURE 20 In a quiescent or nearly quiescent environment, the density of the fuel relative to the atmosphere will dictate the places where fuel vapors accumulate and the zones where combustion is supported. In this example, gasoline which is heavier than air, will settle into a pattern such as shown here, with the zone next to the floor being relatively rich and the zone at the level of the can being relatively lean.

reaction and would have to be in contact with the reaction mixture long enough to ensure the combustion had become self-sustaining. The energy needed to ignite a mixture is usually thermal. Kinetic energy is transferred to the reactants via collisions among the molecules of the mixture. The collisions may be instantaneous, as in the case of someone using a striker and spark to ignite a Bunsen burner, but even the biggest spark will not ignite a mixture that will not support combustion. In other cases, ignition takes much more time, as when a smoldering cigarette is placed between the cushions of a couch. With explosives, pressure can provide sufficient energy for combustion.

3.2 Fire Dynamics

A fire is a time-dependent event that has definable phases and dynamics. After ignition, a fire will grow until it reaches a steady state that depends on the amounts of fuel and oxidant available in the given environment. In the steady state, the fire triangle requirements of fuel–oxidant–heat are met, and the fire burns steadily. As one or more of the key ingredients dissipates, the fire begins to decay and is eventually quenched, although destruction and residual heat linger after the flame itself goes out. Of course, the behavior of any given fire will be more complex even while it follows this general model. How a fire behaves during its lifetime has important consequences for the fire investigator and the forensic chemist. The dynamics of a fire will depend on all the factors described so far, which combine to make each fire event unique, although some generalizations can be made.

In an arson fire, there are one or more **points of origin** for the fire, and an understanding of fire dynamics is essential to locating these points. As noted earlier, conventional fires are diffusion types in which heated gases move upward and outward from the combustion zone. Such flames are also referred to as buoyant flames, since the heated gases are less dense, and thus more buoyant, than the unheated gases. Consequently, flames burn in plumes that can leave distinctive physical and chemical evidence. A simple buoyant flame is shown in the top frame of Figure 21. Since the hot gases are moving upward and outward, a V-shaped burn pattern on adjacent surfaces is often observed. Given that a fire usually burns the longest at the point of origin, the V pattern often marks this spot. If the fire burns in an enclosed room, a ceiling jet will form in which the hot gases and smoke are forced outward.

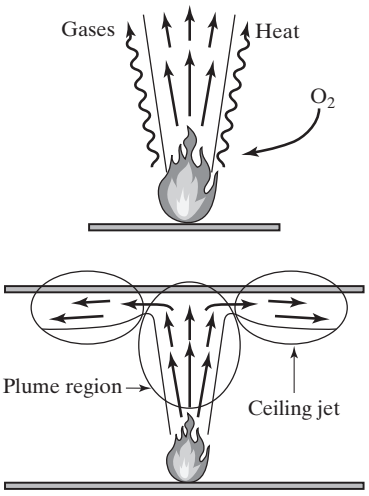


FIGURE 21 Examples of fire dynamics that produce distinctive patterns.

If the fire is sufficiently intense, flashover may occur. In a fire in an enclosed room, for example, flashover is the point at which all the flammable vapors in the room ignite. A fire started in a corner can generate enough heat and vapors from furniture, paint, and flooring that the entire room erupts in flame, and what was a fire burning in one or more isolated locations becomes generalized. Flames extend out through openings, and windows can be broken out by the sudden creation of hot gaseous combustion products. If the room is tightly enclosed, flames may subside to a smoldering state that can erupt again if a door or window is suddenly opened, allowing oxygen in. A similar situation can result if a fire only smolders in an enclosed space, depleting oxygen over time without the telltale flames. A door opened in this situation can cause immediate flashover in a room rich in fuel vapors. Flashover is a significant danger to firefighters and first responders to fire scenes.

4 ARSON AND FIRE INVESTIGATION

During the years 2003–2007, more than 53,000 intentionally set fires were reported in the United States, causing 387 deaths, and more than $900 million in property damage. Half of these were set in homes, and 8% of residential fires were intentionally set.[5]

Intentionally set fires, or arson, usually involve an accelerant of some type, as well as an incendiary device used to ignite it, and these two components create the physical evidence forensic chemists work with. Arson fires are also referred to as incendiary fires. For fires set in homes, a bedroom was the most common point of origin; fires set in public buildings were usually started in bathrooms. From a law enforcement point of view, arson is a difficult crime to clear; in 2008, only 18% of arson cases were cleared by arrest or other means. Nearly half of those arrested were under 18, and 3% were under 10 years of age.[5,6]

One of the challenges of fire investigation is the classification of a fire as natural, accidental, or incendiary (arson). Fire investigators utilize evidence at the scene, as well as forensic analysis, to make such determinations. One of the most important pieces of information required in making a determination of arson is the location of the point or points of origin of the fire. Multiple points of origin are strongly indicative of an intentionally set fire, whereas a point of origin at an electrical outlet suggests an accidental fire. As discussed in Section 3, the behavior of a fire creates predictable damage that is useful in locating a point of origin. Although such fire and fire scene investigations are critical in determinations of arson, we will focus on the chemical analysis aspects of fire and arson investigation. The former is fire investigation; the latter is forensic chemistry.

4.1 Accelerants and Incendiary Devices

An *accelerant* is anything that is intentionally placed at a scene to start and sustain a fire. Accelerants can be solids (wood, paper, road flares), liquids (gasoline), or gases (natural gas, propane). Although a gaseous accelerant will not leave chemical residues, there will always be physical evidence related to the container used to hold or deliver it. For example, if bottled propane is used to start a fire, the bottle will remain even though the propane will not. As with any type of combustion, fuel, oxidant, and a source of ignition are required, and the forensic chemist will be concerned with two of these three: the fuel, such as an accelerant, and the incendiary device. The latter can be as simple as a match, a candle, or a smoldering cigarette. More complex devices are also seen; however, we will focus on the analysis of accelerants, since they represent the bulk of the casework seen by forensic chemists. The most common accelerants are liquid petroleum distillate products. Also frequently seen as physical evidence are gasoline, kerosene, and diesel fuel.

As the name implies, **petroleum distillates** are extracted from crude oil by distillation. The process shown in Figure 22 is one of many available, but for our purposes, it is the most useful because of the parallels between distillation and gas chromatography.[†] As shown in the figure, crude oil is introduced into the **cracking** tower and heated to about 350°C, volatilizing much of its content. The vapors rise, cool, and condense, whereupon they are collected on plates and removed. The heavier fractions, such as diesel and kerosene, are collected lower in the tower than are gasolines and fuel gases. The separation is not complete, and each fraction collected consists of a mixture of hydrocarbon components with similar boiling points. This separation is re-created on a small scale when samples are analyzed by gas chromatography, which separates components on the basis of their volatility and preferential partitioning on a solid phase.

[†]Recall that one measure of the efficiency of a chromatographic column is the number of theoretical plates. This analogy is drawn from distillation: the more collection plates in the column, the more effective is the separation. The maximum number of plates (N) is achieved when the distance between them (i.e., the smallest possible height of a plate, HETP) is minimized.

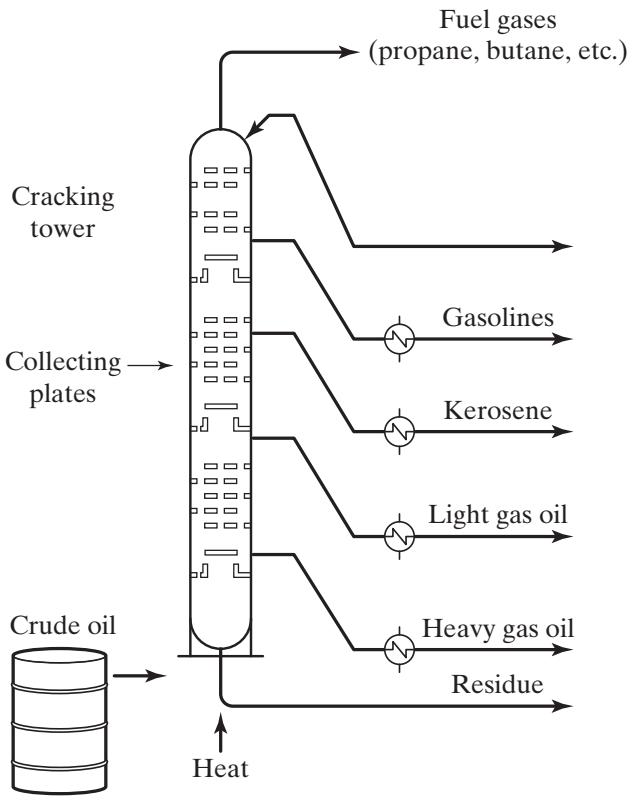


FIGURE 22 Thermal distillation of crude oil. The oil is heated, volatilizing most of the components, which then condense as they cool. Fractions are collected that consist of a mix of hydrocarbons with similar boiling points.

4.2 Forensic Analysis of Fire Debris

The most frequently encountered accelerants are readily available petroleum distillates such as gasoline, jet fuel, and kerosene. ASTM has published a classification for ignitable liquids that is widely used as part of the forensic analysis of fire debris; a summary is presented in Table 3. Each class is further subdivided into three groups based on carbon chain length, which correlates with volatility. As we will see shortly, volatility plays an important role in laboratory analysis.

Fire debris evidence is collected in coated paint cans of various sizes with a predrilled hole sealed with a septum. The debris is placed loosely in the can, leaving plenty of headspace above. As with all paint cans, the can is closed by pounding the lid into the metal lip. This creates an airtight seal that traps vapors, including any residual volatile accelerants. Table 4 and Figure 23 summarize existing ASTM-recommended protocols for fire debris preparation and analysis. They also summarize the evolution of sample preparation methods over the past decades. The first protocols used involved steam distillation. Fire debris (slightly wet) is transferred to a distillation apparatus, and heat is applied. As the vapors rise in the distillation column they are cooled and condense, dropping into a collection thimble. Any petroleum products collect as a layer atop water. This approach is effective in separating and concentrating accelerants but is time consuming and relatively aggressive, meaning that more volatile components can be lost.

The next development in sample preparation was solvent extraction of fire debris with pentane and carbon disulfide (CS_2). Solvent is added to the evidence, collected, and concentrated into a small volume (about 1–5 mL). Both pentane and (CS_2) are nonpolar and well suited to solvation of petroleum hydrocarbons, but both also have limitations. Pentane is extremely volatile, so care must be taken to ensure that the solvent extract does not go to dryness. If it does, the lighter fractions will be lost along with the pentane. Carbon disulfide is not as volatile, but it is relatively toxic and has a foul odor. Additionally, solvent extraction, like steam distillation, is relatively aggressive and can result in a loss of the more volatile fractions of residual accelerants.

Currently, the favored methods of sample preparation are based on headspace. The headspace may be heated or unheated, passive or active. All headspace methods involve concentrating volatiles into a solid phase by adsorption. The effect is the same as a solvent extraction: target analytes are extracted from the fire debris matrix and concentrated on the adsorbent. Generically, this protocol is called *trapping*, and in the case of fire debris, the trap is made of charcoal. In simple passive methods, a polymer strip coated with charcoal is dangled above the fire debris, and the evidence can be resealed. Gentle heating drives the volatiles into the vapor phase, from which they move via diffusion to the charcoal, where they are adsorbed. At the end of the heating time, the charcoal strip is removed and extracted with a

TABLE 3 Classification of Ignitable Liquids (after ASTM)

Class	Light (C_4–C_9)	Medium (C_8–C_{13})	Heavy (C_8+)
Gasoline	Primarily $C_4 - C_{12}$		
Petroleum distillates	Pet ether Lighter fluids (butane)	Charcoal starter fluids	Kerosene, diesel oil
Isoparaffinics	Aviation gas (av gas)	Paint thinners, copier toners	Specialty solvents
Aromatics	Toluenes and xylenes, degreasers	Degreasers, specialty cleaning agents, fuel additives	Industrial cleaning solvents
Naphthenic paraffins	Cyclohexane-based solvents	Lamp oils	Lamp oils, industrial solvents
n-Alkanes	Solvents to heptane	Candle oils	Copier toners
De-aromatized distillates	Camp stove fuels	Some paint thinners	Odorless kerosenes
Oxygenated solvents	Ketones, lacquers	Metal cleaners	
Miscellaneous	Blends	Turpentines	Specialty products

Source: ASTM Standard E 1618, "Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Chromatography–Mass Spectrometry."

solvent such as CS_2. Active or dynamic headspace (DHS), also called *purge-and-trap,* is a methodology widely used in environmental analysis. In DHS, an inert gas constantly flows through the heated container, carrying volatiles downstream to a trap. Because the equilibrium is constantly disturbed by removal of product, the

TABLE 4 ASTM Standards Relevant to Fire Debris Analysis

Number (E-)	Title and Subject
1385	Standard Practice for Separation and Concentration of Ignitable Liquid Residues from Fire Debris Samples by Steam Distillation
1386	Practice for the Separation and Concentration of Ignitable Liquid Residues from Fire Debris Samples by Solvent Extraction
1387	Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography
1388	Practices for Sampling of Headspace Vapors from Fire Debris Samples
1389	Cleanup of Fire Debris Samples Extracts by Acid Stripping[a]
1412	Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Activated Charcoal
1413	Practice for Separation and Concentration for Ignitable Liquid Residues for Fire Debris Samples by Dynamic Headspace Concentration
1618	Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography Mass Spectrometry
2154	Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Solid Phase Microextraction

[a]Used to remove nitrogenous and oxygenated species from a prepared extract.

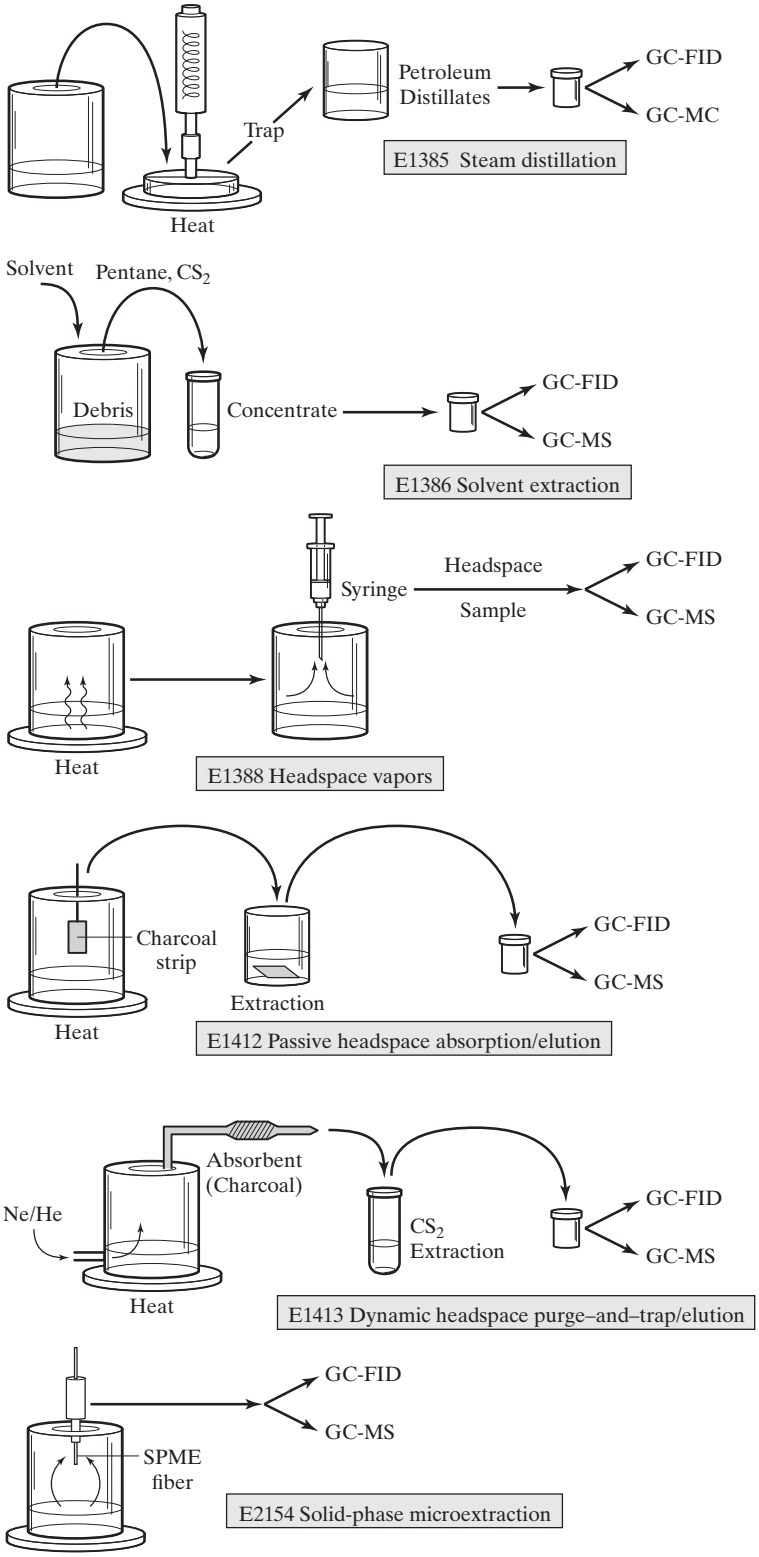


FIGURE 23 Depiction of the sample preparations used in analyzing fire debris.

Suzanne Bell

FIGURE 24 The types of containers used to collect fire debris samples.

volatiles are efficiently extracted and trapped. Solvents are used to desorb the sample traps. DHS methods are particularly effective with low concentrations of residual accelerants.

$$\text{analytes (debris)} \leftrightarrows \text{analytes (vapor)}$$

Another passive method of vapor preconcentration is solid phase microextraction (SPME). The procedure is much like that used for the charcoal strip, except that other adsorbents are used to coat the silica needle. The needle can be directly introduced into the gas chromatograph for thermal desorption, or a solvent extraction can be employed.[7] An added advantage of SPME is versatility: The fiber can be immersed in an aqueous matrix if the fire debris is waterlogged. Currently, ASTM lists as a screening technique standard E 2154-01, but high sensitivity and a solventless approach make the SPME method increasingly attractive. A chromatogram of the same sample prepared by different methods is presented in Figure 25. Note the peak to the far left on the lower two frames, attributable to the solvent used in the extraction. Detection was with a flame ionization detector (FID), and the sample was gasoline on charred carpet.

No matter what sample preparation method is used, discrimination occurs, and there are inherent limitations. Analyses of fire debris samples are designed to detect a wide range of compounds and, as a result, are not optimized for any one compound. Headspace methods will be biased toward the more volatile materials, even under conditions of gentle heating. Excessively aggressive heating can drive off the more volatile fractions, so heating temperature is limited and typically falls into the range of approximately 70°C. The efficiency of a solvent extraction, like any other partitioning, will depend on the relative polarities of the solutes and solvent; in a complex hydrocarbon mixture, discrimination is inevitable, and some compounds will be desorbed more efficiently than others. Similarly, not all components of an accelerant will be adsorbed with equal efficiency onto charcoal or other solid phases.[8] These caveats do not mean that the methods are fatally flawed, but they do mean that the limitations of each technique must be understood and that validated methods are essential. In the case of fire debris, the conditions that are optimal for the collection of gasoline components are likely not optimal for heavy distillates, and vice versa; the discrepancy is even more critical when nonpetroleum products, such as methyl ethyl ketones or industrial cleaning solvents, are involved. However, keep in mind that the transfer (from debris to instrument) does not have to be 100% efficient for every compound that might be in the matrix. Rather, the transfer of each target compound must be in an acceptable range and also must be reproducible. The analysis of fire debris is qualitative, not quantitative, and is based on pattern matching, not the presence or absence of any one component. As we will see, pattern matching is widely used in materials analysis as well.

Regardless of the type of sample preparation, the instrumental method employed for fire debris analysis is gas chromatography, coupled to either a flame ionization detector or a mass spectrometer. Unlike other chromatographic methods used in forensic chemistry, the primary goal (in most cases) is to recognize patterns rather than identify specific compounds. The pattern of gasoline (Figure 25) differs significantly from the pattern of diesel fuel (Figure 26, bottom frame), which is composed of heavier and less volatile hydrocarbons. With the use of mass spectrometry, the patterns can be further analyzed to identify significant groups of compounds within a sample, such as aromatics, alkanes, and branched alkanes.[9–11] In addition to recognizing patterns and groups of compounds, the analyst must consider environmental factors. Accelerants undergo **weathering**, and their composition changes over time. The changes are predictable in that the more volatile a compound is, the more quickly it will be lost. For gasolines, then, weathering is more of an issue than it is for diesel fuel. Understanding weathering and the analysis of weathered samples is essential to interpreting analytical results.

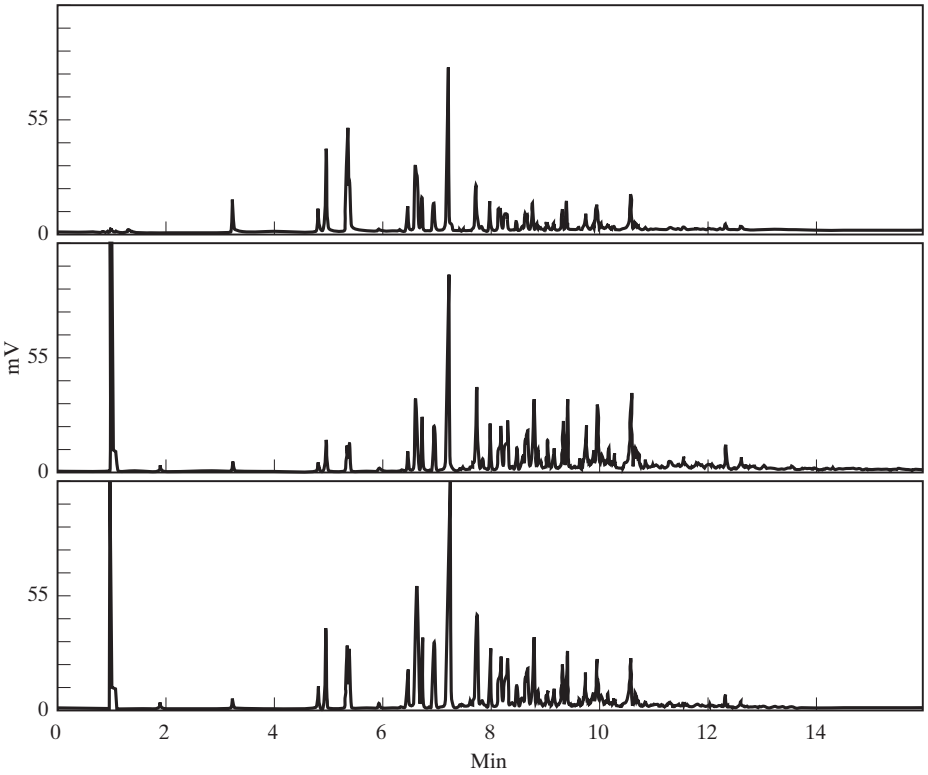


FIGURE 25 Comparison of recovery by three heated headspace enrichment methods. Sample: Fire debris sample, gasoline on a charred matrix of carpet and carpet padding (Instrument 1). Top: SPME, Carboxen/PDMS fiber (70°C, 3 min extraction). Middle: Static headspace enrichment (90°C, 16 h extraction). Bottom: Dynamic headspace enrichment (80°C, 15 min extraction). Reprinted with permission from Ren, Q., and W. Bertsch, "A Comprehensive Sample Preparation Scheme for Accelerants in Suspect Arson Cases." *Journal of Forensic Sciences* 44 (1999): 504–15. Copyright 1999, ASTM International.

Also essential in any fire debris analysis is the collection and analysis of background samples (**matrix controls**).[12–13] For example, if debris suspected of containing an accelerant is collected on a carpet, samples of undamaged carpet should be collected as well, if at all possible. Carpeting is manufactured from synthetic fibers, the raw materials of which are polymers, many derived from petroleum products. As seen in Figure 26, many common materials produce patterns that could be confused with accelerants. The data shown in this figure were collected by means of passive headspace–charcoal strips and heating at 80°C for 16 hours. The instrument used was a GC-MS, but the patterns of the total ion chromatogram (TIC) are comparable to patterns that would be obtained from a GC-FID. The figure illustrates the vital nature of controls in fire debris analysis.

EXAMPLE PROBLEM 4

An officer submitting fire debris requests that you analyze it for the presence of gasoline with ethanol. Would you use GC-FID or GC-MS?

Answer:

GC-MS. The FID detector would respond to ethanol (it is widely used for blood alcohol analysis), but the response is not specific. Because a complex pattern of peaks would be expected from such a sample, it would be difficult to definitively identify one as ethanol, although it would likely be one of the earliest eluting peaks. A mass spectrometer could provide definitive identification of ethanol via the compound's mass spectrum coupled with retention time data and comparison to reference ethanol standards.

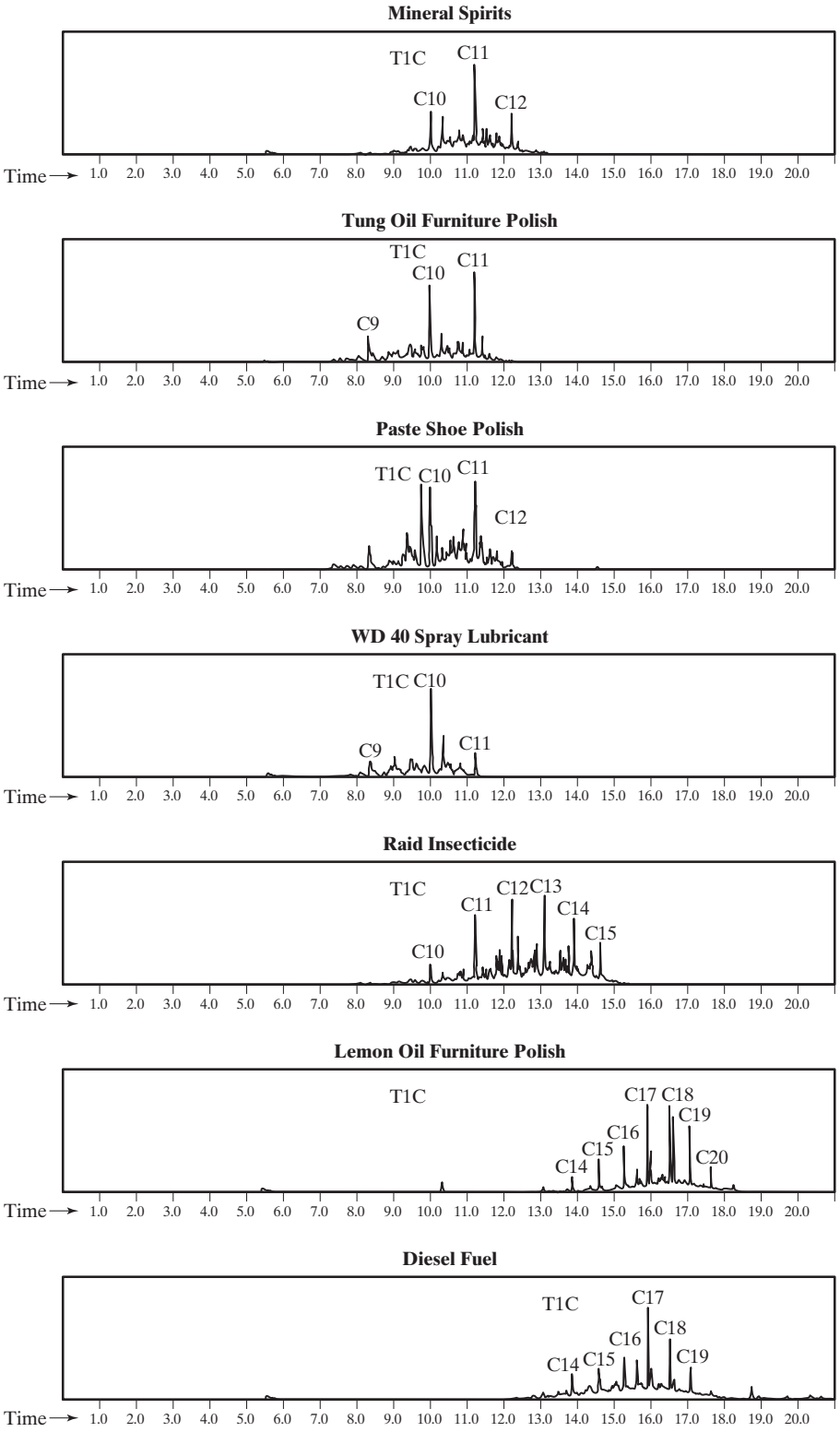


FIGURE 26 An illustration of why control samples are critical in fire debris analysis. Many of the patterns shown here are similar to accelerant patterns. Total ion chromatograms of common household products compared with mineral spirits and diesel fuel. Reprinted with permission from Lentini, J. J., et al., "The Petroleum-Laced Background," *Journal of Forensic Sciences* 45 (2000): 968–89. Copyright 2000, ASTM International.

Whereas identifying residual accelerants is a well-developed forensic protocol, classifying an accelerant beyond gasoline, diesel fuel, and the like is a difficult or impossible task. Part of the difficulty is attributable to weathering and environmental factors, part to the mass-production nature of distillates such as gasoline. Attempts have been made to allow for greater discrimination by targeting compounds other than hydrocarbons, such as oxygenates. Ethanol is added to many gasoline formulations on a seasonal basis, so identifying ethanol in a solvent mixture could provide valuable investigatory information. Oxygenates are added to gasoline for similar reasons, to increase the oxygen balance and improve the efficiency of combustion. Ethanol is a common oxygenate in gasoline and is suitably volatile for headspace detection methods.

Summary

From flame to bomb, the underlying chemistry of combustion is the same: a rapid oxidative decomposition that produces large volumes of hot expanding gases. A slower reaction front produces deflagration, whereas a rapid and confined mixture can detonate. In this chapter, we discussed combustion chemistry generically and then focused on how these principles apply to intentionally set fires in arson. We also discussed how forensic analytical chemistry approaches arson investigations, taking advantage of the volatile nature of many accelerants.

Key Terms and Concepts

Accelerant
Adiabatic combustion
Buoyant flame
Cracking
Deflagration
Detonation
Fuel/air ratio (F/A)
Incendiary device
Laminar flame
Lean mixture
Lower flammability limit (LFL)
Mass transfer
Matrix controls
Oxygen balance
Petroleum distillate
Points of origin
Propellants
Pyrolysis (combustion reactions)
Rich mixture
Saltpeter
Stoichiometric equivalence (STE)
Upper flammability limit (UFL)
Weathering

Problems

FROM THE CHAPTER

1. Suppose that 1.00 gram of nitroglycerin is used in a firearm as a propellant. Suppose also that combustion is 100% efficient and that 65% of the chemical energy is transferred to a bullet that weighs 115 grains. How fast will the bullet be moving? Will it exceed the speed of sound?
2. How is a shotgun like a pipe bomb in terms of energy conversion? How is it different?
3. ANFO is a powerful explosive mixture containing ammonium nitrate and fuel oil. It was used in the 1995 bombing of the Alfred P. Murrah Federal Building in Oklahoma City. Optimal power, related to Q, is obtained with a mixture of about 94% NH_4NO_3 and 6% fuel oil. What is the approximate oxygen balance of fuel oil?
4. Three compounds that have been used as fuel in cars and racing cars are ethanol, nitromethane (CH_3NO_2), and gasoline. Using octane as representative of gasoline, calculate the following quantities for each compound: ΔH: of combustion per mole of the fuel, moles of gas produced per mole of fuel at 25°C and 1 atm pressure, Q produced per gram of fuel, liters of gas produced per gram of fuel at 25°C and 1 atm, and the QV value. Assume that all components are in the gas phase. Which fuel would be the best choice based on these considerations? You may need to look up some heat of formation values.

5. Through a balanced equation of combustion, calculate the oxygen balance of the following compounds. Assume that the combustion is complete and produces fully oxygenated species.
   a) Ammonium nitrate
   b) HMX (Octogen)
   c) Picric acid
6. The propellant used in the solid rocket boosters during the space shuttle program was based on aluminum metal and ammonium perchlorate. If these were the only two ingredients, what composition would produce a net zero oxygen balance?
7. Assume that the two main ingredients in a gunpowder formulation are nitrocellulose (NC) and nitroglycerin (NG). If these were the only active ingredients, what composition would produce a net zero oxygen balance? Assume that the oxygen balance of NC is −24%.
8. According to the UFL and LFL for flammables listed in Table 2, which would be "better" choices for setting an arson fire based strictly on these criteria? Why?
9. You are called to a fire scene in which the point of origin appears to be in a restroom in a high school. The dimensions of the room are 25 ft × 10 ft × 10 ft. In one corner, you find the burned remains of a small tank labeled acetylene that appears to have been stolen from the high school welding shop. You call the supplier and learn that a full tank contains 25 lb of acetylene. The shop teacher says the tank was new and barely used. Estimate the LFL and UFL in pounds and determine if a combustible mixture would be supported. Assume 1.0 atm pressure and a typical indoor temperature of 25°C.
10. Is SPME a destructive analysis? Justify your answer.
11. Two common solvents used in clandestine drug laboratories are diethyl ether and acetone. Being less than vigilant in laboratory and safety practices, clandestine chemists often work with leaky equipment. If a person was brought to an emergency room under suspicious circumstances, where would you predict the burn patterns on the person's body to be most pronounced if he or she was injured by a fire or explosion at a clandestine laboratory?

FOOD FOR THOUGHT

1. Hydrogen is billed as "the fuel of the future" for automobiles. A popular misconception, mostly owing to films of the *Hindenburg* disaster, is that cars that store hydrogen as fuel will be more likely to explode in an accident than current cars that use gasoline. Why is this a misconception?

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Explosives

1 Explosions, Detonations, and Explosives
2 Detonation
3 Explosive Power
4 Forensic Analysis of Explosives

OVERVIEW AND ORIENTATION

In the previous chapter, we discussed combustion in the sense that most of us are familiar with it—as a reaction between a fuel and oxidant that generates a flame and heat. From a forensic perspective, we are interested specifically in purposely set fires (incendiary fires) and the collection, analysis, and interpretation of associated evidence. In this chapter, we move to the other extreme of the combustion continuum to discuss explosives. Propellants and firearms involve characteristics of both.

Sadly, bombings and explosives have become a high priority area for law enforcement and forensic chemistry. Bombings, even terrorist bombings were not unknown before September 11, 2001, but the nature, scope, and threat of bombing attacks has changed (Figures 1–6). Some famous pre-9/11 cases involving explosives include the Centennial Olympic Park bombing in Atlanta (July 27, 1996; 2 dead, more than 100 injured), the first World Trade Center bombing (February 26, 1993; 7 killed, more than 1000 injured), and the Oklahoma City bombing (April 19, 1995; 168 killed and nearly 700 injured). After 9/11, the list of bombings includes international incidents such as the Madrid train bombings on March 11, 2004, in which 10 bombs exploded on four different trains, killing 191 and injuring more than 2000; and the London Underground bombings on July 7, 2005, in which 52 were killed and nearly 800 injured. The explosives used in these attacks varied, as did the device construction, but all did enormous damage and caused immeasurable suffering. It is no surprise that the forensic analysis and investigation of bombings and explosives has become one of the discipline's most pressing challenges.

Federal Emergency Management Agency

FIGURE 1 Aftermath of the Oklahoma City Bombing.

Kathy Willens/AP Photo

FIGURE 2 The Olympic Bombing, 1996

Richard Drew/AP Photo

FIGURE 3 World Trade Center, 1993

Dylan Martinez/REUTERS

FIGURE 4 London, 2005.

CHRISTOPHE SIMON/AFP/Getty Images/Newscom

FIGURE 5 Madrid, 2004

Itsuo Inouye/AP Photo

FIGURE 6 Bali, 2002. A bombing at a nightclub killed 202 people.

1 EXPLOSIONS, DETONATIONS, AND EXPLOSIVES

The term **explosion** is used informally to describe different events, some of which involve burning and others, detonations. There are many types of explosions, not just chemical ones. For example, steam explosions can cause significant damage, as was the case during the Chernobyl nuclear plant accident in 1986. Thus, we need to define explosion more generally as an event that generates a pressure wave as a result of a sudden increase in volume. Explosives as we are used to describing them, such as TNT, generate the increase in volume by production of gases through a chemical reaction, but this is not the only way in which a pressure wave can be produced. For example, the explosion in a nuclear weapon is created by a nuclear reaction, not a chemical one. In this chapter we focus on chemical explosions, but there are other means to generate a sudden increase in volume.

One way to categorize an explosion is based on the velocity of the pressure waves produced by the sudden expansion of volume. If the wave travels at a speed that is less than the speed of sound (331 m/s, subsonic), the explosion is categorized as a **deflagration**. If the wave travels faster than the speed of sound (supersonic), the explosion is categorized a **detonation**. We can further generalize these terms by defining a burn as an event in which the wave (flame front) velocity is typically on the order of mm/s, deflagration as an event in which the wave velocity is on the order of m/s, and a detonation as an event with a velocity on the order of km/s. We will describe detonation in detail shortly.

Recall that fires affect their environment and cause damage principally as a result of the heat generated by the combustion reaction. Hot expanding gases are also created but are not purposely harnessed or exploited to do work. Explosives (and propellants) are designed specifically to exploit the gases and the heat produced to do. Explosives can cause damage through two general mechanisms—the compressive force of the pressure wave or through generation of fragments of the container imparted with high levels of kinetic energy, defined by $\frac{1}{2}mv^2$. The pressure wave effects are sometimes referred to as the **pushing power** of an explosive. The destructive fragmentation capability of an explosive is referred to as **brisance**. Explosives and explosive devices can be purposely engineered to favor one effect over the other, and the effects depend on many factors in addition to the chemical composition of an explosive or the formulation of an explosive mixture (Figure 7).

We can view an explosive reaction in the same terms. Fuel and an oxidant are still present, but now atmospheric oxygen cannot supply sufficient oxygen to the reaction at a fast enough rate to sustain it. Thus, chemical explosives or their formulations must contain oxygen within the molecular structures. The oxygen balance of explosive compounds is rarely zero and is often negative, meaning that even with oxygen in the formulation (as with TNT for example), there is still insufficient oxygen to attain a net zero oxygen balance for a single explosive compound. The oxygen balance values are useful for designing explosive formulations that come close to having a net zero oxygen balance. You can perform oxygen balance calculations for several types of compounds. Now, we can simplify this calculation. If a compound is written in the form $C_aH_bN_cO_d$, we can determine the oxygen balance as:[1]

$$\text{Oxygen balance} = d - 2a - 1/2b \qquad \textbf{(1)}$$

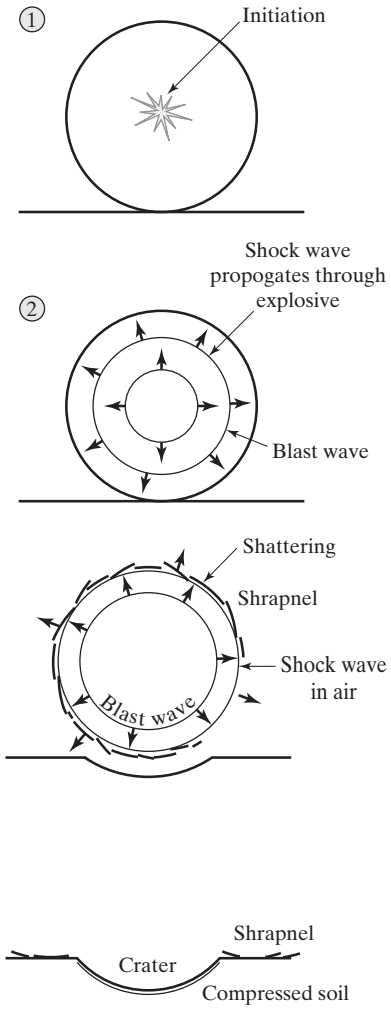


FIGURE 7 Effects of the detonation of a bomb. Note the shrapnel and compression of the soil beneath the bomb; these constitute the crater created by the expanding hot gases.

TABLE 1 Oxygen Balance of Common Explosives

Ammonium nitrate NH_4NO_3	+20.0	Nitrocellulose (guncotton, insoluble)	−28.6
Potassium nitrate KNO_3	+39.6	Picric acid	−45.4
Sodium chlorate $NaClO_3$	+45	TNT	−74.0
Nitroglycerin NG	+3.5	Carbon	−266.7
Ammonium perchlorate NH_4ClO_4	+34.0	Sulfur	−100
Barium nitrate $BaNO_3$	+30.6	RDX (Hexogen)	−21.6
Potassium chlorate $KClO_3$	+39.2	HMX (Octagen)	−21.6
Sodium nitrate $NaNO_3$	+47.0	TATP (Triacetone triperoxide)	−151.2

Source: Meyer, R., J. Kohler, and A. Homburg. *Explosives*. 6th ed. Weinheim, Germany: Wiley-VCH, 2007.

This shortcut makes a few assumptions, but reasonable ones for the present application. When the calculation is applied, the number that results represents the number of oxygen atoms in excess or deficit per molecule of explosive. The oxygen balance of some common components of explosive formulations is given in Table 1.

EXAMPLE PROBLEM 1

Calculate the oxygen balance for nitroglycerin (NG) (see Table 2 for structure using equation 1).

Answer: NG has a formula of

$$\underset{a}{C_3}\underset{b}{H_5}N_3\underset{c}{O_9}\overset{d}{\overset{\circ}{\overset{\circ}{\overset{\circ}{9}}}} - 2(3) - \frac{1}{2}(5) = \frac{1}{2}$$

or $\frac{1}{2}$ of an oxygen atom extra per molecule of NG. As a weight percent:

$$\frac{\frac{1}{2}(16)}{230} \times 100 = 3.5\%$$

which agrees with Table 1.

Even with an understanding of oxygen balance and creation of explosive formulations with a near-zero net oxygen balance, the products of an explosion/detonation are difficult to predict. This does not mean we cannot do so, but it is important to understand the strengths as well as the limitations of any given approach, approximation, and calculation that we use in the complex world of detonation and explosives.

As we have seen with other materials such as drugs, there are numerous methods used to classify explosive materials. For example, the United States Department of Transportation classifies explosives into three categories for determination of safe shipping methods:

- Class A: Detonation hazard/maximum hazard. Examples include nitroglycerin and lead azide.
- Class B: Flammable hazard. Examples include many propellants and pyrotechnic powders.
- Class C: Contain A and B explosives as part of a formulation, but in small quantities.

Another classification method, one frequently used in forensic settings, is presented in Figure 8. Here, the first delineation is made between explosives and

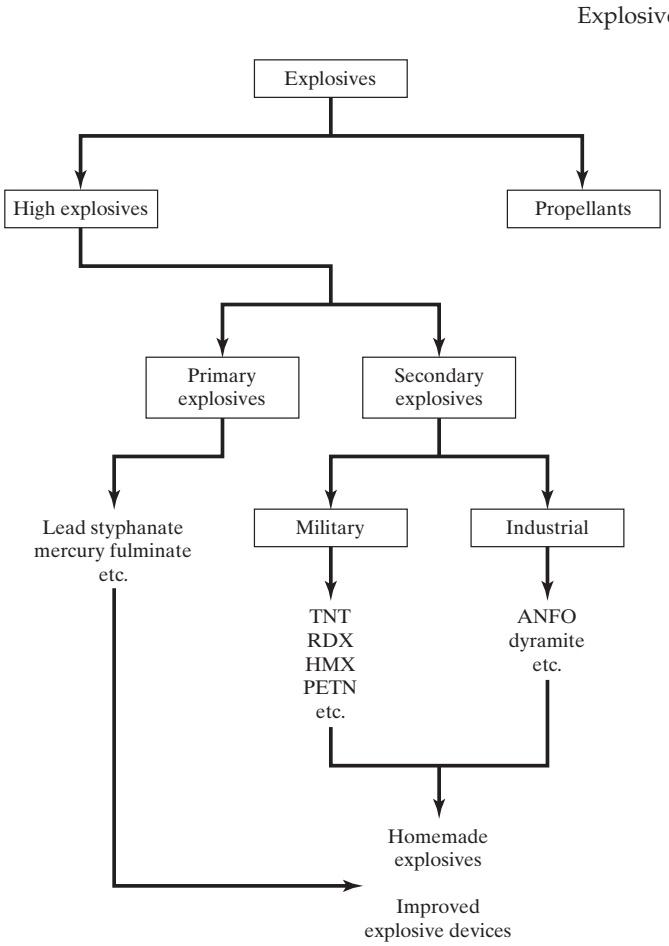


FIGURE 8 One method of classifying explosives.

propellants. Under explosives, there are **primary** and **secondary explosives**. Primary explosives are much more sensitive than secondary explosives and are easily detonated by sparks, mechanical force, and flames. Primary explosives are often used to detonate secondary high explosives. Secondary high explosives are relatively insensitive, and detonation within them is typically initiated using a primary explosive. Occasionally,

EXHIBIT A

TWA Flight 800

Federal Emergency Management Agency

This accident marked a historic milestone in forensic science and its interface with forensic engineering and homeland security. TWA Flight 800 departed from John F. Kennedy International Airport in New York at 8:00 P.M. July 17, 1996, bound for Paris. At 8:31 P.M.,

(continued)

and wreckage, littering a 12-square mile area off the coast of Long Island. Two hundred three people died. The accident lit up the sky before hundreds of witnesses on the ground, on the water, and in the air.

Eyewitness accounts of fiery streaks heading upward in the sky before the explosion led credence to the theory that a shoulder-launched missile had brought down the wreckage and supported the theory of a missile or a bomb. Despite the preponderance of early evidence pointing toward a criminal act, investigators from the National Transportation Safety Board (NTSB) converged on the site. The NTSB would assume responsibility for the investigation if no criminal activity was involved.

As the investigation proceeded, it quickly became evident that the cause of the crash was an explosion in the center fuel tank, but the reason for the explosion was not clear. On the night of July 17th, the center fuel tank of Flight 800, a massive hollow space the size of a garage, contained only 50 gallons of fuel. Complicating the matter was the heat of an east-coast summer day, which meant that air-conditioning units below the tank were running nonstop while the jet was on the ground. These units generated considerable heat, some of which was transferred to the fuel tank. The hotter the jet fuel becomes, the more of it vaporizes, and the more vapor, the more explosive is the atmosphere. The flash point of jet fuel is close to 100°F, a temperature easily reachable in the tank, although a source of ignition would still be required.

Bombs and missile impacts leave distinctive traces on aircraft, such as characteristic structural damage and explosive residues. Structural damage would still be detectable even after the wreckage was submerged for some time, but explosives residues were another matter. A study conducted as part of the investigation revealed that explosives, which are somewhat water soluble, are quickly dissipated by immersion in salt water. However, a quick recovery of the debris was impossible, as it was scattered over an area approximately 4 miles by 3½ at a depth of 120 feet. The area was divided into three "debris fields," and wreckage recovered was assigned a color code based on the field from which it was recovered. Computer models were used to reconstruct the last few seconds of Flight 800 and to account for the pattern of dispersal of the wreckage. Simulations indicated that the section of the plane where the center fuel tank was located was the first to separate from the fuselage, further evidence that the initial catastrophic event took place there. Seconds later, the front portion of the plane, including the cockpit, was ripped away, falling to the sea farther east. The rear of the plane, including the wings and engines, considerably lighter with the loss of the other two sections, apparently shot upward in flame until the engines exhausted their fuel. This, investigators felt, could explain many of the eyewitness accounts of flaming streaks ascending in the sky. The wreckage continued to break apart and plummeted to the sea far away from the airport.

At the wreckage site, visibility in the water was poor and the water temperature at various depths was cold and grew colder as recovery work moved into the fall. As the underwater recovery effort was getting under way, the FBI organized hundreds of agents to interview witnesses and chase down leads. Early speculation that a "friendly fire" accident involving U.S. armed forces was discounted, as was the possibility of large missiles. A smaller, shoulder-launched missile remained a possibility, but an increasingly remote one, as the FBI learned more about the limitations of such a weapon. At the altitude of the plane when the explosion occurred, nearly 14,000 feet, only a missile fired from a boat directly under the plane would have had a chance to hit the target, and even then the odds were slim. In addition, anyone below on the water would have been showered with flaming debris and fuel. Thus, despite the convincing and numerous eyewitness accounts of flaming streaks in the sky, the investigation turned more toward the possibility of a bomb.

Bombs that had been used to bring down aircraft before—for example, the Pan Am 103 bomb—were composed of chemical compounds such as RDX, residues of which investigators hoped would remain on materials that were near the detonation. However, the long immersion times concerned everyone and made the task of detecting, confirming, and interpreting findings exceedingly difficult. As wreckage was recovered and delivered to the hangar, forensic chemists and explosives experts from the FBI and ATF used dogs and portable equipment to comb the pieces for any traces of explosives. Promising pieces of debris were swabbed or transported whole to the FBI laboratory in Washington, DC, for further testing. Explosives were found but were traced to a dog-training exercise months earlier. As the investigation continued and more pieces of wreckage were recovered, no structural or metallurgical evidence of a bomb

or missile was found. Similarly, the FBI probe did not uncover definitive evidence of criminal activity. The NTSB began leaning toward an accidental cause of the explosion in the center fuel tank and pursued forensic engineering tests to examine possible accident scenarios.

Diving operations ended in November 1996, but since a full reconstruction of the plane had been approved, trawling operations were conducted until April 1997. When those operations ended, an amazing 98% of the aircraft had been retrieved from the bottom, wreckage that was transported to the hangar, examined, tested, and eventually reconstructed. No convincing evidence of a bomb or missile was found, either in the form of chemical residues or characteristic structural damage. This finding coupled with other evidence and the flammability of the vapors in the center tank, led to the investigation's closing within a few months. The NTSB listed the probable cause as ignition of these vapors, but even after months of work, the agency was not able to pinpoint the source of the spark or flame that ignited the vapors.

Sources: Milton, P. *In the Blink of an Eye: The FBI Investigation of TWA Flight 800*. New York: Random House, 1999.

National Transportation Safety Board, In-Flight Breakup over the Atlantic Ocean, Trans World Airlines Flight 800, Boeing 747-131, N93119, Near East Moriches, New York, July 17, 1996.

Aircraft Accident Report NTSB/AAR-00/03. Washington, DC. Notation 6788G, August 23, 2000. Available online at *www.ntsb.org.*

the term **tertiary explosive** is used to describe materials that are relatively insensitive to this type of detonation; ammonium nitrate (NH_4NO_3) is an example. These materials often require a booster charge to initiate detonation. An **explosive train** is a linked series of combustible or explosive materials that are organized from most sensitive to least. The function of the train is to generate a detonation wave that will reach the main charge with sufficient energy to initiate detonation.

The terms **improvised explosive** and **improvised explosive device (IED)** are terms used to describe explosives that are made clandestinely, either from readily available precursors (much as methamphetamine is made from precursors in clandestine drug laboratories) or from diverted or stolen sources such as military munitions. **Homemade explosives** are those made from precursors in clandestine settings; peroxide explosives such as were used in the London Underground attack are examples. In this sense, homemade explosives are a subset of improvised explosives. The terms **high explosive** and **low explosive** are also frequently seen. In this scheme, primary and secondary explosives are considered to be high explosives, whereas many propellants are low explosives. The difference is that low explosives generally deflagrate, and high explosives detonate.

Finally, we can classify explosives based on chemical structure and functional groups:

- nitric compounds such as TNT
- aromatic nitramines (Tetryl)
- nitrate esters (nitroglycerin)
- peroxides (TATP)
- initiating explosives (lead styphnate)
- salt formulations (urea nitrate UN)
- fuel/oxidant formulations (ammonium nitrate/fuel oil, or ANFO)

2 DETONATION

Detonation is a complex process, the scope of which exceeds this text. However, a rudimentary understanding of the process is essential for an understanding of explosives and the forensic evidence created by explosions and detonations. A key criterion for detonation is the reaction front that propagates at speeds that exceed the speed of sound.

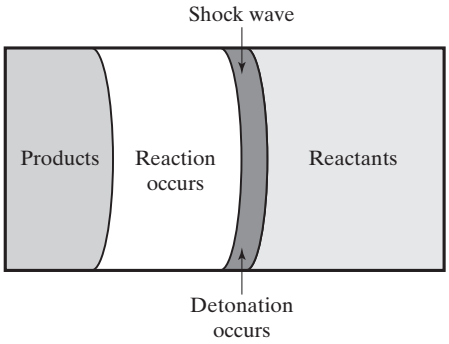


FIGURE 9 The detonation zone is the zone where combustion is initiated by mechanical pressure.

Imagine a tube filled with air and equipped with a piston at one end. Pushing this piston forward generates a pressure wave in the tube. At the front of this wave, molecules are compressed and heating occurs in this region. If you could push hard enough on the piston, you could generate a wave that moved faster than the speed of sound. This pressure front is called a **shock wave**, and extreme heating occurs in the compressed region. If this shock wave is propagating through an explosive material rather than air, the heat generated by the passage of the compressed region may be sufficient to cause thermal decomposition of the explosive materials. This reaction occurs just behind the shock wave front and generates additional heat and gases that increase the force behind the shock wave, which is now called a **detonation shock wave**. This concept is illustrated in Figure 9.

Another conceptual view of detonation is illustrated in Figure 10. Here, we consider a closed tube filled with a flammable/explosive mixture. If the mixture is ignited, we can visualize a combustive wave moving outward. The wave is reinforced by additional heat and gas. If the tube is long enough, the compression wave created by combustion can accelerate sufficiently to become a **detonation wave**.[2] Under suitable conditions, the compression waves can catch up with earlier waves, surpass the speed of sound, and establish a detonation wave.

A detonation wave results from a positive feedback cycle of increasing acceleration. When simple combustion begins, the first event generates heat and a wave of hot compressed gases that propagates outward. Increasing the pressure increases the heat, and as a result, more of the energy released is available, since less is needed to pre-heat the reaction mixture. This process establishes a positive feedback such that each new pressure wave moves faster than the previous one because the gases produced in successive combustions are hotter. Eventually, one of the waves exceeds the speed of sound and passes the initial wave at supersonic speed. This shock wave ignites the fuel–air mixture and sustains the detonation via extreme compression.[2] This process is usually incredibly fast. Nitroglycerine for example, if ignited under suitable conditions, can reach detonation-sustaining temperature, pressure, and density in about 10^{-12} s.[3]

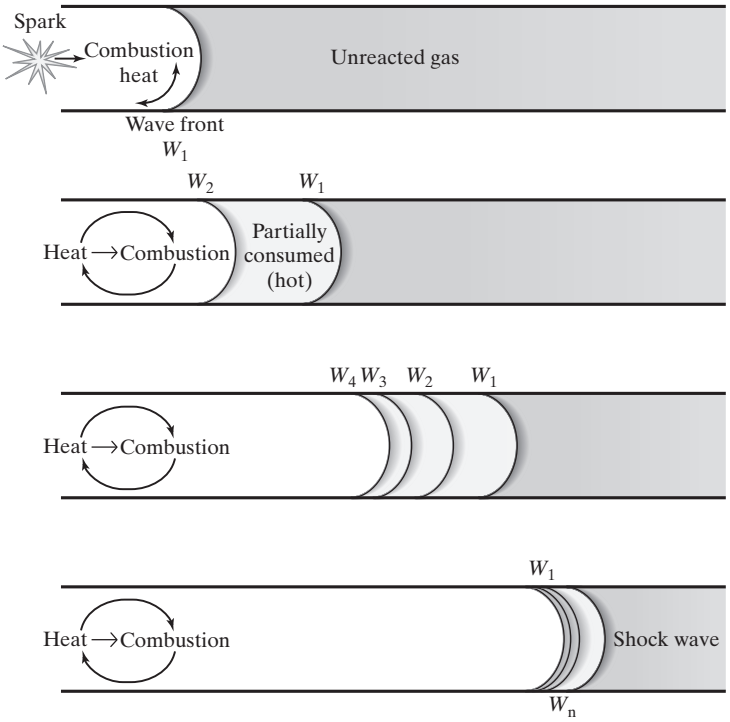


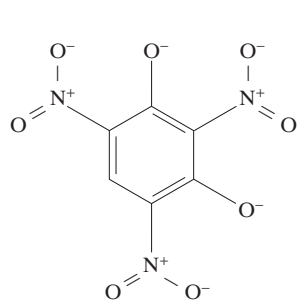
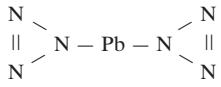
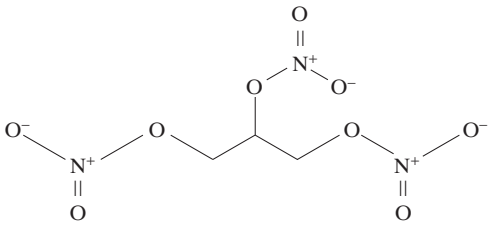
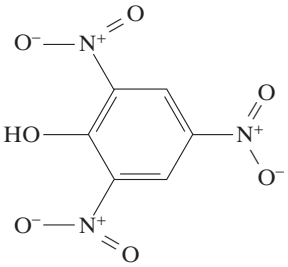
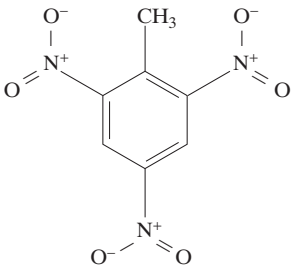
FIGURE 10 How a confined deflagration can progress to a detonation.

The speed of propagation of an explosion is in the range of 1500 to 9000 m/s.[7] The speed of sound depends on the medium through which the pressure wave is propagating and the temperature, since temperature influences density. The relationship is

$$v = (331.4 + 0.6T)\text{m s}^{-1} \qquad \textbf{(2)}$$

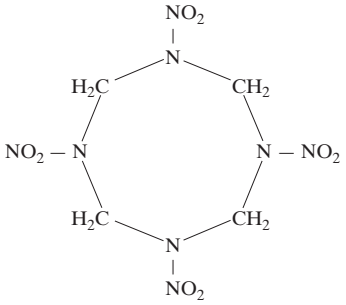
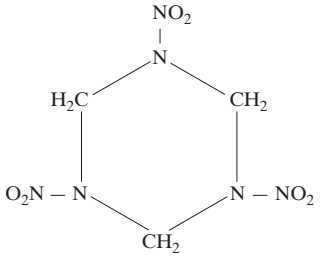
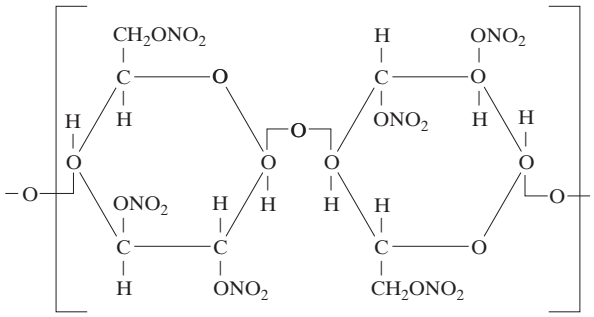
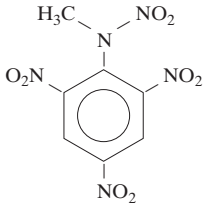
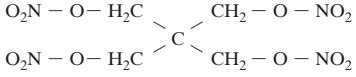
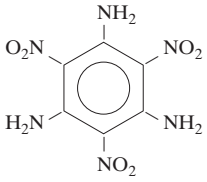
where v is the speed of sound, and T is the temperature in °C. At 25°C in air, v = 347 m/s. However, during detonation, the wave is propagating not through air, but through the reaction mixture and through the solid explosive, both of which are denser than air. The greater the density of the propagating medium, the faster the sound propagates.

TABLE 2 Detonation Speeds (m/s) and Structures of Selected Explosives

Explosive	Structure	Density (g/cm^3)	Speed	Power Index
Mercury fulminate	$Hg(C \equiv N^+ - O^-)_2$	1.25	2300	14
		3.07	3925	14
Lead styphnate		2.9	5200	21
Lead azide		3.8	4500	13
Nitroglycerin		1.60	7750	171
Picric acid		1.60	7900	100
TNT 2,4,6-Trinitrotoluene		1.55	6850	331.2

(continued)

TABLE 2 Detonation Speeds (m/s) and Structures of Selected Explosives *(continued)*

Explosive	Structure	Density (g/cm^3)	Speed	Power Index
HMX Octogen; cyclotetramethylene-tetranitramine		1.89	9100	455
RDX Hexogen; cyclotrimethylene-trinitramine		1.70	8440	457
Nitrocellulose Nitrated cellulose $–NO_3$ replacing –OH groups		1.15	7300	variable
Tetryl 2,4,6-trinitrophenyl-methylnitramine		1.55	7080	355
PETN Pentaerythritol tetranitrate		1.60	7920	452
TATB 1,3,5-triamino-2,4,6-trinitrobenzene		1.88	7760	273

Data Source: From "Chapter 3: Combustion, Deflagration, and Detonation" in *The Chemistry of Explosives.* by J. Akhavan, 1998. Reproduced by permission of The Royal Society of Chemistry.

Similarly, the denser the explosive, the faster the detonation wave propagates. Table 2 summarizes the detonation speeds and structures of some common explosives.

Among the common chemical components of explosives are carbon, oxygen, hydrogen, and nitrogen. Because an explosion is a combustion, the principal products of the reaction are familiar (CO_2, H_2O) with some additions such as N_2. Unlike typical fuels, most explosives contain oxygen, but many (such as TNT) are oxygen deficient. Others, such as the azides, contain no oxygen. Other sources of oxygen for explosive mixtures include chlorates (ClO_3^-) and perchlorates (ClO_4^-). Rocket fuels and improvised explosives can be made from combinations of oxidizers such as perchlorates and hydrocarbon fuels; an example of an improvised formulation would be sugar (a fuel) and ammonium perchlorate (the oxidant).

Most commercial and military explosives are mixtures of materials (fuel and oxidants) combined with components called **sensitizers**. The role of the sensitizer is as the name implies, although different sensitizers are sensitive to different stimuli. One may impart shock sensitivity, and another may create sensitivity to electrical currents or heat.

3 EXPLOSIVE POWER

The explosive power of a bomb depends fundamentally on the heat evolved *(Q)* and the volume of gaseous products produced *(V)* and is expressed as the product of these two quantities. One way to express the relative power of one explosive compared with another is obtained through the power index

$$\text{PI} = \frac{QV_{\text{explosive}}}{QV_{\text{picric acid}}} \times 100 \tag{3}$$

The **explosive index** of some common explosives is given in Table 2; typically, the index is calculated on the basis of 1.00 g of material.

A second and similar method of comparing explosives, also based on QV, is the **relative explosive power**, or REP value. In this approach, the value is not normalized to picric acid but rather is expressed as the square of the mass:

$$\text{REP} = \frac{QV}{m^2} \tag{4}$$

Where m is the mass in grams and Q and V are as previously defined. The units for REP are typically kJ·L g^2.

At this point, we run into an issue we can no longer ignore. You have learned to calculate Q and V based on the assumption that combustion was complete, occurred at stoichiometric equivalence, and that only gaseous water and CO_2 were produced. With explosives, these assumptions become too far removed from reality to be of practical use. Now we have to paint a more realistic picture of the products produced to obtain a more realistic estimate of Q and V. There are many methods for doing so; we will look at a simple rule-based approach. In such a method, we still balance the combustion equation, but now we apply some rules to better approximate the product mix that is produced by a complex detonation process. The rule set we will use

TABLE 3 Springall Roberts Rules for Determining the Gaseous Products of Explosives

1. C is converted to CO.
2. Any O remaining after the implementation of rule 1 is incorporated into water.
3. If any O remains after the implementation of rule 2, CO from rule 1 is converted to CO_2.
4. N is converted to N_2.
5. One-third of the CO formed is oxidized to CO_2.
6. One-sixth of the original amount of CO (prior to the implementation of rule 5) is converted to C and water.

is called the **Springall Roberts rules**, summarized in Table 3. An example application of the rules is provided in Example Problem 2. Keep in mind that the result of applying the rules remains an approximation, but a better one than we would have assuming a simple combustion. Now, we can proceed with calculations of explosive power index and the REP.

EXAMPLE PROBLEM 2

Use the Springhall Roberts rules (Table 2) to predict the products of an explosion of nitroglycerin ($C_3H_5N_3O_9$)

Answer: Since there are odd numbers in the subscripts, balance starting with 2 moles to avoid fractions.

Rule 1: $6\ C \longrightarrow 6\ CO$

Oxygen left = 18 – 6 = 12

Rule 2: Since there is oxygen left, we can make H_2O until we run out of H or O.
Here, we are limited by H, of which we have 10.

Thus, we make $5\ H_2O$

Oxygen left = 12 – 5 = 7

Rule 3: We have 70 left, so we can

Oxidize all CO $\longrightarrow$ CO_2

$6\ CO \longrightarrow 6\ CO_2$, 10 left

Rule 4: $6\ N \longrightarrow 3\ N_2$

Rule 5 and Rule 6 don't apply because we don't have any CO left, all C $\longrightarrow$ CO_2

Collect terms and confirm:

$$2\ C_3H_5N_3O\ g \longrightarrow 6\ CO_2(g) + 5\ H_2O(g) + 3\ N_2(g) + \frac{1}{2}\ O_2(g)$$

Clear fractions and check:

$$4\ C_3H_5N_3O\ g \longrightarrow 12\ CO_2(g) + 10\ H_2O(g) + 6\ N_2(g) + O_2(g)$$

C: $4 \times 3 = 12$		C: 12
H: $4 \times 5 = 20$	$\longrightarrow$	H: $10 \times 2 = 20$
N: $4 \times 3 = 12$		N: $6 \times 2 = 12$
O: $4 \times 9 = 36$		O: $12 \times 2 + 10 + 2 = 24 + 10 + 2 = 36$

EXAMPLE PROBLEM 3

Calculate the REP of nitroglycerin using data from the previous problem. Assume $\Delta H°$ for NG is $\frac{-384.4\ \text{kJ}}{\text{mole}}$

Answer: $\text{REP} = \frac{QV}{m^2}$. We can use the balanced equation to determine both Q and V, and we will account for 4 moles as we go.

First, calculate V, volume of gas:

Gases produced per 4 moles NG are:

$$12\ CO_2 + 10\ H_2O + 6\ N_2 + O_2$$

$$12 + 10 + 6 + 1 = \frac{29\ \text{moles gas}}{4\ \text{moles NG}}$$

$$= \frac{7.25\ \text{moles gas}}{\text{mole NG}} \times \frac{22.4\ \text{L}}{\text{mole}} = \frac{162.4\ \text{L}}{\text{mole NG}}$$

Next, calculate Q by calculation the $\Delta H°_{RXN}$:

$\Delta H°_{RXN} = \Sigma\ \Delta H°_{products} - \text{the}\ \Sigma\ \Delta H°_{reactants}$
All are in kJ; calculate for reaction as balanced

$$\Delta H°_{RXN} = \left[12\ \text{moles}\left(\frac{-393.5\ \text{kJ}}{\text{mole}}\right) + 10\ \text{moles}\left(\frac{-241.8\ \text{kJ}}{\text{mole}}\right)\right] - \left[4\ \text{moles}\left(\frac{-384.4\ \text{kJ}}{\text{mole}}\right)\right]$$

$$= [-7140.0\ \text{kJ}] - [-1537.6\ \text{kJ}] = \frac{-5602.4\ \text{kJ}}{4\ \text{moles NG}} = \frac{-1400.6\ \text{kJ}}{\text{mole NG}}$$

$$\text{REP} = \frac{(1400.6\ \text{kJ})(162.4\ \text{L})}{\left(\frac{\text{mole NG} \times 227.1\ \text{g}}{\text{mole}}\right)^2} = \frac{4.4\ \text{kJ L}}{\text{g}^2}$$

4 FORENSIC ANALYSIS OF EXPLOSIVES

Bombs create a wealth of physical evidence ranging from the container to the explosives and postblast residue, with forensic chemistry contributing important information at all stages. We will focus on the analysis of the explosives and residues here. As was the case in seized-drug analysis, we can roughly divide analytical methodology into field and lab—presumptive versus confirmatory analysis—although the line between field and laboratory methods is not hard and fast. If you have traveled by air recently, you have seen and perhaps experienced some of the field testing protocols used to detect explosives as part of passenger and luggage screening. There are many new developments in explosives analysis and detection, so we will just get a sampling of it here. For more information, check the "References" and "Further Reading" sections at the end of the chapter.

4.1 Field Screening Methods

Broadly speaking, we can consider field testing methods based on two protocols. The first is the familiar direct sampling. An example would be swabbing luggage such as occurs during airport security screening. A second method is **stand-off detection** which has many desirable characteristics, not the least of which is physical separation from a potentially dangerous sample. As shown in Figure 11, there are ways to

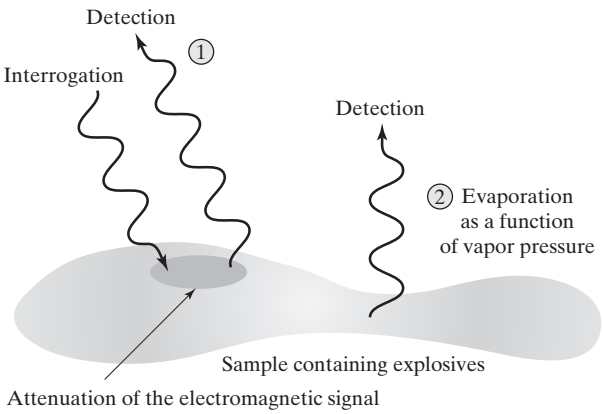


FIGURE 11 Two methods of stand-off detection.

remotely sample a material suspected of containing explosives. The first is direction of some form of energy such as electromagnetic energy or sound pulses into a sample where some interaction occurs that attenuates or changes the signal as a function of the amount and type of materials present. The signal is returned to a detector and the composition of the mixture deduced from the attenuation. Examples of this include the use of tetrahertz (THz)[4–7] and Raman spectroscopy.[8,9] The second stand-off method can be applied to explosives that have a relatively high vapor pressure such as TNT and TATP. Appreciable amounts of these explosives will be in the gas phase and thus available for detection using a variety of methods. Typically, concentration of the explosive vapor and the detection limits of the instrumentation or sensor system are the factors that dictate utility of this approach. A preconcentration or enrichment step is often needed such as solid-phase microextraction (SPME) or heated headspace, a method we last discussed in the context of blood alcohol testing.

4.1.1 IMMUNOASSAY AND BIOSENSORS **Biosensors** are devices that sense a binding event with high specificity and selectivity. The principles have been successfully extended to explosives. In many cases, environmental contamination has been the driving concern, and as a result, explosives that are also common environmental contaminants such as TNT and RDX have received the greatest attention. As in toxicology, immunoassays for explosives may be competitive or noncompetitive, heterogeneous or homogeneous. Detection can be indirect, through the introduction of a substrate that leads to color formation, or direct via fluorescence or chemoluminescence. Recently, quantum dots have also been used, a methodology already used in forensic science for latent print development. We will address both of these topics.

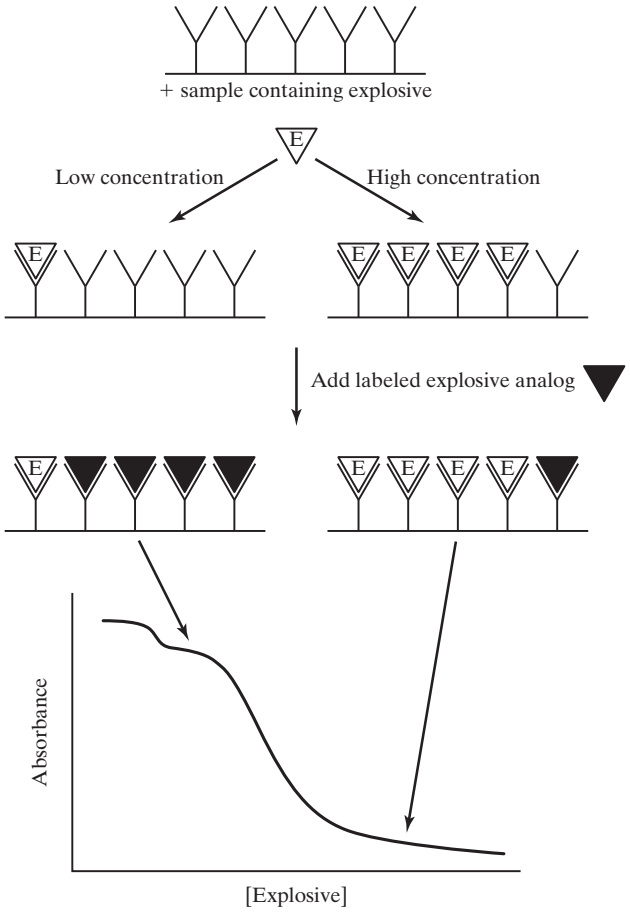


FIGURE 12 A generic immunoassay for explosives.

An example competitive assay is shown in Figure 12. Here, antibodies are bound to a surface that is exposed to a solution containing an explosive E that will bind selectively with the antibody. When the concentration of explosive is relatively low, binding sites will remain open, and when the concentration is relatively high, most binding sites will be occupied by E. A solution is then added that contains an explosive analog that will also bind with the antibody. The analog in this example is capable of participating in a color-generating reaction. The labeled analog will compete for binding sites. The higher the original concentration of E, the fewer sites available for the labeled analog; the lower the original concentration of E, the more sites will be available. This is illustrated in the center of Figure 12. At the conclusion of the binding stage, unbound materials are washed away and a substrate added. This substrate will react with the bound explosive analog to produce a color that is measured using a spectrometer. The intensity of the color is a function of how much of the analog was bound to the antibody, which is in turn a function of the concentration of explosive E in the original sample. The more intense the color, the lower the original concentration of the explosive.

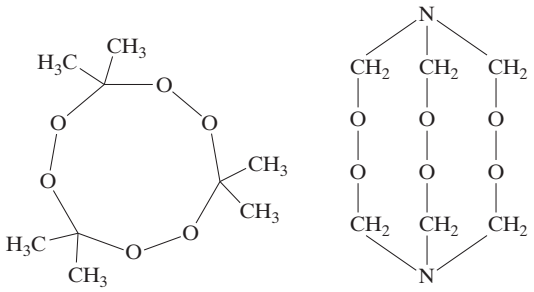


FIGURE 13 Two peroxide explosives.

Many immunological-based explosives sensors have been reported in the literature.[10,11] A significant proportion of these protocols rely on fluorescence as the detectable signal given the high intensity and correspondingly low detection limits. Other methods of signal generation include chemiluminescence and electrochemiluminescence. From a forensic perspective, one of the most interesting approaches is the use of quantum dots as the signal generation system.[10] Quantum dots are a type of nanomaterial made of a semiconductor such as CdS. Their size is on the order of 10 nm or less. The unique feature of quantum dots is that the emitted color is not a function of the material but rather the size and crystal structure of the particle, owing to unique quantum effects that are beyond the scope of this text. There are many excellent references available for further exploration of this topic. One of the advantages of the quantum dot approach is the ability to perform **multiplex assays** or to test for more than one analyte in the same assay. In one example, a quantum dot assay was developed for simultaneous detection of three explosives (TNT, RDX, and PETN).[12]

A particular challenge for field detection are the peroxide-based explosives such as TATP and HMTD (Figure 13). Although not widely used militarily or commercially, peroxide explosives have become a significant threat because of the ease of synthesis and availability of precursors. We saw the same phenomenon in the context of clandestine drug laboratories. Peroxide-based explosives were used in the London Underground bombing and were planned to be used in 2006 to bring down trans-Atlantic flights. This last incident led to the restriction in the amount of liquids that can be carried aboard flights within or destined for the United States. A number of analytical methods are based on the detection of H_2O_2 residuals in the explosive. Because peroxide is such a strong oxidizing agent, many instruments exploit this property to generate colored or fluorescent materials. As one example, a polymeric sensor was developed in which the peroxide reacts with a precursor to fluorescein, resulting in the production of the dye which fluoresces strongly in the visible range (521 nm).[11] Another biosensor, this one based on an enzymatic reaction, has also been described and commercialized.[10,11] In this scheme, the sample is first dissolved in an acidic organic solution to release the peroxide, which reacts with a peroxidase enzyme that catalyzes conversion of an added substrate, creating a detectable color change.

4.1.2 ION MOBILITY SPECTROMETRY IMS 8 remains one of the most widely used field detection instruments for explosives. It can be used to sense vapors in a stand-off mode or as a direct sensor through the thermal desorption of wipes.

IMS works by separating ion–molecule clusters on the basis of their size-to-charge ratio, and the instrument can operate in the positive- or negative-ion mode. Most explosives detection work is conducted in the negative-ion mode, in which nitrates or nitrate groups are targeted. Explosives such as nitroglycerin (NG), RDX (cyclonite), and pentaerythritol tetranitrate (PETN) are electronegative and amenable to analysis by IMS. Typically, such systems employ methylene chloride as the "doping agent," which functions as a source of dopant ions that increase selectivity and reduce background interference.[12,13-16]

When a sample is introduced into an ion mobility spectrometer, a soft ionization occurs via interactions with beta particles emitted by a ^{63}Ni source.[†] Molecules in

[†]This is the same technique used to create ions in an electron capture detector (ECD). There are other ionization methods used in IMS, but currently is used in most field-deployable instruments.

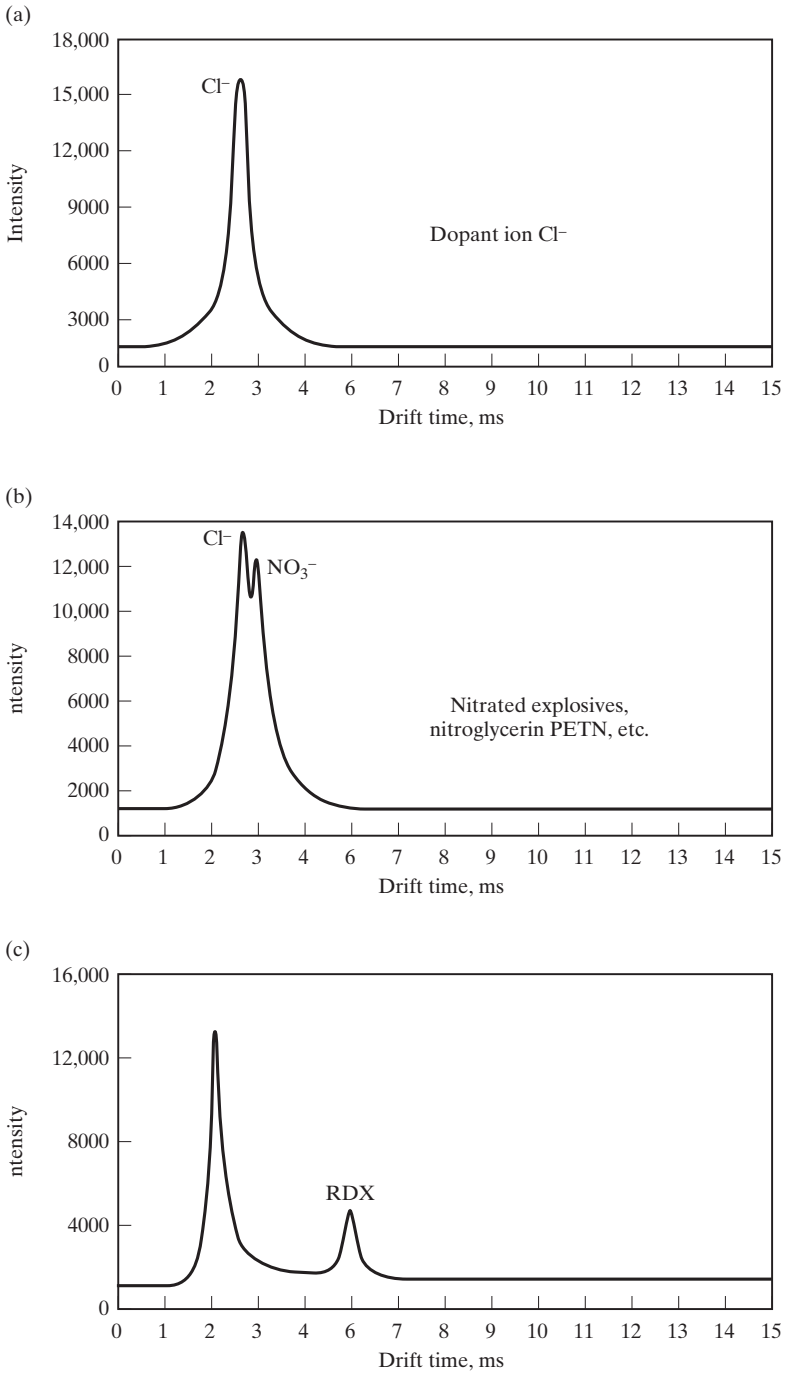


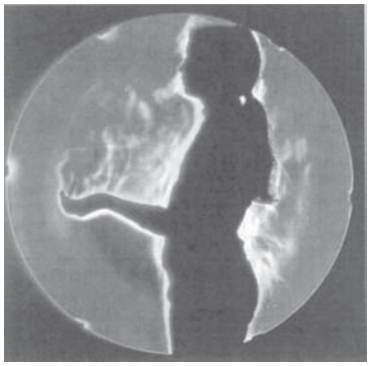
FIGURE 14 ^{63}Ni IMS spectra of 100 ng of (a) RDX, (b) NG, and (c) PETN. Spectra were obtained at 200°C with methylene chloride as dopant. Reprinted with permission from Tam, M., and H. H. Hill, "Secondary Electrospray Ionization–Ion Mobility Spectrometry for Explosive Vapor Detection." *Analytical Chemistry* 76 (2004): 2741. Copyright 2004 American Chemical Society.

air form clusters of ions or molecules, and in the negative-ion mode, these are usually species such as $O_2^-(H_2O)_n$ where n, the number of associated water molecules, depends on the humidity and other factors. These ions are referred to as reactant ions, because they always exist under atmospheric conditions. The components of the clusters are associated with each other but can undergo further exchange reactions when a sample (generically, M) is introduced. The reactions can be complex, involving proton abstraction, fragmentation, and charge transfer[32] and creating products such as M^-, $(M—1)^-$, and NO_2^-. When methylene chloride is present as the dopant, Cl^- ions are also present and participate in the reactions as well. The pool of potential product ions is reduced to species such as M ¥ $Cl^-(H_2O)_n$ For example, if the explosive TNT is present, the ion produced will be $C_5H_7(NO_2)_3Cl^-$ or $(TNT)Cl^-$. This ion is introduced into the drift region of the instrument, where it will separate from others based on the time it takes to reach the detector, which is held at a positive potential to detect negative ions. Mobility spectra of three explosives are shown in Figure 14. Note that the RDX cluster is much farther to the right, corresponding to the longer drift time of the RDX ¥ Cl^- cluster relative to the nitrate. PETN dissociates and shows a nitrate peak, but not an M ¥ Cl^- peak. A recent advance in IMS is the development of miniaturized mobility spectrometers such as differential mobility spectrometers (DMS), but these have yet to find widespread use in field screening.

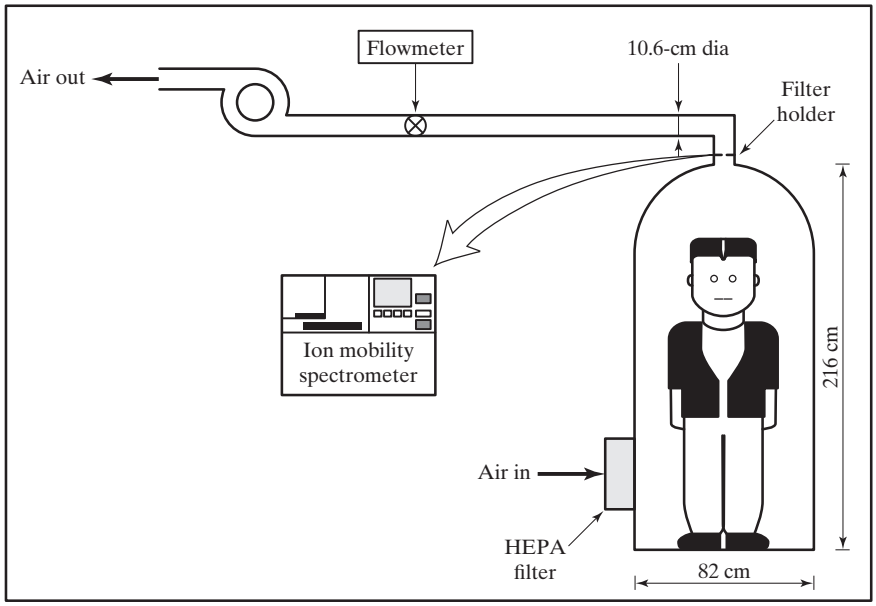
Recently, IMS has been coupled to an electrospray ionization source (ESI) with promising results for homemade explosives. IMS has generally been successfully used to detect peroxide based-explosives.[17]

Applying the Science 1 Human Heat Plumes

Airport screening for explosives has taken on new urgency since September 11, 2001. To facilitate rapid and noninvasive screening, walk-through portals or other "no-touch" systems are preferred. Systems that use techniques such as ion mobility spectrometry detect explosive vapors and work well when the explosives have a high vapor pressure. These systems are more problematic, however, when the explosives have low vapor pressures, as does RDX. To enhance the efficiency of portal detection, it is imperative to maximize the transfer of residuals from the person to the sampling inlet and detection system. Because our bodies are warmer than their surroundings, a heat plume surrounds us, as shown in the accompanying figure. Just as buoyant flames produce a rising column of heated air that carries soot upward, the heat plume of the human body can do the same, albeit on a much smaller and less dramatic scale. Explosive residues clinging to skin or clothing can be caught in this plume, so the more completely the plume is sampled, the lower are the limits of detection and the more effective is the screening. In the report cited here, researchers studied the effect of clothing, motion, room temperature, and other variables on the detectability of TNT and RDX from patches worn by volunteers as they stood in a portal. Among the interesting findings were significant variations from person to person. Findings regarding explosives are applicable to other portal screening devices targeting other materials, such as drugs.



Schlieren photo of human thermal convection.



Dispersal chamber and associated apparatus.

Source: Gowadia, H., and G. S. Settles. "The Natural Sampling of Airborne Trace Signals from Explosives Concealed upon the Human Body." *Journal of Forensic Sciences* 46 (2001): 1324–31. Figures and captions reproduced with permission from this source; copyright 2001, ASTM International.

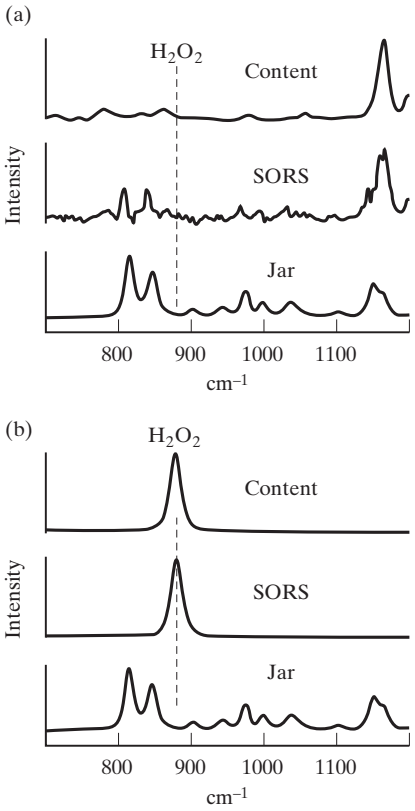


FIGURE 15 Conventional (CR) and SORS Raman spectra of Olay facial moisturizer cream, filled with (a) its original content and (b) 30% H_2O_2 (aq) respectively. The bottle was made of semi-transparent hard plastic material with a thickness of 6.5 mm. The dotted line indicates the signature band of H_2O_2 (aq) at 876 cm^{-1}. Adapted from reference number 27.

4.2 Laboratory Methods

Instrumentation available for the analysis of explosives is similar to that used for drug analysis. Chromatography, hyphenated techniques, and spectroscopy are all widely used. Most of the analytical capability is directed toward organic compounds, but appreciable effort has been invested in the detection of inorganics and ions such as ammonium, perchlorate, chlorate, and nitrate; compounds introduced in Table 2. The only family of methods not widely used are elemental analysis techniques such as ICP-MS, owing simply to the type of analytes that are of interest. When forensic analysis extends to the materials used in a bomb, then elemental analysis is a vital tool.

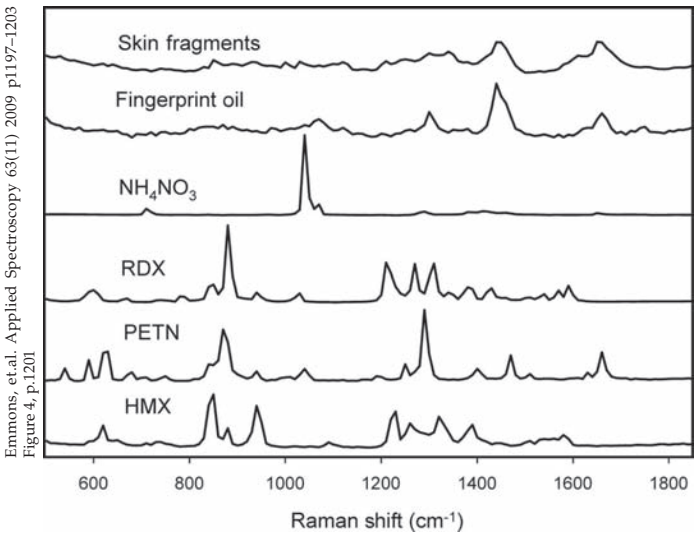
Until recently, analysis of ions and polyatomic species such as NH_4^+ were performed using ion chromatography.[18,19] However, recent advances in instrumentation, particularly in the realm of capillary electrophoresis (CE)[20-26] and mass spectrometry have significantly reduced (but not eliminated) the use of ion chromatography. In particular, CE is increasingly being used in microfluidic devices and chips that could prove invaluable for field detection.

4.2.1 SPECTROSCOPY IR and Raman spectroscopy are versatile detectors for explosives. Raman in particular has been exploited for direct and stand-off detection purposes, and both types of instrument are now commercially available in small portable configurations.[27-32] One of the advantages of Raman instrumentation is the ability to probe materials through many types of containers. The need for this capability became apparent after the attempted destruction of trans-Atlantic airliners in 2006 mentioned above. An example of a Raman application developed in response to this threat is shown in Figure 15.

One of the advantages of a Raman system is the ability to interrogate the sample at an oblique angle. **SORS** (spatially offset Raman spectroscopy) involves collection of signal at locations that are physically offset from the point of incidence as opposed to signal scattered directly from this point as in conventional Raman (CR). The wider the lateral diffusion of collected signal, the greater the return signals from photons emerging from deeper parts of the sample. The effect is to reduce the relative contribution to the signal of the surface relative to deeper levels of the sample.[27] The advantage of the offset is shown in Figure 15, where the peroxide peak signal is much cleaner when SORS is employed. Note that the sample was collected through the jar; this is an example of stand-off detection using remote interrogation, as was shown in Figure 11.

Applying the Science 2 Chemical Imaging of Fingerprints

Spectroscopic techniques can be designed to generate visible images of molecules on surfaces, a technique referred to as **chemical imaging**. Not surprisingly, fingerprints have become the target of chemical imaging studies in an attempt to find materials such as drugs and explosives. In this study, Raman chemical imaging (RCI) was deposited along with the latent print. The Raman spectra of the target analytes are distinct from the skin



Reference spectra of explosives and fingerprint residue. From E. D. Emmons et al. in Applied Spectroscopy, 63(11) 2009, p. 1199. Used by permission of the Society for Applied Spectroscopy.

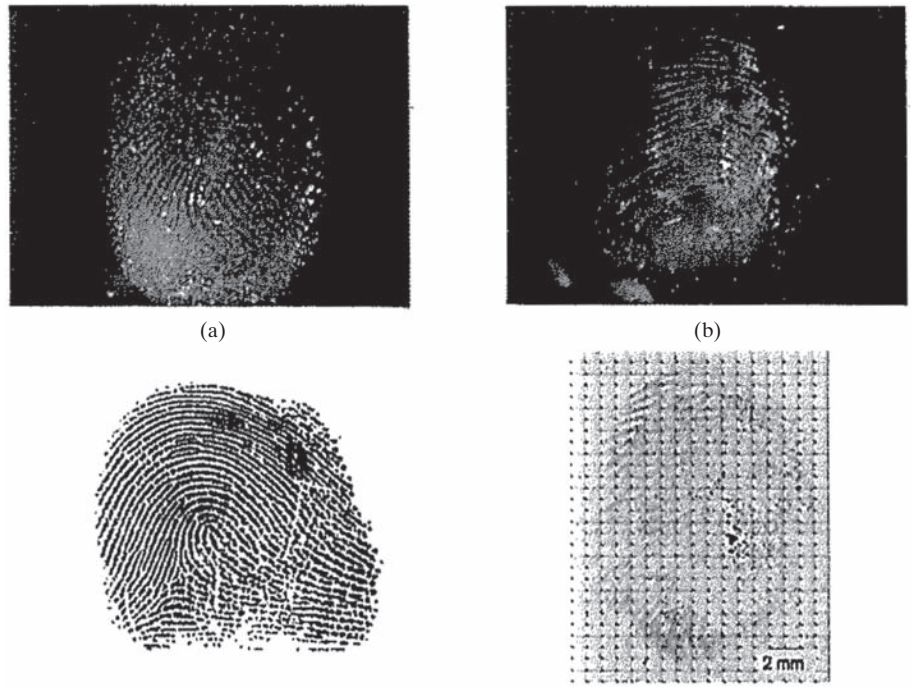


Figure 2 (a) Image of an uncontaminated fingerprint on an aluminum slide. (b) Image of a fingerprint from the same finger contaminated with explosives. (c) Image of the same finger from a fingerprint card obtained professionally from a police agency. (d) Montage of images of the explosive-contaminated fingerprint in (b). From E. D. Emmons et al. in Applied Spectroscopy, 63(11) 2009, p. 1201. Used by permission of the Society for Applied Spectroscopy..

background, as seen in the first figure. In the second figure, the image of the latent print was combined with the Raman results to generate a "map" of the dispersion of explosives within the fingerprint.

Source: Emmons, E. D., A. Tripathi, J. A. Guicheteau, S. D. Christesen, and A. W. Fountain. "Raman Chemical Imaging of Explosive-Contaminated Fingerprints." *Applied Spectroscopy* 63, no. 11 (2009): 1197–1203.

4.2.2 CHROMATOGRAPHY AND MASS SPECTROMETRY Probably the most significant recent advances in the analysis of explosives have been in mass spectrometry, specifically liquid chromatography and tandem-MS instrumentation and methodology. GC-MS, the workhorse instrument of seized-drug analysis is not as versatile in the explosives arena owing to limitations imposed by molecular weight, volatility, polarity, and thermal decomposition issues. This is not to say that GC is not used, but in general, it has taken second place to liquid chromatographic methods.

GC with electron capture detection (**ECD**, Figure 16) and cool on-column injection has been used for environmental analysis of explosives, and the coupling of GC to ECD and various sample introduction methods have been reported for explosives detection.[33–40] A detector that until recently was widely used in explosives analysis is the thermal emission analysis detector or TEA. Headspace GC-MS has been applied to the study of peroxide explosives and the associated degradation products and to plastic explosives.[41–42]

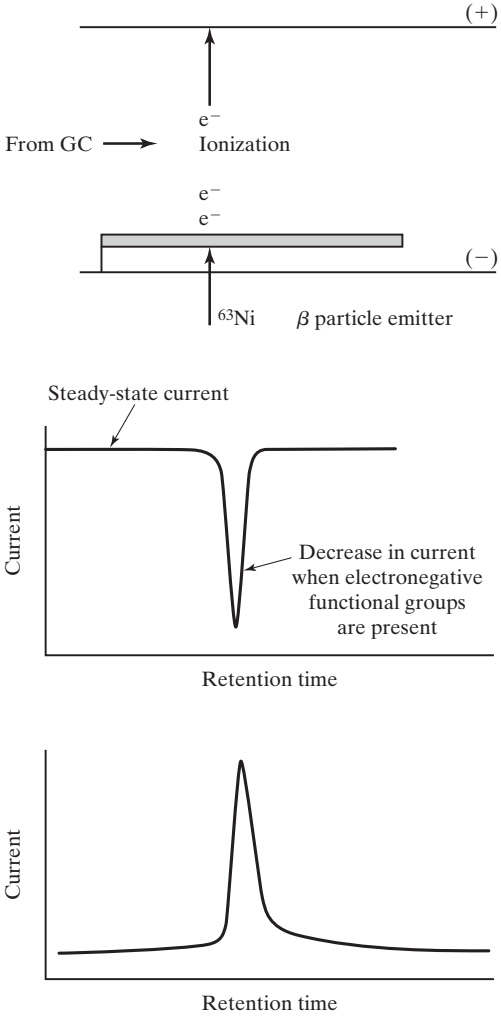


FIGURE 16 Schematic of an ECD. Eluent enters the detector, where it encounters electrons (β particles) emitted by radioactive ^{63}Ni. A series of ionizations occur, releasing electrons that are drawn toward the anode (+). A steady current will exist until the composition of the eluent changes, as when a compound elutes. If that compound is electronegative, the current will decrease. The plot is inverted to generate the chromatogram.

HPLC has been used for environmental and forensic analysis of explosives since the 1980s. The Environmental Protection Agency has published a method (EPA 8330 and updates) that uses HPLC with UV detection and a dual column methodology to identify and quantify 14 nitroaromatic explosives including TNT, RDX, and degradation products. We have seen this protocol before: recall that blood alcohol analysis is conducted using a headspace GC-FID and two columns. The versatility of LC methods was increased with the introduction of photodiode array detectors (PDA), which allow a complete UV spectrum of eluting analytes to be collected. However, the breakthrough in LC instrumentation was the emergence of affordable LC-MS systems. Tandem-MS systems and time-of-flight (ToF) instruments coupled to electrospray ionization sources (ESI) have significantly improved detection limits, improved confidence of identification, and expanded the list of potential analytes.[43–55]

ToF systems are used to gather exact mass data and identify compounds that would be difficult or impossible to do using tandem-MS systems. The latter are best suited for trace quantitative assays in which the analytes are known and standards obtained. In contrast, ToF systems are designed to obtain the mass of analytes accurate to within a few parts per million (i.e., in the fourth to fifth decimal place of the formula weight). This information, coupled with knowledge of natural isotope abundances, is used to postulate the likely combination of atoms that constitute the detected species. In the examples shown here (Figure 17 and 18), mass spectra were obtained for a propellant mixture using an HPLC coupled to an ESI source. The detector system illustrated in this example is a quadrupole combined with a ToF analyzer (**Q-ToF**). The quadrupole serves to filter ions such that only those in a very narrow mass range enter the ToF analyzer at

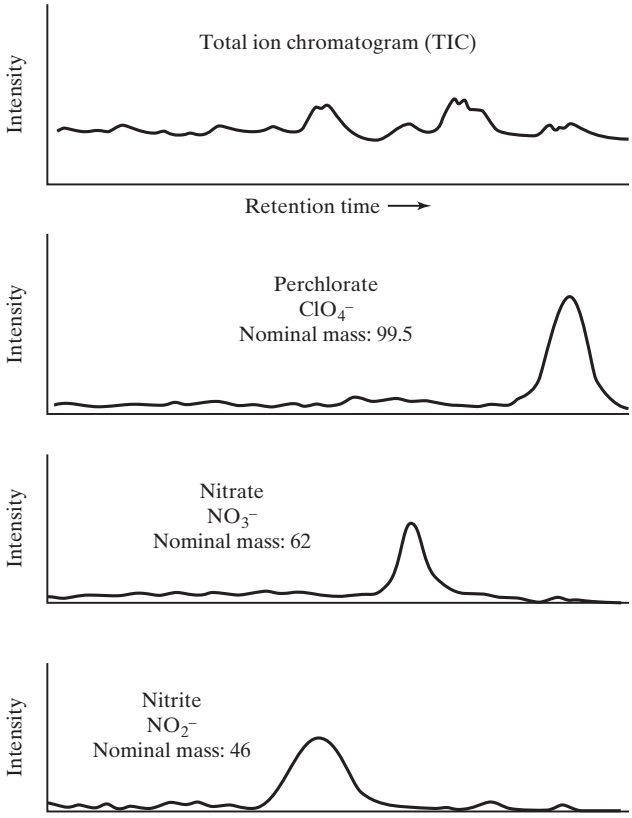


FIGURE 17 Hypothetical Q-ToF analysis of an improvised explosive mixture. The total ion chromatogram is unremarkable, but the extracted ion chromatograms clearly show the presence of the target analytes. The mass spectra (Figure 18) confirm the identification. Adapted from Bottegal, M., L. Lang, M. Miller, and B. McCord. "Analysis of ascorbic acid based black powder substitutes by high-performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry." *Rapid Commun. Mass Spectrom*. 24(2010): 1377–1386.

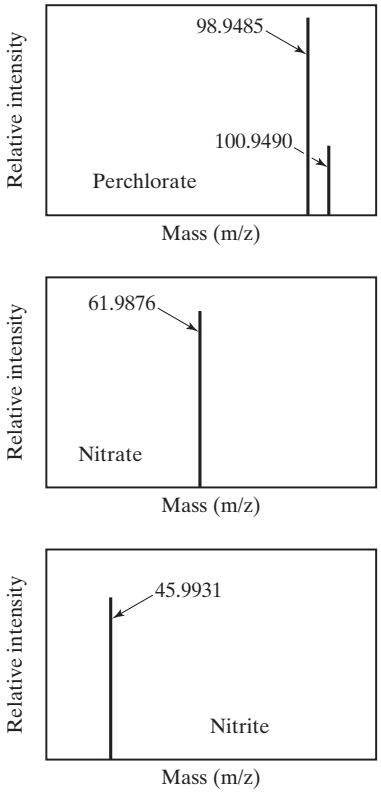


FIGURE 18 The Q-ToF mass spectra corresponding to the extracted ion chromatograms in the previous figure. Note the exact mass values obtained from the time-of-flight detector. Adapted from Bottegal, M., L. Lang, M. Miller, and B. McCord. "Analysis of ascorbic acid based black powder substitutes by high-performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry." *Rapid Commun. Mass Spectrom*. 24(2010): 1377–1386.

a given time; scanning the quad allows a complete mass spectrum to be collected. The total ion chromatogram and extracted ion chromatograms are shown in Figure 17 and the corresponding mass spectra in Figure 18. Notice the number of decimals on the *m/z* values; this exact mass determination is the strength of ToF systems. Nitrite (NO_2^-, second spectrum from the top), for example, has a calculated formula weight of 14.00674 + 2(15.9994) or 45.9947. The *m/z* peak on the spectrum falls at 45.9931, very nearly identical with the calculated value. Remember also that the recorded spectrum will be the result of combinations of naturally occurring isotopes, the knowledge of which is used to generate possible formulas for unknown mass peaks. Note that the analytes here are all anions, an indication of the versatility of these emerging LC-MS systems and instruments.

Summary

Explosives and detonation are extreme examples of combustion in which hot gases and pressure are exploited to do work and cause damage. Detonation is a unique type of reaction in which compression initiates the process. Blast effects can be related to the pressure wave or to shattering, or to combinations of these effects. Analytically, ion mobility spectrometry and liquid chromatography are the perhaps the most widely used, but recent developments in biosensors, spectroscopy, and mass spectrometry are being exploited in field and laboratory applications. Propellants share many characteristics of explosives but that also have unique differences and present different forensic challenges.

Key Terms and Concepts

Biosensor
Brisance
Chemical imaging
Detonation shock wave
Deflagration
Detonation
Detonation wave
Explosive index
ECD
Explosion
Explosive train
High explosive
Homemade explosives
IED
Improvised explosive
Low explosive
Multiplex assay
Primary explosive
Pushing power
Q-ToF
Relative explosive power (REP)
Secondary explosive
Sensitizers
Shock wave
SORS
Springall Roberts rules
Stand-off detection
Tertiary explosive

Problems

FROM THE CHAPTER

1. Can you make a bomb with dry ice? If so, explain how. If not, explain why not.
2. Why is nitrogen not included in the shortcut equation for determining oxygen balance (eq. 1)? Do you see any potential problems with this omission?
3. The molecule N_5 has recently been suggested as a new explosive. What are the features of this structure that suggest this application?
4. Use the data in Table 1 to suggest optimal formulations for the following compositions:
   a. Barium nitrate and lead styphnate, oxygen balance of −18.8. This is a crude approximation of the materials used in primers for ammunition.
   b. TATP and ammonium nitrate
5. Use the Springall Roberts rules to predict the products of the explosive decomposition of the following:
   a. PETN, $C_3H_8N_4O_{12}$
   b. Ethylene glycol dinitrate, EGDN, $C_2H_4N_2O_6$
   c. TATP
   d. Picric acid, $C_6H_3N_3O_7$
6. Calculate the oxygen balance for each of the explosives in the previous problem using the shortcut method (eq. 1). Could any of these be used alone based only on oxygen balance considerations?
7. Using the balanced chemical equations obtained in the previous question, estimate the Q value for each explosive on a per gram basis. Use the following heats of formation ($\Delta H°$) values in kJ/mol.
   a. PETN: −520
   b. EGDN: −227
   c. TATP: −116
   d. Picric acid: −245
8. Using the balanced chemical equations obtained in problem 6, calculate the volume of gas produced by the detonation of 100.0 g of each substance (PETN, EGDN, TATP, and picric acid).
9. Using data calculated in previous problems, calculate the REP value for each substance (PETN, EGDN, TATP, and picric acid).
10. What do IMS and electron capture detectors have in common?

11. Do a little reading and research and describe the basic principles of ion chromatography. Draw a box diagram and explain how a signal is produced for anions and cations.

INTEGRATIVE

1. What do propellants and explosives have in common? How to they differ?

Further Reading

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Firearms and Associated Chemical Evidence

OVERVIEW AND ORIENTATION

Now, we turn to propellants and firearms, which lie in the middle of our combustion continuum model. Firearms create a wealth of evidence that can be analyzed using techniques of forensic chemistry such as color tests and elemental analysis. As we will see, firearms exploit rapid burning to generate large amounts of hot gas that in turn generates the force necessary to accelerate a bullet. The propellant is ignited via a sensitive primary explosive such as lead styphnate. Firearms as a topic afford an excellent review and practical application of thermodynamics and kinetics, and you might want to refresh your memory of these topics using an introductory chemistry text before tackling this chapter. We will discuss several color tests that have been used in the context of firearm evidence; these are now used mostly in the context of distance determinations (weapon to target). Finally, we will explore the topic of serial number restoration, a challenge that frequently arises with guns, and that allows us to explore interesting applications of oxidation–reduction chemistry. Again, it is a good idea to crack open your introductory chemistry text before tackling this section.

1 HOW GUNS WORK

In this section, we will paint a more complete picture of this process. Recall the first law of thermodynamics, which is often paraphrased as "energy is neither created nor

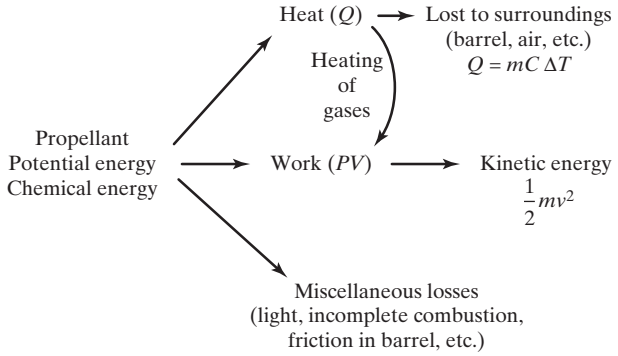


FIGURE 1 Ways in which chemical energy of the propellant is converted to other forms of energy by a firearm.

destroyed; it only changes form." Algebraically, we can express this law as

$$\Delta E = Q + w \quad \textbf{(1)}$$

Where E is the internal energy of the system in question, Q is heat, and w is work, here PV work. The operation of a firearm makes this relationship tangible (literally). Think about what happens when a gun is fired—chemical energy is converted to kinetic energy and heat, as evidenced by the barrel's becoming noticeably hotter with each round fired. Heat is also lost to the surroundings, and some of the energy is converted to light, known as *muzzle flash*. Some of the energy is also dissipated as friction as the bullet moves down the barrel. Thus, the potential or chemical energy stored in the propellant is converted to many different types of energy, as seen in Figure 1.

Guns are surprisingly simple devices, be they handheld or large artillery pieces. We will focus on small arms, specifically pistols and revolvers, since these are commonly involved in crime. Figure 2 shows a revolver in which cartridge cases are loaded into a rotating cylinder. When the trigger is pulled, the hammer moves back and then forward, using the spring to accelerate the firing pin into the base of the cartridge, where the primer is located. The material in the primer ignites, and a flame is directed into the propellant, igniting it and initiating the production of hot expanding gases. Once sufficient force is built up, the bullet begins to move down the barrel, which is **rifled**. This means that grooves have been cut at an angle in the barrel. These features, also called **lands** (the elevated portion) and **grooves**, grip the bullet (softened slightly by the heat), imparting spin to the bullet as it exits the barrel.

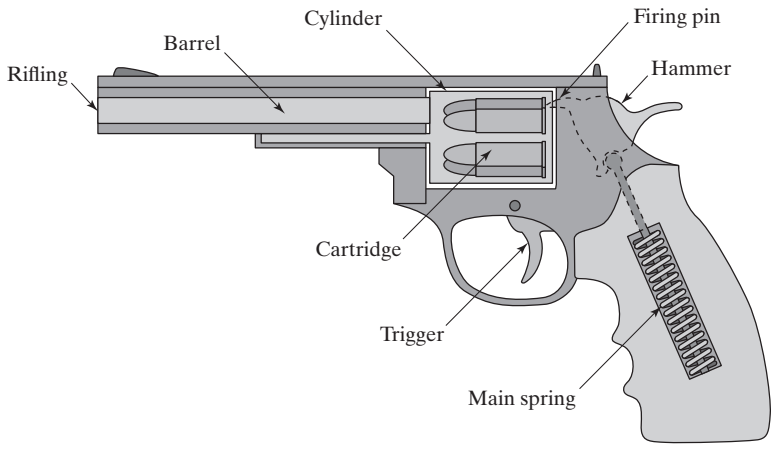


FIGURE 2 Simplified schematic of a revolver.

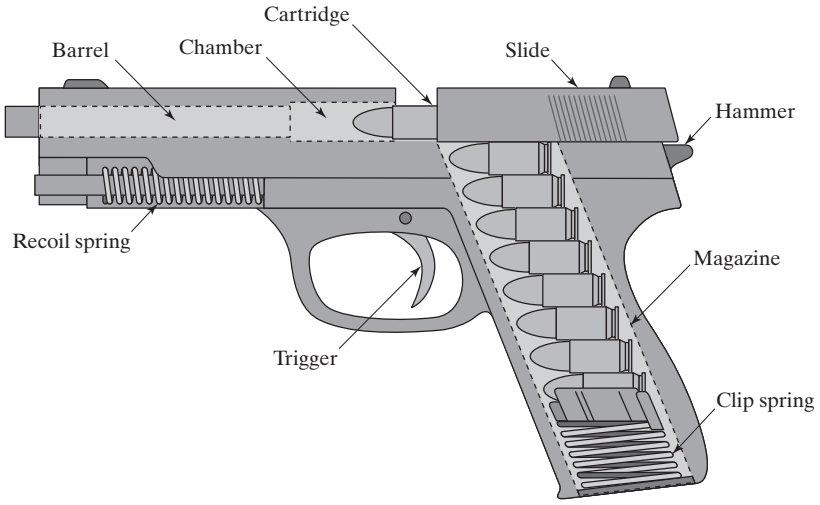


FIGURE 3 Simplified schematic of a semiautomatic pistol.

Suzanne Bell

FIGURE 4 View down the barrel of a semiautomatic pistol. The rifling is clearly visible, as is a residue towards the back of the barrel.

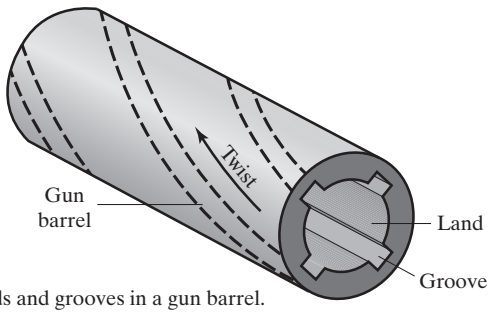
A semiautomatic pistol (Figure 3) is more complex because portions of the expanding gases are harnessed to move the slide and eject the spent casing. A spring in the magazine pushes the next round into the chamber. Pulling the trigger results in the same sequence of events as described for the revolver. Figure 4 shows the view down

EXHIBIT A

Caliber and Gauge

The *caliber* of a gun originally referred to the diameter of the barrel of a rifled pistol or rifle; however, the term can also refer to the size of cartridges used in firearms. Caliber is measured from the tops of the lands and is given in hundredths or thousandths of an inch or in millimeters. Common calibers include 9 mm and .22, .38, .40, and .45 for pistols and .22 and .30–06 for rifles. The caliber of a gun is considered to be a nominal measurement, meaning that the actual barrel diameter may vary slightly from the caliber measure used to describe it.

Originally, the gauge of a pellet referred to how many pellets of a given size (the same as the barrel diameter) were needed to reach a weight of 1 pound. Twelve gauge pellets weighed approximately $\frac{1}{12}$ of a pound each and would fit in the barrel of a 12-gauge shotgun. Now the term *gauge* is similar to *caliber* and describes the size of the shotgun barrel. Higher gauge numbers mean smaller barrels, so a 12-gauge shotgun has a barrel of larger diameter than does a 16-gauge shotgun, just as 12-gauge shot is larger than 16-gauge shot.



Lands and grooves in a gun barrel.

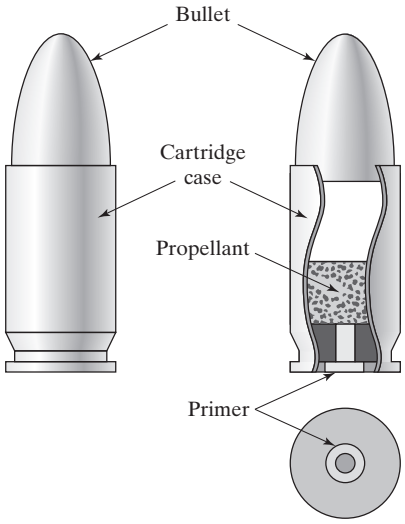


FIGURE 5 Modern ammunition.

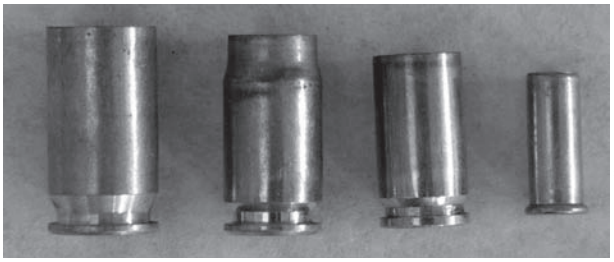

Suzanne Bell

FIGURE 6 Examples of cartridge cases. The three on the left are for semiautomatic pistols, as evidenced by the tapering of the base. This taper affords a place for the ejector mechanism to grasp the case. All three of these are center fire design. The cartridge on the right is a rimfire cartridge used in a revolver.

the barrel of a pistol with the rifling clearly visible. If you look closely, you can see residue at the far end; this discharge and residue will be discussed in detail shortly, as it is among the most important types of chemical evidence produced by firearms.

Modern cartridge cases, in which the bullet, propellant, and primer are integrated into a single unit, were first introduced in the latter part of the nineteenth century and have evolved and been refined since. Figure 5 depicts the basic design of ammunition for small arms, referred to as a **cartridge**. The case can be made of different metals, the most common being brass, steel, and aluminum. Because brass is the most common, the term "brass" is sometimes used generically to refer to a cartridge case regardless of the material of which it is made. The case is partially filled with propellant, and the bullet is seated at the top of the case using a crimp seal. The primer fits into the base of the cartridge. Several types of casings are shown in Figure 6. Note that the cartridge on the far right has a different design than the other three; this is due to differences in primer design.

As shown in Figure 7, there are two types of primer configurations. The most common is **center fire,** in which the primer is centered in the base of the cartridge with vents leading to the propellant; this is how the flame from ignition or detonation of the primer reaches and ignites the propellant. The **rimfire** design is found in small-**caliber** ammunition such as .22 (referring to the nominal barrel diameter of .22 inch). Here, the primer material is wrapped around the base of the propellant in direct contact with it. When the firing pin strikes the base of the cartridge, the primer material is crushed and ignited.

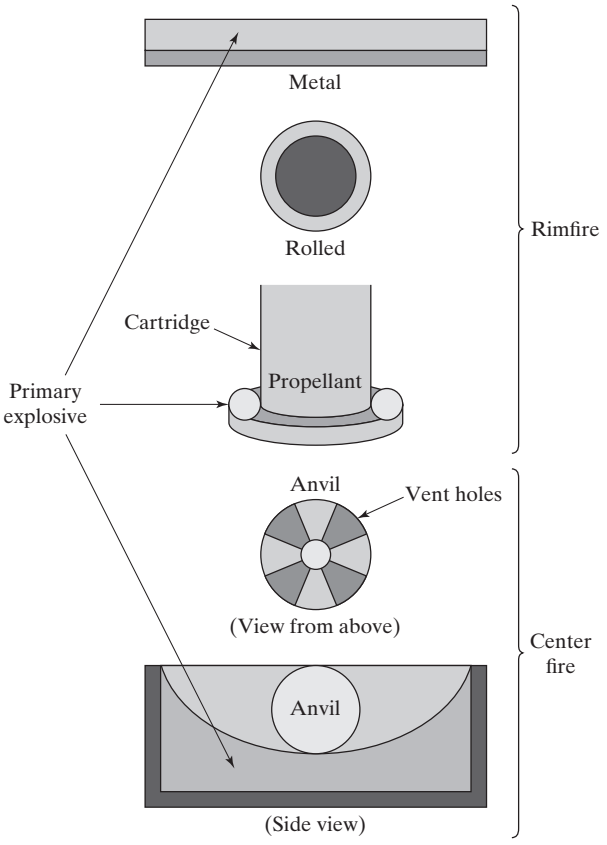


FIGURE 7 The difference between rimfire and center fire primer designs.

2 PRIMERS AND PROPELLANTS

Primers and propellants are the source of much of the chemical evidence associated with firearms examination. A list of common ingredients of propellants is provided in Table 1. Additionally, primers often contain antimony sulfide (SbS, oxygen balance ~−42%) and oxidizing salts such as barium nitrate ($Ba(NO_3)_2$) and potassium nitrate (KNO_3). Most of the stabilizers work by scavenging the nitric acid (HNO_3) that is formed as the nitrated energetics such as nitrocellulose or nitroglycerin degrade.[1,2]

Early primers were simple sparks. Flintlock muskets used a flint to generate a spark that ignited black powder propellants. The first chemical explosives used were compounds such as mercury fulminate and potassium chlorate ($KClO_3$). Modern primers contain percussive explosives previously mentioned such as lead azide, lead styphnate, tetracene, and compounds of barium and antimony. Primer residues are forensically important constituents of gunshot residue, a topic we will discuss in detail in Section 3.

The first propellant used in firearms was black powder (now called gunpowder), the invention of which is generally credited to the Chinese. Gunpowder is a simple formulation of charcoal (~5–15% w/w) sulfur(~5–15%), and **saltpeter** (~75% or more; potassium nitrate, KNO_3). This powdered formulation burns to produce nitrogen and carbon dioxide gases in various combinations depending on the powder's specific formulation. One example is shown below:

$$8C(s) + 3S(s) + 10KNO_3(s) \longrightarrow 3K_2SO_4(s) + 2K_2CO_3(s) + 6CO_2(g) + 5N_2(g) \quad \textbf{(2)}$$

The two salts formed are solids and contribute to the copious smoke (much of which is unburnt carbon) associated with black powder.

Variations of the preceding formula were used for crude cannons and pyrotechnics (fireworks) until the mid-1800s, when several important products were invented. One of these was guncotton, in the 1830s. **Guncotton**, also called **nitrocellulose** (NC) or cellulose nitrate, is produced by treating cotton with nitric and sulfuric acids. This treatment parallels that of nitroglycerin (NG), in that –OH groups are nitrated, but in guncotton the conversion is not complete. Guncottons are rated according to their

EXAMPLE PROBLEM 1

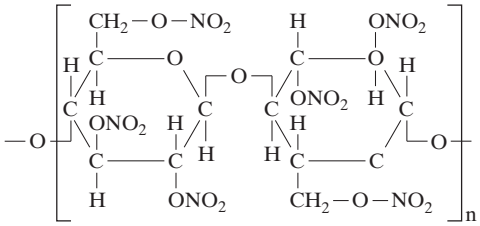
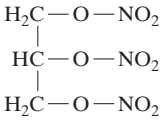
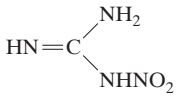
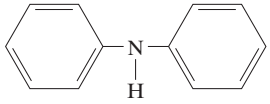
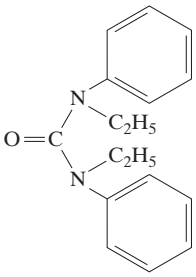
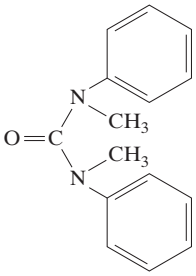
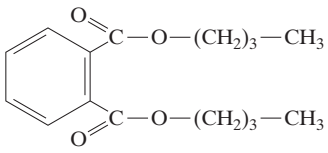
Suppose the main active ingredients in a primer are barium nitrate and lead styphnate. What would be the ideal composition?

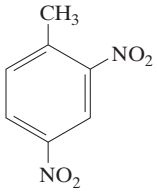
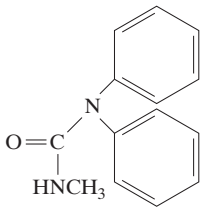
Answer:
As with explosives, the best combination is that which produces a net oxygen balance of zero. The calculation is straightforward as long as the oxygen balance of each component is known. These can be calculated or obtained from a table. Barium nitrate has an oxygen balance of approximately +31%, and lead styphnate, −19%. If we define the fraction of the mixture that is $BaNO_3$ as x and the fraction that is lead styphnate as $1-x$, the calculation is

$$\begin{aligned} 31x - 19(1 - x) &= 0 \\ 31x - 19 + 19x &= 0 \\ 50x &= 19 \\ x &= 0.038, \text{ or } 38\% \end{aligned}$$

The ideal combination would be 38% $BaNO_3$ and 62% lead styphnate.

TABLE 1 Components of Propellants

Compound	Structure	Approximate Oxygen balance	Function and Notes
Nitrocellulose		−24%	Polymeric binder, energetic
Nitroglycerin (NG)		4%	Energetic plasticizer
Nitroguanidine		−31%	Used in triple-base powders; not commonly seen in forensic analysis
Diphenylamine DPA		−279%	Stabilizer and antioxidant
Ethyl centralite (Centralite I)		−256%	Stabilizer and antioxidant
Methyl centralite (Centralite II)		−246%	Stabilizer and antioxidant
Dibutyl phthalate		−224%	Plasticizer (inert)

Dinitrotoluene (DNT, 2,4- and 2,6-; 2,4-shown)		−114%	Burning rate control, muzzle flash suppression
Akardite II		−240%	Stabilizer and antioxidant

Source: ICT Database of Thermochemical Values, demonstration version, supplied with Meyer, R., J. Kohler, and A. Homburg. *Explosives*, 6th ed. Weinheim, Germany: Wiley-VCH Verlag, 2007; Original edition, 1977.

degree of nitration, which, expressed as a percentage, falls into the range of 12%–14%. The first gunpowder using guncotton was introduced by the French Army in the late 1870s and was made with thick guncotton gelatin that used ethanol and ether. The slurry was extruded into flat sheets that were allowed to dry and then chopped into small grains for use in ammunition. This simple nitrocellulose-based propellant is called **single-base smokeless powder**. Alfred Nobel played a role in the development of propellants by introducing **double-base smokeless powder** in 1888.[2] The additional ingredient was nitroglycerin. **Triple-base powders** included a third explosive, nitroguanidine. Triple-base powder is used in large-caliber weapons not typically encountered in forensic contexts.

EXHIBIT B

Gunpowder

The invention of gunpowder (black powder) has been attributed to many cultures, including the Greeks and the Chinese. Although gunpowder has been used for centuries, on a battlefield the thick smoke either quickly obscured the view or gave away the position of those firing. Smokeless powder was developed for use by the French Army in 1876 and has replaced black powder in commercial ammunition, although the latter is still used by collectors and hobbyists. Smokeless powder contains cellulose nitrate and organic stabilizers and is manufactured to carefully control the size of the grains. Powder does not explode when ignited (it is considered a low explosive) but, rather, burns very rapidly, and since burning occurs at the surface of particles, the size of those particles dictates how much surface area is available and how fast the burning will occur. The term "gunpowder" now commonly refers to smokeless powder.

Modern firearms—at least the type seen most frequently in forensic laboratories—employ double-base powder formulations. The shape of the propellant grains is an important variable and a key consideration for use in ammunition given that the burn rate or burn time has to be controlled to impart the maximum kinetic energy to the projectile. The rate of burn is adjusted by adding deterrents and by shaping of the

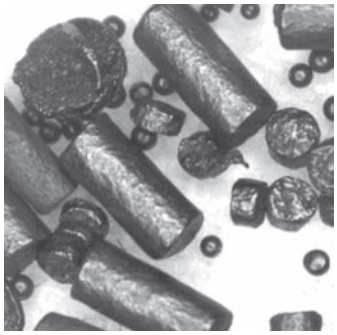

Suzanne Bell

FIGURE 8 Micrograph of double-base powders.

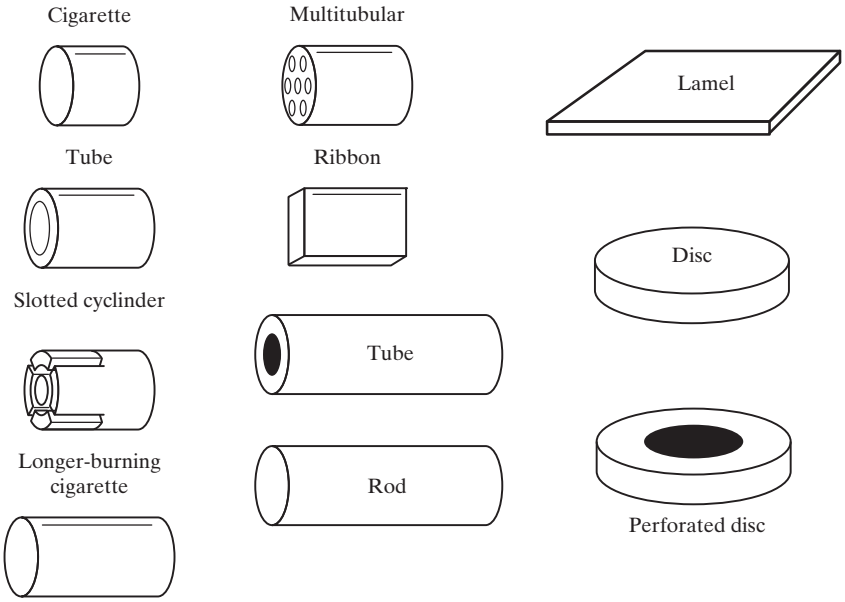


FIGURE 9 Powder granules are shaped to optimize the surface area for given applications.

powder particles, as illustrated in Figures 8 and 9. So-called ball powders are manufactured by creating spheres in an emulsion that dries to form spherical granules of propellant.[4]

Figure 10 illustrates powder granules burning end to end and outside in, but this is not the only burn pattern employed. Powders that burn from outside to inside are categorized as **degressive-burning powders**, whereas **neutral-burning powders** burn evenly. Neutral burning is accomplished by creating holes and pores in the powder granules, exposing more of their surface. A **progressive-burning powder** results from coating the propellant with a **deterrent**. At first, burning is inhibited, but it increases in speed as the deterrent is consumed.[4]

Now that we have a basic understanding of how firearms work and how the chemical energy in propellants is converted into the kinetic energy of the bullet, we can perform some simple estimations of the muzzle velocity of bullets, which is the v term in the expression for kinetic energy ($1/2mv^2$). The mass is the mass of the bullet, which is usually expressed in grains. This is a somewhat archaic unit, but a common one in firearms; 1 g = 15.43 grains. As we will see, these types of calculations are nothing more than complex dimensional analysis and unit-conversion questions, typically anchored around the units of energy, joules or calories. We can begin with this expression:

$$CE = KE \quad \textbf{(3)}$$

or the chemical energy of the propellant is equivalent to the kinetic energy imparted to the bullet. However, we know this is not true (Figure 1) and that there are several paths and fates for the chemical energy produced. In this type of problem, we are

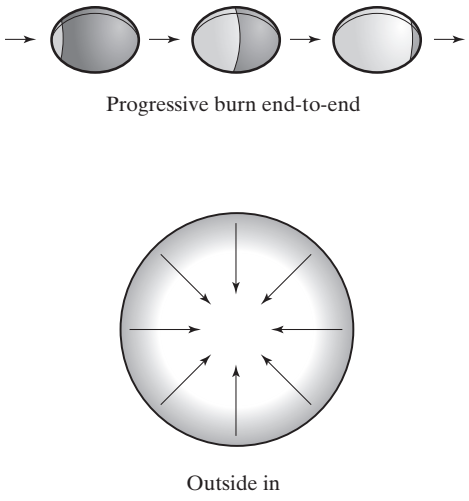


FIGURE 10 Different burning patterns in powder particles.

concerned only with the *PV* work that generates the force that imparts the kinetic energy to the projectile. Thus,

$$CE \times \text{efficiency} = KE \quad \textbf{(4)}$$

We correct the expression so that only the chemical energy that is converted to kinetic energy is taken into consideration. The available chemical energy is a function of the energy stored in the propellant (usually measured in J/g, kcal/kg, or cal/g) and the mass of the propellant in the cartridge, also typically expressed in grains. Now we can write:

$$m_{\text{propellant}} \times \text{energy}_{\text{propellant}} \times \text{efficiency} = 1/2 m_{\text{bullet}} v^2 \quad \textbf{(5)}$$

This equation can be rearranged as necessary to calculate the muzzle velocity *(v)*. This is an approximation, but a useful one. For example, a "hot" load may be sufficient to accelerate the bullet to a velocity that exceeds the speed of sound (~341 m/s or 1,126 ft/s or 770 mph) which will generate a sonic boom. It is important to realize that this process is not the same as detonation and the speed of sound discussed in that context. Here, a burning reaction (rapid as it is) generates hot expanding gases that have sufficient energy to get the bullet moving faster than the speed of sound, but the process of propellant combustion is not a detonation. If it does become a detonation, that is usually a bad situation for the person holding the weapon.

Now we can tie all these elements together, as shown in Figure 11. Prior to ignition of the primer and propellant, the pressure inside the cartridge is negligible. When

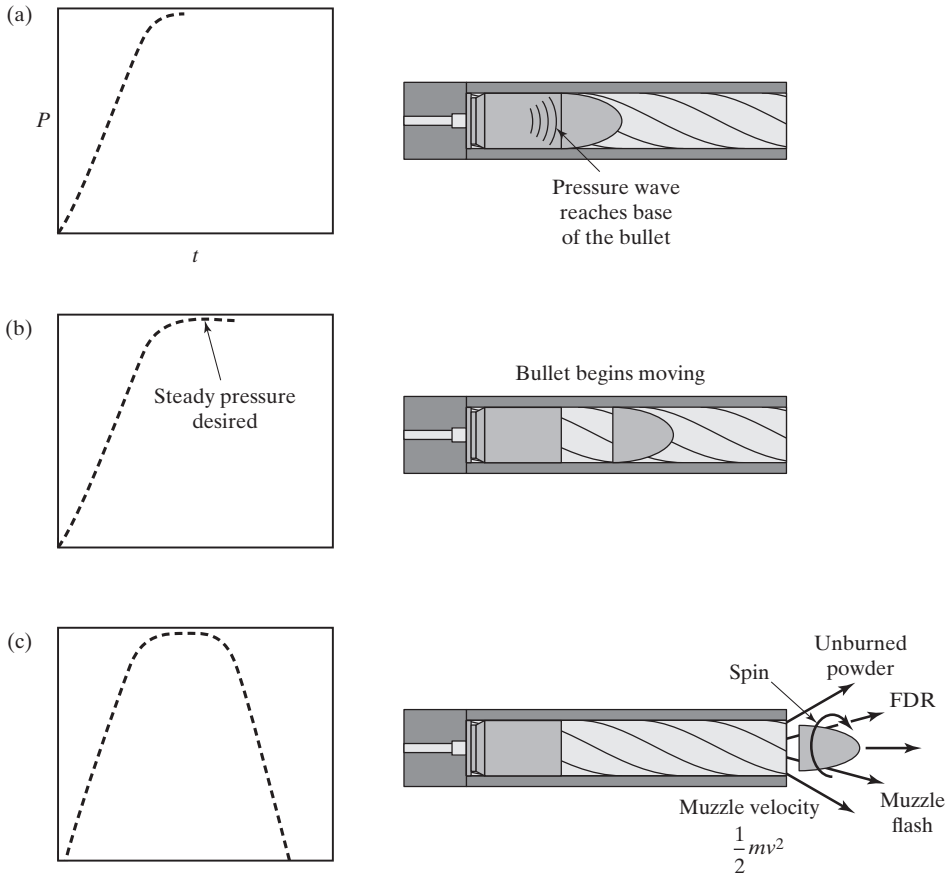
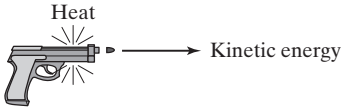


FIGURE 11 Buildup of pressure as propellant burns.

the reaction starts, pressure builds rapidly as the combustion reaction generates hot expanding gases (CO_2, H_2O, N_2, etc.) Once sufficient pressure builds and there is sufficient force to move the bullet (held in place by a crimp seal), the bullet starts to move down the barrel. As noted earlier, the speed of the burn is critical: ideally, once the bullet starts moving, sufficient gas is still being generated to sustain an even pressure until the bullet exits the muzzle. Too fast a burn will cause the pressure curve to drop before the bullet leaves the barrel, reducing the kinetic energy imparted to the bullet. If the burn is too slow, the bullet will be gone before all the propellant is consumed. Any burning that occurs once the bullet has left the muzzle is wasted energy (at least in terms of bullet velocity). Example Problem 2 shows an integrated example of firearms-related calculations. One of the practical limitations of these types of calculations is related to significant figures and equipment. Scales that are used to deliver powder have a readability of 0.1 grain at best and bullet weights are usually supplied in values such as 115 grains. How do we deal with this? Again, by realizing that these calculations are approximations by nature and being realistic about what assumptions are made and how we report the results of such calculations.

EXAMPLE PROBLEM 2

A .40 caliber pistol is loaded with a cartridge containing 3.2 grains of propellant and a bullet that weighs 100.0 grains.

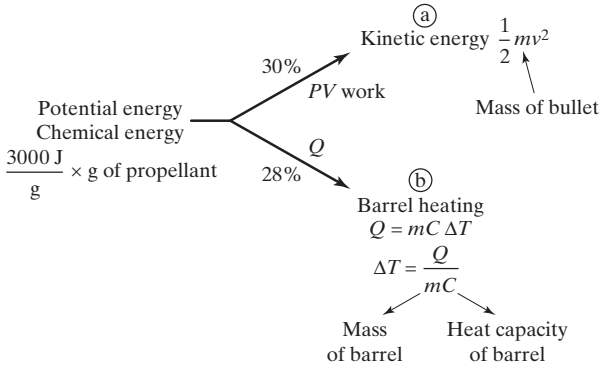


Assume that the chemical energy is converted to kinetic energy (30% efficiency) with a significant loss to heat that is absorbed by the barrel. The barrel, which is carbon steel, has a heat capacity of 0.12 kcal/kg°C, and the propellant is rated at 3000 J/g. In addition, 28% of the energy is lost to the barrel as heat.

a. Calculate the estimated muzzle velocity of the bullet.
b. Estimate how much hotter the barrel gets after one shot. Assume the barrel weighs 3 oz.

Answer:

Draw a picture to help track the potential energy as it is converted to kinetic energy (*PV* work) and heat (*Q*):



In this problem, we are not concerned with the remaining ~ 40% of the available chemical energy.

This becomes a unit-conversion problem. Because energy is in joules, this is a good place to anchor the calculation:

$$1\ \text{J} = 1\frac{\text{kg} \cdot \text{m}^2}{\text{s}^2}$$

a. available $CE \times 0.30$ will dictate muzzle velocity. Extra digits are shown here for the intermediate calculations for illustrative purposes.

$$CE = \frac{3.2\ \cancel{\text{grains}}}{\text{of propellant}} \times \frac{1\ \text{g}}{15.43\ \cancel{\text{grains}}} \times \frac{3000\ \text{J}}{\text{g}} = 622.16\ \text{J}$$

$$622.16\ \text{J} \times 0.30 = 186.65\ \text{J} = KE = \frac{1}{2}mv^2$$

(Bullet)

$$m_{\text{bullet}} = 100.0\ \cancel{\text{grains}} \times \frac{1\ \text{g}}{15.43\ \cancel{\text{grains}}} \times \frac{1\ \text{kg}}{1000\ \text{g}} = 0.00648\ \text{kg}$$

This conversion is required because $\text{J} = \frac{\text{kg}\cdot\text{m}^2}{\text{s}^2}$.

$$\frac{1}{2} \times 0.00648\ \text{kg} \times v^2 = 186.65\ \text{J} = 186.65\ \frac{\text{kg}\cdot\text{m}^2}{\text{s}^2}$$

$$v^2 = \frac{186.65\ \frac{\text{kg}\cdot\text{m}^2}{\text{s}^2} \times 2}{0.00648\ \text{kg}}$$

$$v = 240.0\ \text{m/s}$$

A reasonable way to report this answer would be to say that the muzzle velocity is approximately 240 m/s. This accounts for the significant figure issues described above.

b. From part a, we know that the total energy available is 622.16 J and that 28% is lost as heat to the barrel:

$$622.16\ \text{J} \times 0.28 = 174.21\ \text{J}$$

This value is Q in $Q = mC\,\Delta T$.

$$\frac{174.21\ \text{J}}{m_{\text{barrel}}\ C_{\text{barrel}}} = \Delta T$$

Again, convert units to reconcile kcal and J.

$$13.3\ \text{oz} \times \frac{28.35\ \text{g}}{\text{oz}} = 377.1\ \text{g} \qquad \frac{0.12\ \text{kcal}}{\text{kg}\cdot^\circ\text{C}}$$

$$\Delta T = \frac{174.21\ \cancel{\text{J}}}{(377.1\ \text{g})\left(\frac{0.5021\ \cancel{\text{J}}}{\cancel{\text{g}}\cdot^\circ\text{C}}\right)} \qquad 0.12\ \text{kcal} \times \frac{4.184\ \text{kJ}}{\text{kcal}}$$

$$= \frac{0.5021\ \text{kJ}}{\text{kg}\bullet^\circ\text{C}} = \frac{\text{J}}{\text{g}\bullet^\circ\text{C}}$$

$$\Delta T = 0.9\ ^\circ\text{C}$$

The barrel temperature increases by about 1 degree.

3 FORENSIC CHEMICAL ANALYSIS OF FIREARMS EVIDENCE

3.1 Definitions

Several terms are used to describe evidence created when a firearm is discharged.[2] Inclusive terms for this material are **firearm discharge residue (FDR)** and **cartridge discharge residue (CDR)**, which can further be divided into organic and inorganic categories. Not surprisingly, the organic portion of FDR is referred to as organic FDR or, in some

contexts, **organic gunshot residue (OGSR**). The organic residues consist of pieces of unburned powder, components of propellant such as given in Table 1, and combustion by-products. To date, the organic constituents have received less attention than the inorganic constituents, but this situation appears to be changing.

Within the inorganic residue category are residues created from the primer and from the propellant, cartridge case, and bullet. **Gunshot residue (GSR)** strictly defined refers to the particulates containing barium, antimony, and lead originating from the primer. As we will see, there are other metals and metal components, but these three are the focus. Chemical analysis of GSR has focused almost exclusively on GSR, but as we will see, there are difficulties associated with this methodology, and anecdotal reports suggests that this type of evidence is being used less than in the past.

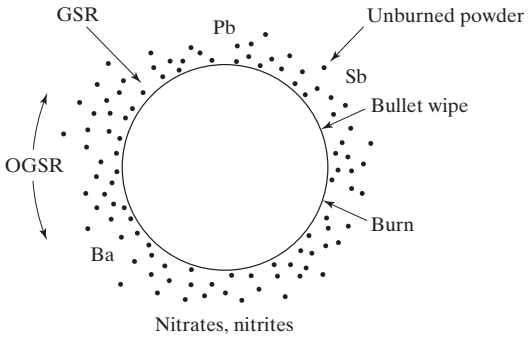


FIGURE 12 The different types of physical evidence produced by firing a gun into a target at relatively close range.

When a gun is fired and a bullet strikes and passes through a target, several forms of evidence are transferred, as shown in Figure 12. If the muzzle is extremely close to or touching the target, burn patterns may be evident. A dark ring called *bullet wipe* is created by lubricants and other foreign matter transferred from the bullet to the target as the bullet enters. Grains of unburned or partially burned propellant can adhere to the target as well, as can organic and inorganic combustion products and by-products.

As seen in Figure 13, GSR travels a significant distance from the muzzle of the gun, but a distance that is still measured in inches. In general, GSR is expected to be found when the muzzle is within a foot or so of the target. Beyond about 18", the amount of GSR transferred to a target falls dramatically to undetectable levels. Variables of importance include the caliber of the weapon, the ammunition, and wind conditions. Figure 13

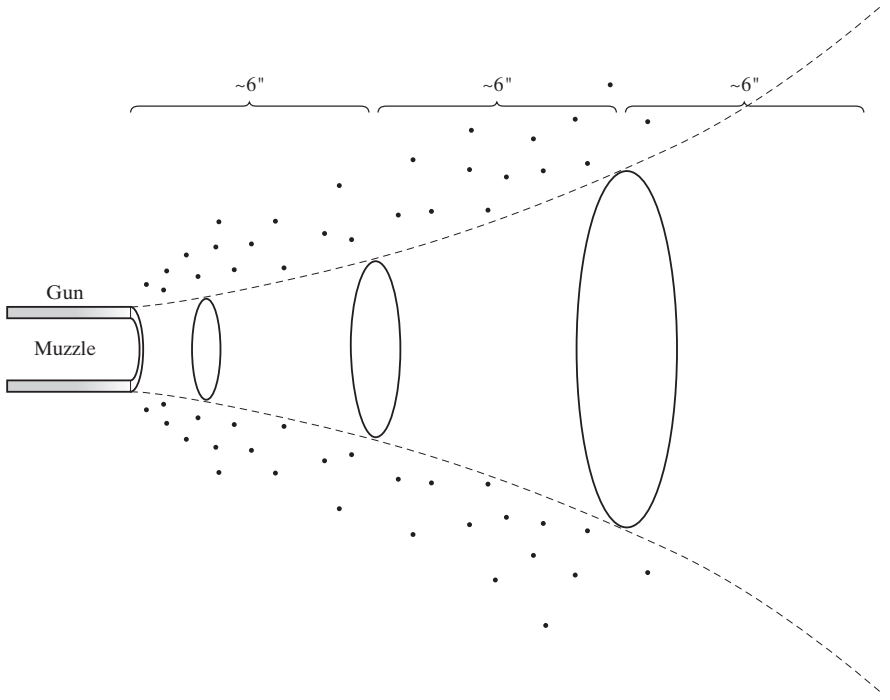


FIGURE 13 The cone-shaped dispersal of GSR as a function of distance from the muzzle.

illustrates how GSR and other products of the firing event propagate outward in a cone-shaped zone away from the muzzle of the gun. Particulates settle out of the plume based on their morphology and weight. When larger particles of unburned powder or other residue are found associated with a bullet hole, they indicate a short distance between the muzzle and the target. Estimating the distance from the muzzle to the target **(distance determinations)** is an important aspect of firearms evidence, and it is accomplished using visualizing agents (i.e., color tests).

EXHIBIT C

Guns

It is not surprising that the culture credited with inventing gunpowder engineered the first guns. The Chinese were utilizing gunpowder to hurl projectiles at their enemies. Their original designs, called fire lances, were used as crude flamethrowers, with small projectiles added in. Later versions were optimized into what could be called crude shotguns. One version was called "Nine-Arrow Heart-Piercing Magic Poison Thunderous Fire Erupter."

Source: Kelly, J., "Fire Drug." Chap. 1 in *Gunpowder: Alchemy, Bombards, and Pyrotechnics: The History of the Explosive That Changed the World.* New York: Basic Books, 2004.

3.2 Color Tests and Visualizing Agents

Although no longer frequently employed, analyses targeting anions (nitrates and nitrites) were once routinely used as tests for GSR. Nitrates are found in the oxidizers of fuels and propellants and are easily detected with the use of simple reagents. However, nitrates are nearly ubiquitous, being found in cosmetics, fertilizers, and numerous commercial products. Nitrites are less common in the environment. Reagents used for presumptive tests for nitrates include diphenylamine (DPA) and naphthylamine (Figures 14 and 15). The paraffin test, or dermal nitrate test, involved dipping a suspect's hand in warm wax and allowing it to set. The peeled wax was then treated with diphenylamine or similar reagents to reveal deposition patterns of nitrates. This test has largely been abandoned owing to excessive false positive rates. The **Walker test**

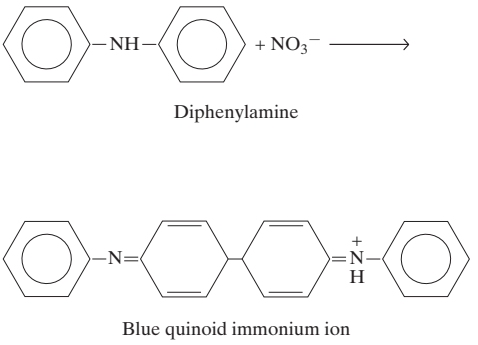


FIGURE 14 The reaction with diphenylamine and nitrate to produce a blue color.

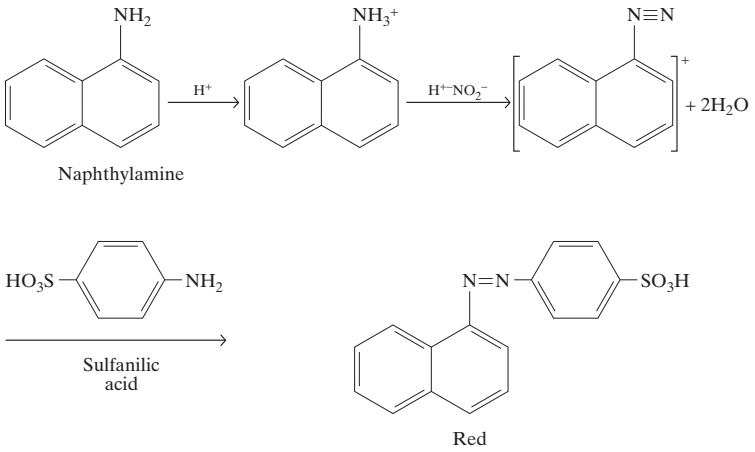


FIGURE 15 The naphthylamine test.

used in distance determinations uses naphthylamine–sulfanilic acid–impregnated photographic paper. The target surface—typically clothing—is placed in contact with the paper. An iron is used to press the fabric into the paper. The resulting reddish patterns mark nitrite deposits.

Another presumptive test for nitrates is the Griess test (or modified Griess test[5,6]). This test is widely used in many fields to detect nitrates and nitrite ions. In environmental analysis, for example, the nitrate ion is often of greatest concern, but since the Griess reagent itself responds to nitrite, elemental zinc or cadmium may be used in this context to assist in the conversion of nitrate to nitrite. The mechanism is summarized in Figure 17. The **Griess reagent** consists of sulfanilamide and naphthylamine in acidic solution, although there are many variations of the recipe. The nitrite reacts with the reagents to form the characteristic azo dye, as shown in Figure 17. Lead and barium residues are detected using sodium rhodizonate (Figure 18). Concerns about lead toxicity have led to the introduction of lead-free primers, making the rhodizonate test less useful when such ammunition is employed. An alternative is rubeanic acid, which will react with copper.[7] The copper typically originates from the cartridge casing, bullet, or primer casing, but not from the chemical compounds in the primer (Figure 19).

In distance determination, these reagents can be used to visualize the nitrites, metals, or other compounds on garments or other types of evidence through which a shot has been fired, and the distance from weapon to target is an issue. Most commonly used for distance estimations are the Walker and Griess tests. An example is shown in the color insert.

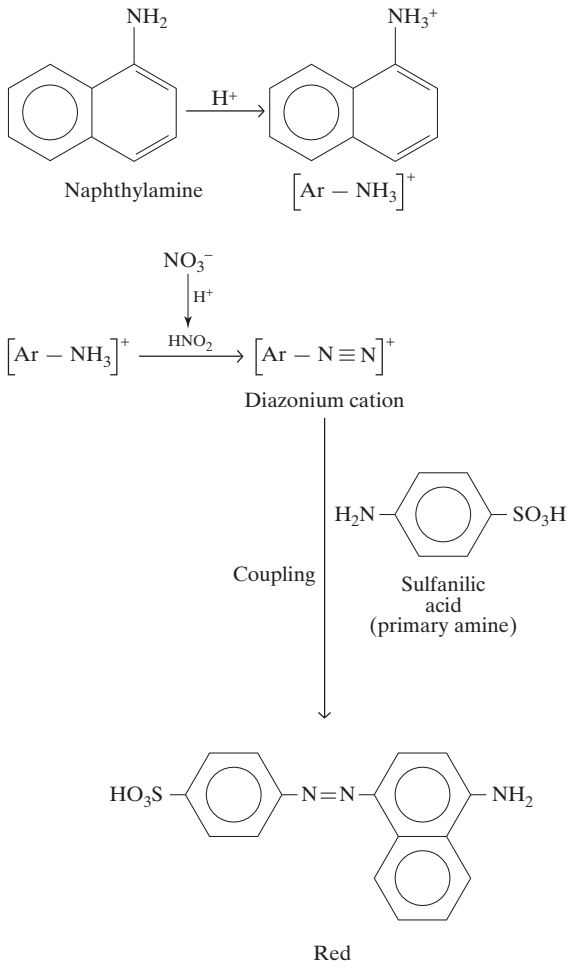


FIGURE 16 The Walker test.

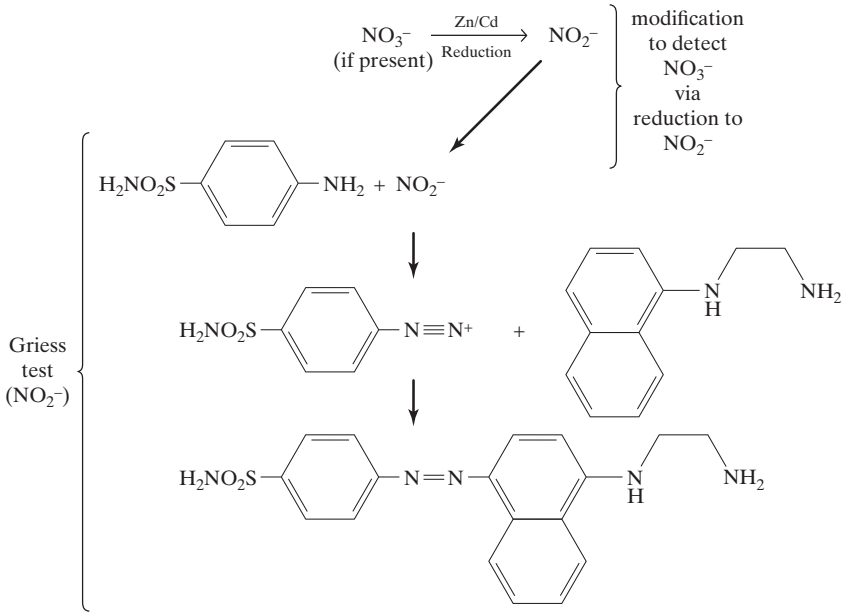


FIGURE 17 The Griess test and variants. The Griess reagent reacts with nitrites, but the test can be modified to react with nitrates if these are first converted to nitrites via a reduction, typically with elemental zinc or cadmium.

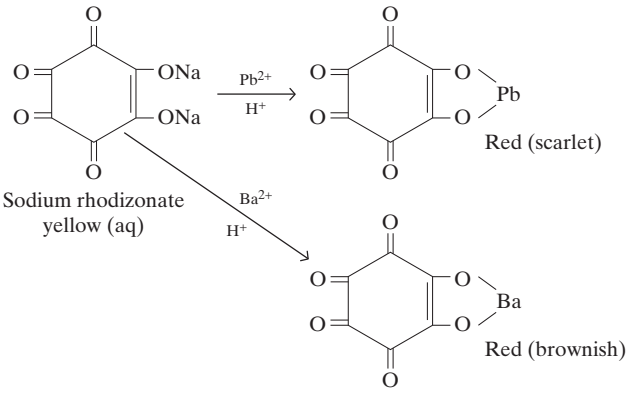


FIGURE 18 The sodium rhodizonate test. An example is shown in the color insert.

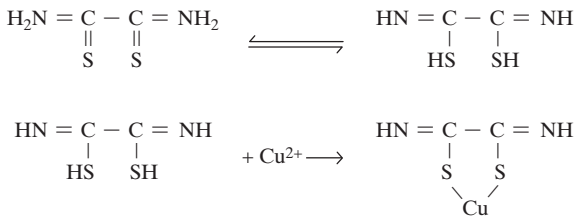


FIGURE 19 Rubeanic acid and its reaction with copper.

3.3 GSR

The compounds that definitively identify material as GSR are particulates containing lead, antimony, and barium. The morphology of the particulates is also important. These distinctive particles are not simply unburned residues of the primer, but particulates that form under the unique combustive and explosive environment created when the primer is detonated by the impact of a firing pin.[8,9] As the primer detonates or deflagrates, gases are released into a hot, oxidizing, and high-pressure environment. Elemental metals are vaporized and condense with cooling, resulting in particulates ranging in size from about 10 to 100 μm. Many, but not all, of these are spherical. Just as occurs in solution, smaller particles can aggregate to form larger ones that will appear lumpy and irregular. The key feature is that these particles are condensates, a fact that defines their morphology.[9] Currently, particulates containing Pb–Sb–Ba are considered characteristic of GSR,[2,8,10,11] as are those containing Sb and Ba together.[10] Other combinations are considered to be consistent with GSR, but not uniquely so; these combinations include elements such as Ca, S, and Si.

Methods of analysis for GSR have evolved along with the instrumentation available for elemental analysis. Prior to the advent of scanning electron microscopy–energy-dispersive X-ray (SEM–EDX) techniques, (flame and graphite furnace) atomic absorption was the principal analytical technique employed. Suspected GSR was collected

EXHIBIT D

Bullet Lead

GSR is the most common subject of chemical analysis applied to firearms, but it is not the only one. Bullets can be characterized with inorganic analysis methods such as inductively coupled plasma mass spectroscopy (ICP-MS). As with many other characterization protocols described in this text, one aspect of interest is comparing surface characterization with bulk characterization. For example, a paint chip can be studied layer by layer, or the whole chip can be analyzed by means of pyrolysis GC. Both approaches have advantages and disadvantages, strengths and limitations. A recent report noted work using laser ablation ICP-MS applied to bullets for an elemental characterization, with the goal of categorizing bullets more effectively on the basis of their elemental profiles.

Recent applications of bullet profiling have been controversial. Forensic chemists have employed elemental data to link bullets to boxes of ammunition and to original batches of

smelted lead. The controversy swirls around an issue we have seen and will see again in this text: the characterization of inter- and intrasample variation in mass-produced items. Bullets are such items; in the second article cited in the sources, it was noted that about 100,000 pounds of lead are used to make millions of bullets. The lead contains many minor and trace constituents that could conceivably be used to characterize each batch of bullets. However, to date, little data is available concerning normal expected variations in these trace and minor constituents in bullets. These types of data are obtained by repetitive measurements with validated methods that yield defensible mean values as well as the critical uncertainties. Knowing the range of expected values (the uncertainty) is essential for comparison of data sets. Without this foundational database, the utility of bullet analysis will remain problematic.

Sources: Smith, W. D. "Controversy Over Forensic ICPMS Method." *Analytical Chemistry* 74, no. 15 (2002): 411A, Randich, E. and Grant, P.M. "Proper Assessment of the JFK Assassination Bullet Lead Evidence from Metallurgical and Statistical Perspectives." *Journal of Forensic Sciences* 51, no. 4 (2006): 717–28.

with the use of wipes or swabs moistened with 1% nitric acid, and the residue collected was introduced into the instrument. Less frequently used were neutron activation analysis (NAA), anodic stripping voltammetry, and photoluminescence techniques.[11] ICP methods (AES and MS) appear, with ICP-MS methods receiving the most attention.[2]

Given the importance of particulate morphology, it is no surprise that SEM–EDX is the preferred method for GSR analysis. The disadvantages of the technique are high cost and limited availability. SEM facilitates examination of the particulates under high magnification, whereas EDX allows for elemental analysis. ASTM has published and recently reapproved a GSR standard (*E 1588*) for SEM–EDX. As shown in Figure 20, the morphology of condensed, mostly spherical, particulates is distinctive under SEM imaging conditions. Samples for SEM can be collected using tape or other adhesives. Advances in SEM techniques in recent years were the advent of automated GSR particulate searches and research into elemental ratios to further classify ammunition.[12,13] However, given the dearth of components in GSR particles and the relative scarcity of instrumentation, improved discrimination of FDR is likely to come from the organic components that are present.

3.4 Organic Analysis

Organic compounds are found in all smokeless powders and are used as fuel, as deterrents to control burn rate, and as ingredients to control consistency. A number of these compounds were listed in Table 1. The last decade witnessed increasing interest in the characterization of OGSR. Many of the same techniques for explosives are used for propellant analysis, including gas chromatography with various detectors, IMS, HPLC, capillary electrophoresis, and, recently, tandem MS methods ($LCMS^n$). We will discuss just a few of these.

Several detectors have potential applicability to OGSR; MS, not surprisingly dominates, but applications using TEA and nitrogen–phosphorus detectors have also been noted. Unlike TNT and other similar explosives, organic constituents of propellants (and even primers) are not as prone to thermal degradation in the injector port; as a result, GC-MS is useful

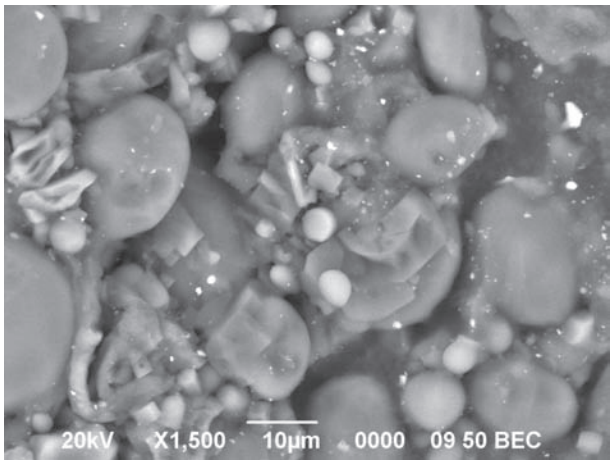


FIGURE 20 The small, bright, smooth particles are typical of GSR.

in OGSR analysis. Recently, GC-MS and GC with a nitrogen–phosphorus detector have been coupled with solid-phase microextraction[14,15] to detect various components of propellant, and GC-TEA has been used to characterize propellants.[16] GC has also been used in conjunction with FTIR spectroscopy for distance determination estimations. HPLC using UV detection has long been used in explosives analysis, and many of the methods reported work well for components of propellants such as the dinitrotoluenes. Capillary electrophoresis (CE) has been used to characterize propellants, including the ionic constituents,[17-19] as has IMS.[20] Finally, as in explosives, drugs, and toxicology, significant strides are being made in the application of $LCMS^n$ to the analysis of propellants.[21,22] LC–triple quadrupole mass spectrometry has been used to detect the energetics in propellants (negative-ion mode) and stabilizers in positive-ion mode. It is likely that MS^n methods will find much greater application in OGSR analysis as methods are developed and the instruments become more commonplace in forensic laboratories.[23]

4 SERIAL NUMBER RESTORATION

We conclude this chapter with a discussion of a topic that might at first seem unrelated—the restoration of serial numbers. It is included here for two reasons; first, it is not uncommon for a gun submitted as evidence to have had the serial number filed off to prevent tracing the weapon. Second, the process of serial number restoration exploits oxidation–reduction chemistry and electrochemical reactions in a simple yet highly effective restoration process.[24-30]

Recall from introductory chemistry the concept of paired redox reactions. We will approach these types of reactions from the perspective of loss and gain of electrons. Oxidation is defined as a loss of electrons, and reduction as a gain of electrons. Because electrons are transferred, we can also treat these reactions using the basic tenets of electrochemistry (voltaic cells, batteries, etc.). We can use standard half-cell potentials ($E°$) values to predict which reactions will be spontaneous. In serial number restoration, acids and other reagents are used to selectively dissolve metals such as steel; thus, with a bit of knowledge regarding the reagents, metal, reduction potentials, and how the serial number was created, it is possible to understand how the restoration process works.

Serial numbers can be applied to metal in a number of ways, including etching and stamping. When a serial number is stamped into metal (Figure 21), pressure is applied to the stamp, creating an indentation in the metal. A closer look at the metal shows that the structure is disrupted where the stamping occurred. The existence of the damaged region is exploited in serial number restoration methods, as shown in Figure 22.

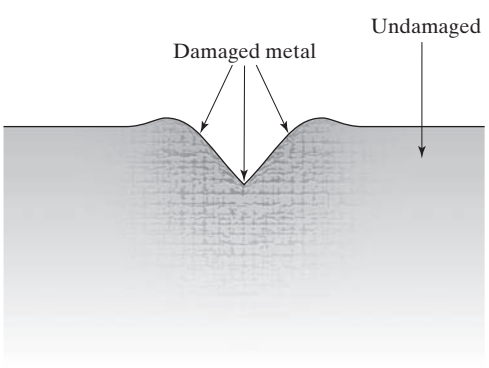


FIGURE 21 Damaged metal under a stamped serial number.

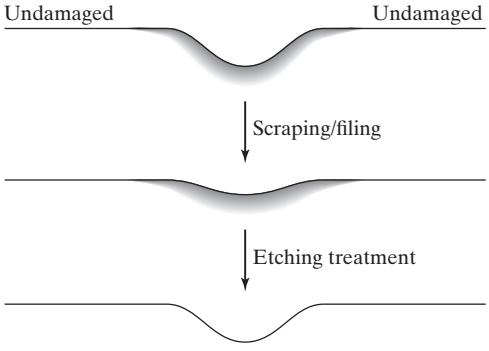


FIGURE 22 Process of etching to recover a serial number. The damaged metal is etched more rapidly than the undamaged metal.

Here, the serial number has been filed away so that the surface appears clear, but unless the filing is taken to a sufficient depth, regions of damaged metal remain even if the serial number itself is not visible. The damaged areas are more susceptible to acid and chemical etching. If the etching is conducted carefully, it is often possible to selectively dissolve the damaged area away, leaving some portion of the original serial number visible.

A simple example is shown in Figure 23. In this case, the metal surface is zinc, and the reagent selected is a hydrochloric acid solution. The metal will dissolve (oxidation) according to the half-reaction $Zn \rightarrow Zn^{2+} + 2e^-$ if an electron acceptor is available (reduction) and if the coupled redox is spontaneous. The only available electron acceptor is H^+, so if the coupling results in a positive cell potential, we have good evidence that this pairing is feasible. From Table 2, in which the reactions are listed as reductions, we see that we can use hydrogen as a reference. Any metal below hydrogen will be oxidized preferentially to the hydrogen and in this example, zinc is below hydrogen. We can also calculate the cell potential; if the resulting potential is positive, the reaction is spontaneous, although we must always keep in mind the assumptions of standard half-cell potentials: the relative concentrations of components must be taken into consideration, using the Nernst equation, but that discussion is beyond the scope of our goals here.

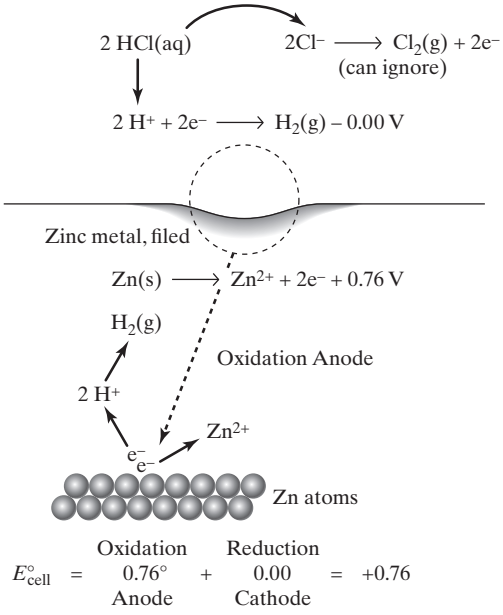


FIGURE 23 Detailed view of zinc metal dissolving in acid. The electron exchange occurs at the surface.

In the example in Figure 23, the reduction of hydrogen, being listed higher in Table 2 will go as written, and the zinc reaction will go in the reverse direction, which means we must also reverse the sign of its half-cell potential. When we add the two half-cell values, a positive result is obtained, and we can predict with confidence that we can use HCl to dissolve zinc. However, we cannot use this acid to dissolve copper, because it is located above the hydrogen reduction. Similar methods are used to design other etching reagents. The metal or alloy metals are always the substance that must undergo oxidation (loss of electrons) for this type of restoration to work. Depending on where that metal falls in the electrochemical activity series, selecting an electron acceptor is straightforward. The caveat is that with alloys, more than one reaction is possible and must be taken into consideration.

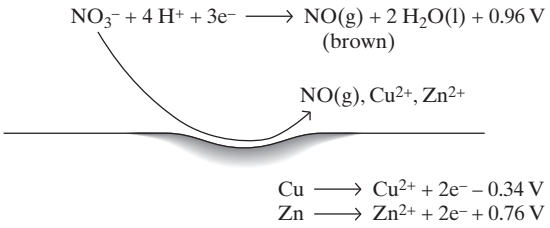
TABLE 2 Selected Standard Half-Cell Potentials

E°, volts (as reduction)	Half-Reaction (all species aqueous unless otherwise noted)
+1.36	$Cl_2(g) + 2e^- \rightarrow 2Cl^-$
+0.96	$NO_3^- + 4H^+ + 3e^- \rightarrow NO(g) + 2H_2O$
+0.77	$Fe^{3+} + e^- \rightarrow Fe^{2+}$
+0.34	$Cu^{2+} + 2e^- \rightarrow Cu(s)$
0	$2H^+ + 2e^- \rightarrow H_2(g)$
−0.28	$Ni^{2+} + 2e^- \rightarrow Ni(s)$
−0.44	$Fe^{2+} + 2e^- \rightarrow Fe(s)$
−0.76	$Zn^{2+} + 2e^- \rightarrow Zn(s)$

EXAMPLE PROBLEM 3

Suppose you have a brass surface (~67% Cu(s), 33% Zn(s)) from which a serial number has been filed away. What would happen if this surface was treated with nitric acid?

Answer:
Use the standard reduction potentials and note the nitrate reduction:



Both metals can be dissolved this way.

Summary

This chapter concludes our discussion of combustion chemistry, although we strayed a bit at the very end into chemical etching. Firearms exploit deflagration as well as primary explosives in the primer. As a result, a wealth of forensic evidence is created including organic and inorganic residues. The tools of analysis range from the highly sophisticated (SEM and LC-MS^n) to simple color tests, a pattern that we have seen in several different contexts.

Key Terms and Concepts

Caliber
Cartridge
Cartridge discharge residue (CDR)
Center fire
Degressive-burning powders
Deterrent
Distance determinations
Double-base smokeless powder
Firearm discharge residue (FDR)
Greiss reagent
Guncotton
Gunshot residue (GSR)
Lands and grooves
Neutral-burning powders
Nitrocellulose (NC)
Organic gunshot residue (OGSR)
Progressive-burning powder
Rifled
Rimfire
Saltpeter
Single-base smokeless powder
Triple-base powder
Walker test

Questions

1. Study Table 1, noting in particular the oxygen balance figures. Predict which energetic is likely to be the largest component of propellant and explain your reasoning.
2. Based on your knowledge of how firearms work, explain in general terms how silencers work.
3. Why do so many of the presumptive color tests described in this chapter target nitrate and nitrite ions?

4. The following data was reported for bullets and bullet fragments associated with the assassination of President John F. Kennedy in 1963 (for a complete list, see the table reference). For the sake of this exercise, assume that five replicate analyses were used to generate the standard deviation.

	ppm by Weight, Mean (standard deviation)		
Fragment	Sb	Ag	Cu
A	638 (4)	8.3 (0.3)	44 (2)
B	647 (4)	7.5 (0.5)	42 (2)

Source: Randich, E., and P. M. Grant. "Proper Assessment of the JFK Assassination Bullet Lead Evidence from Metallurgical and Statistical Perspectives." *Journal of Forensic Sciences* 51, no. 4 (2006): 717–28.

Use hypothesis tests to evaluate and interpret the data in light of the forensic question, Are these fragments from the same source (inclusionary evidence) or from different sources (exclusionary evidence)?

5. Discuss the mechanism of color formation in the Walker test (Figure 16).
6. A commercial etching paste used for serial number restoration from copper consists of hydrochloric and nitric acids along with ferric chloride. Assuming the redox reaction(s) are spontaneous, describe the likely mechanism of copper metal dissolution.
7. Estimate the muzzle velocity of a .38 caliber bullet weighing 90 grains if the cartridge is loaded with 3.2 grains of propellant with an efficiency of 29% and available energy of 3000 J/g. Report your result in feet per second. Does the bullet exceed the speed of sound?

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The Chemistry of Color and Colorants

OVERVIEW AND ORIENTATION

This chapter talks about color quantitatively and analytically. Colorants (substances that impart color) of forensic interest are dyes and pigments, and they are applied to or are part of a wide range of evidence, including fibers, inks, and paint. There are numerous chemical structures in this chapter, but do not be daunted or overwhelmed. The underlying theme is color. As you look through the structures, you will note that these molecules contain features such as highly conjugated systems. This serves as a reminder that color arises from chemical structure.

1 COLOR: A QUANTITATIVE VIEW

Earlier, basic elements of color chemistry were introduced in the context of spot tests. When we move into evidence such as inks, paints, and fibers, a quantitative description of color is essential. The problem, however, is that color is a difficult concept to quantify or describe in a common language. For example, one person's perception of "red" may be different from another person's. A descriptor such as "fire-engine" red or "stop-sign" red makes it easier to imagine a color, but each person still perceives color differently. One person may be a bit more sensitive to reds and less sensitive to blues than another, so what the eye sees and what the brain registers are different and inherently impossible to describe with words. Some people are partially or completely color-blind. Color can be quantitated on the basis of spectral characteristics, removing the viewer's subjectivity from descriptions of color.[†]

[†]Note that many of the grayscale figures in this chapter are also shown in the color insert.

1.1 CIE System

Humans sense electromagnetic radiation in the range of ~380–700 nm as color. Physiologically, the sensation of color is created by the response of receptor cells in the retina of the eye. There are tens of thousands of such cells present, and they are divided into rods and cones. The rods, which are present in the greatest number, are responsible for night and peripheral vision. The cones, which require more intense light for activation, provide color sensing. There are three types of cone cells, each responding to a different one of the primary colors—red, green, and blue (**RGB**), each with a different range of sensitivities (Figure 1). The number, location, and mix of cones varies among individuals and, as a result, so does color perception. In forensic applications, this subjectivity is problematic.

Many systems and techniques have been used to describe color in objective quantitative terms. We will focus on one commonly used in forensic science, one developed by the **Commission Internationale de l'Eclairage** (**CIE**, www.cie.co.at). The CIE colorimetry system (and variants) is based on human color perception and allows for color to be expressed on a two-dimensional plot of the chromaticity coordinates (x,y). The chromaticity coordinates x and y are mathematically derived from a reflectance spectrum (typically) that is expressed as a function of combinations of the primary colors. For example, the reflectance spectrum of a material that appears purple could be generated by a 50/50 mixture of red and blue, so an RGB combination for purple could be expressed as 0.50R + 0.0G + 0.50B. This is called an **additive color system** because the three primary colors are added together to match other colors. Note that this does not mean that the purple color of this material is generated by a mixture of red and blue dyes; all it means is that we can *match* this color with combinations of red and blue. The goal of the CIE algorithm is to express any color as a form of a weighted average of RGB contributions and to convert this expression into a point that can be plotted in two dimensions.

The steps required to convert a reflectance spectrum to plottable points is outlined in Figure 2. The spectrum, reported as a numerical data file of percent reflectance (%R) at each scanned wavelength is the starting point. Next, the type of illumination is selected;

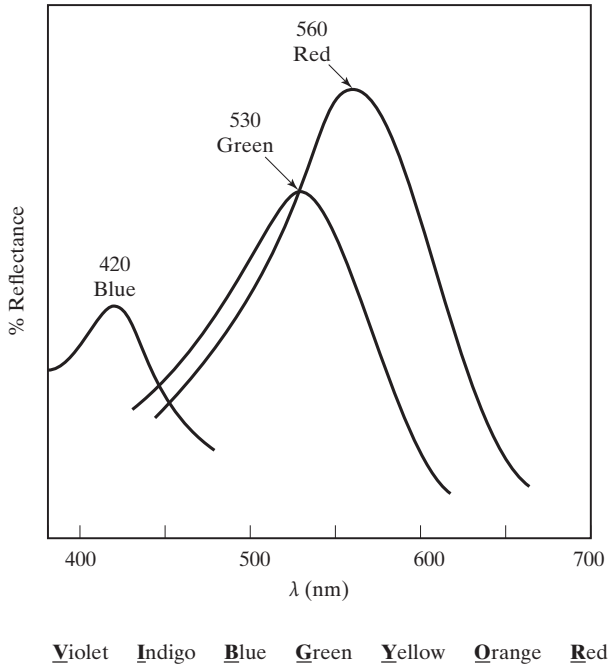


FIGURE 1 The approximate sensitivity ranges of the three types of cone receptors in the retina.

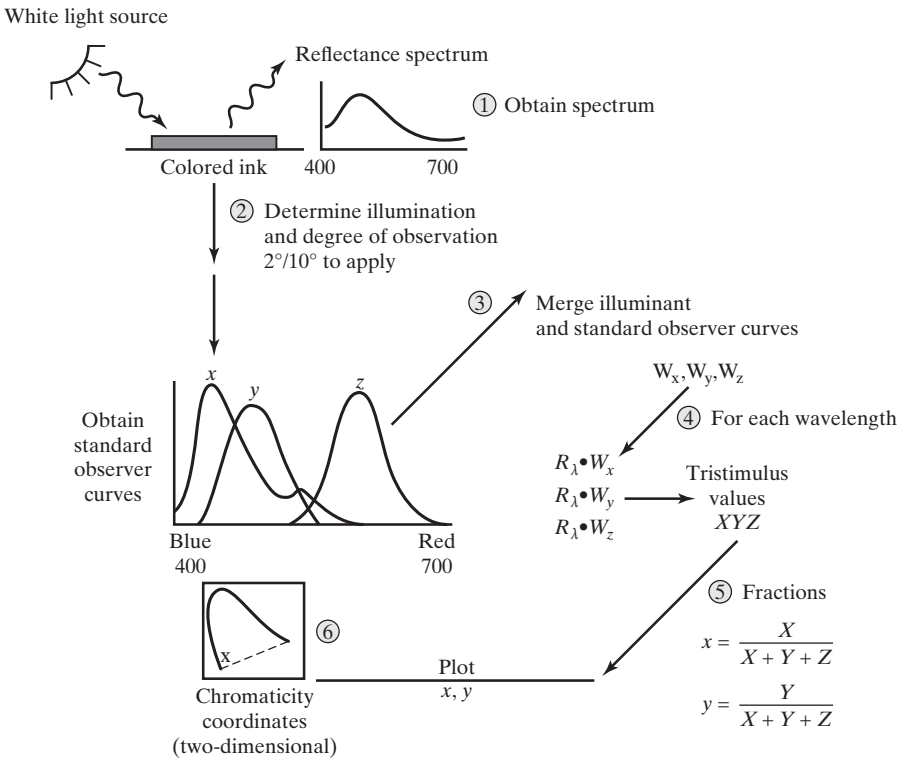


FIGURE 2 The steps involved in converting a reflectance spectrum into two plottable points using the CIE methods discussed in the text.

this is necessary because color perception depends on the lighting type; the color of an object outdoors at noon in daylight is perceived differently than if the object is indoors under low lighting conditions. The angle of observation is also selected, 2° or 10°. With these criteria selected, the next step is to obtain the appropriate CIE tables. Currently two versions are being used, data from 1931 and 1964. Within these two categories, the tables are organized as values applied to a wavelength, in different wavelength increments, such as one reflectance value for every wavelength, one for every 10 nm, and one for every 20 nm. If the CIE tables used provide data from 400 to 700 nm in increments of 5 nm, then the reflectance spectra must cover this range of wavelengths in 5-nm increments. We will see why when we work through an example (Example Problem 1). It is worth noting that in forensic application the choice of conditions is usually not as critical as the consistent use of those conditions, since the data are typically used to compare colors. For example, if there is a known blue ink sample (*K*) to be compared with a questioned ink sample (*Q*), the conditions under which the reflectance spectra are obtained and processed must be identical. This includes the selection of the illumination source tables.

One method of standardizing illumination is to consider it relative to the radiation released by an object called a **blackbody radiator**. Blackbody radiation correlates with the spectral emission profile of a perfect blackbody radiation source when it is heated to a given temperature. The term "white hot" originates here if an object such as an iron rod is heated sufficiently, it glows red, then yellow, and then, at the hottest, white to blue. When light has the same spectral spread as a blackbody emitter at a given temperature, the light is said to have that **temperature**. Here, "temperature" is a descriptor, but it does not have any physical correlate. A filament in a lightbulb is hot, but the actual temperature of the filament is not the same as the temperature of the emitted light. Some example temperatures are 1500K for candlelight, 3400K for a tungsten lamp, and 5500K for noon on a sunny day.

Recall our example with the purple color in which we noted that we could create this color by combining red and blue in the proper proportions to match it. The CIE system depends on this color matching concept. Here we could write:

$$0.50\text{R} + 0.0\text{G} + 0.50\text{B} \rightarrow \text{stimulus (purple)}$$

where the arrow is read as "matches" the stimulus that generates the perceived color purple. The CIE color matching tables provide the weighing factors necessary to match a perceived color using a weighted combination of RGB. These factors are called $x(\lambda)$, $y(\lambda)$, and $z(\lambda)$ and are provided as tables. The **tristimulus** values are calculated using these factors, information regarding the **illuminant** (E), and the reflectance value at each wavelength:

$$X = \sum E(\lambda)\, R(\lambda)\, x(\lambda) \quad \textbf{(1)}$$

$$Y = \sum E(\lambda)\, R(\lambda)\, y(\lambda) \quad \textbf{(2)}$$

$$Z = \sum E(\lambda)\, R(\lambda)\, z(\lambda) \quad \textbf{(3)}$$

Often, tables combine the illuminant E values with the color matching values, and the expressions simplify to

$$X = \sum W_x(\lambda)\, R_x(\lambda) \quad \textbf{(4)}$$

$$Y = \sum W_y(\lambda)\, R_y(\lambda) \quad \textbf{(5)}$$

$$Z = \sum W_z(\lambda)\, R_z(\lambda) \quad \textbf{(6)}$$

where W is the weighting function. An example of such a combined table is shown in Table 1 and plotted in Figure 3. The final step is to reduce the XYZ data points to the chromaticity coordinates x and y:

$$x = \frac{X}{X + Y + Z} \quad \textbf{(7)}$$

$$y = \frac{Y}{X + Y + Z} \quad \textbf{(8)}$$

$$z = \frac{Z}{X + Y + Z} \quad \textbf{(9)}$$

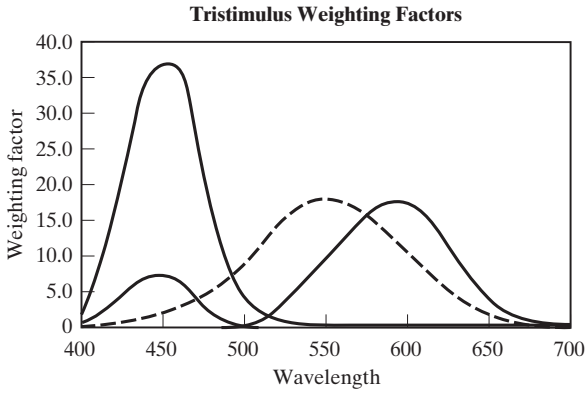


FIGURE 3 Weighting factors for each standard observer curve.

TABLE 1 Tristimulus Weighting Factors (Normalized to $W_y = 100$)

Wavelength (λ)	$X(\lambda)$	$Y(\lambda)$	$Z(\lambda)$
400	0.019	0.002	0.086
420	0.204	0.021	0.973
440	0.384	0.062	1.967
460	0.302	0.128	1.745
480	0.081	0.254	0.772
500	0.004	0.461	0.219
520	0.118	0.762	0.061
540	0.377	0.962	0.014
560	0.705	0.997	0.000
580	1.014	0.869	0.000
600	1.124	0.658	0.000
620	0.856	0.398	0.000
640	0.432	0.180	0.000
660	0.153	0.060	0.000
680	0.041	0.016	0.000
700	0.010	0.004	0.000
720	0.002	0.001	0.000
740	0.001	0.000	0.000
760	0.000	0.000	0.000
780	0.000	0.000	0.000
SUM:	5.823	5.834	5.836

Adapted from: International Commission on Illumination (CIE) Downloads, "Selected Colorimetric Tables, www.cie.co.at, last accessed Nov 2011. This is a free Excel file that includes data on illuminants and standard observed curves.

Because $x + y + z = 1$, there are only two degrees of freedom, leading to a two-dimensional plot of x and y on a **chromaticity diagram** (Figure 4).

The choice of illuminants and degree of observation depends on application. For example, a retailer interested in how clothing appears under indoor lighting would use a different set of table values than someone interested in how paint appears outdoors. In forensic applications, colors such as a known and questioned ink (Q versus K)are usually compared. In these cases, the most important point is to use the same conditions and tables for all samples.

The chromaticity diagram (Figure 4) conveys significant information about a color in a concise and easily interpretable way. The chromaticity coordinates of a color (x,y) describe the color but do not distinguish light and darkness of that color. A dark red and light red will have identical chromaticity coordinates, as long as the base red hue is the same. The more saturated a color, the closer to the edge of the parabola it will fall, whereas paler colors will plot more to the interior. The depth of color (light versus dark) is not the same as the **saturation**. One way to think of the concept is in terms of applying watercolors to a piece of paper. If you start with a tube of color and apply one layer, it may appear light, but application of subsequent layers of the same color from the same tube will make the color darker. The **hue** does not change, since the same tube of paint is used, but the color becomes darker with each application, increasing the saturation.

When the illuminant source is taken into consideration, even more information can be extracted. Figure 5 is the same as Figure 4 with the addition of the incandescence curve superimposed over the chromaticity chart. The curve shows the temperature of the illuminant and a few of the available CIE standard illuminants such as D65 (daylight 6500K equivalent). The white point W is also called *achromatic*, since it consists of equal amounts of RGB and is perceived as white. The incandescence curve and the chromaticity coordinates, together provide additional information and descriptors of a color.

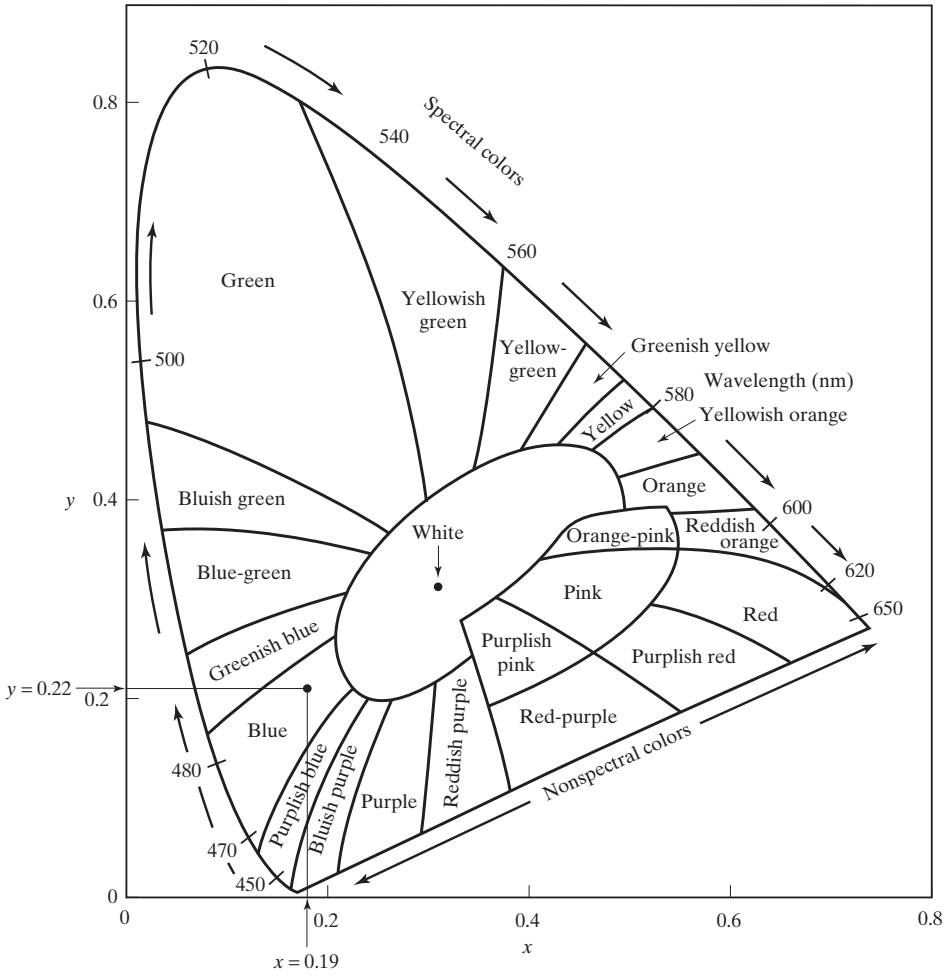


FIGURE 4 Chromaticity diagram or chart. Color as encoded in a VIS spectrum is described by plotting the chromaticity coordinates x and y on the chart. The points that are plotted are from Example Problem 1. The outer band contains spectral colors (ROYGBIV), and the connecting line consists of nonspectral colors, such as pink and purple. A colored version is found in the color insert.

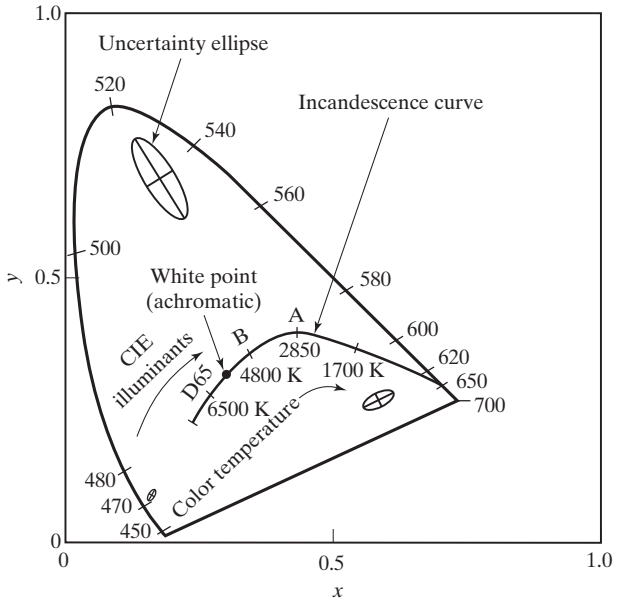
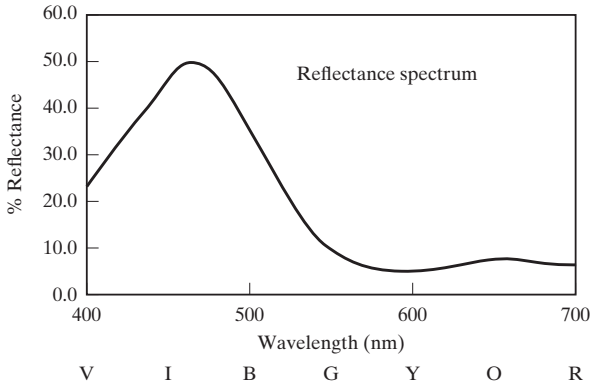


FIGURE 5 The chromaticity chart with the incandescence curve added. The Kelvin temperature of the illuminants is given. D65, B, and A are standard CIE illuminants.

EXAMPLE PROBLEM 1

Calculate the tristimulus values for the following spectrum based on a 10° observer angle. Determine the chromaticity coordinates. What color is the sample?



Answer:

We know from the spectrum that this color will be perceived as blue because the majority of the reflected light is blue. We will use this knowledge as a check on the calculations. The data used to plot the spectrum must be available as %*R* at each wavelength. We also need a table of **color matching functions** and illuminants to carry out the necessary calculations. We will use Table 1 values here and sum the corrected value over all wavelengths. The final step is to calculate the chromaticity coordinates using equations 7–9. Note that this calculation is best done as a spreadsheet and this approach was used here. Significant figure issues are addressed at the end of the calculation.

Calculations:

Wavelength	%Reflectance	$X(\lambda)$	$Y(\lambda)$	$Z(\lambda)$	W_x(%R)	W_y(%R)	W_z(%R)
400	23.3	0.019	0.002	0.086	0.45	0.05	2.00
420	33.0	0.204	0.021	0.973	6.75	0.71	32.09
440	41.7	0.384	0.062	1.967	16.00	2.59	82.04
460	50.0	0.302	0.128	1.745	15.11	6.41	87.27
480	47.2	0.081	0.254	0.772	3.80	11.97	36.44
500	36.5	0.004	0.461	0.219	0.14	16.82	7.98
520	24.0	0.118	0.762	0.061	2.83	18.28	1.46
540	13.5	0.377	0.962	0.014	5.09	12.99	0.18
560	7.9	0.705	0.997	0.000	5.57	7.88	0.00
580	6.0	1.014	0.869	0.000	6.08	5.21	0.00
600	5.5	1.124	0.658	0.000	6.18	3.62	0.00
620	6.0	0.856	0.398	0.000	5.14	2.39	0.00
640	7.2	0.432	0.180	0.000	3.11	1.29	0.00
660	8.2	0.153	0.060	0.000	1.25	0.49	0.00
680	7.4	0.041	0.016	0.000	0.30	0.12	0.00
700	7.0	0.010	0.004	0.000	0.07	0.03	0.00
				Sums:	**77.86**	**90.84**	**249.46**

The total $X + Y + Z$ is 418.2 and the chromaticity coordinates of x, y, and z are:
$x = 77.86/418.2 = 0.19$
$y = 90.84/418.2 = 0.22$
$z = 249.46/418.2 = 0.60$
Note that the sum $x + y + z = 0.19 + 0.22 + 0.60 = 1.0$, as expected. The two points to be plotted are $x = 0.19$ and $y = 0.22$. This point is plotted on Figure 4; note that it falls exactly where we predicted based on the spectrum, in the blue range.

EXHIBIT A

Human Color Vision and the Tristimulus System

The tristimulus system is based on the mechanism by which humans perceive color. The eye contains receptor cells called rods and cones. The rods respond to all wavelengths of light and are quite sensitive and so are utilized in night vision and peripheral vision. They are not sensitive to different colors, so if a person had only rods, he or she would see the world in shades of black, white, and gray. The cones are far less sensitive but do respond to color via the reaction of light with pigments. The cones contain three different light-sensitive pigment complexes that correlate with the RGB of the tristimulus system. Although the physiology and chemistry of vision is complex, the key to color perception is a simple photo-induced change in pigment molecules from the cis to the trans form. The so-called blue cones respond optimally to a wavelength of ~420 nm, the green cones to ~530 nm, and the red cones to ~560 nm (Figure 1). When we perceive a mixed color such as orange, both the red and green cones are stimulated. The degree to which each type of cone is stimulated determines the shade of color we see, such as yellow (little green contribution) to deep orange (strong green contribution). If all three types of cones are stimulated equally, we see white.

Source: Marieb, E. N, "The Special Senses." Chap. 15 in *Human Anatomy & Physiology*, 6th ed. Upper Saddle River, NJ: Pearson Benjamin Cummings, 2004, 554–602.

As useful as the chromaticity diagrams are, they have limitations. Because the chromaticity parabola is asymmetric, calculations and comparisons of color differences are not uniform. Consider, for example, two pairs of inks, one pair blue and one pair red. Suppose that the perceived color difference between the two blue inks is the same as the perceived difference between the reds. If the chromaticity diagram were uniform, then on a plot of the x- and y-values for the four samples, the Euclidean distance between the two blues would be the same as the distance between the two reds. The problem is illustrated in Figure 6. The elliptical regions are drawn around a point centered on a standard color, and the region around the point is that region in which an x, y plot of a colored sample will be perceived (by most people) as identical with the central color. The region represents the zone in which the eye cannot perceive a difference. The zones are not uniform, and distortion is introduced.

To address the distortion, a mathematical transformation is applied to the tristimulus values *XYZ*. The algorithm begins with considering color from the perspective of how someone would describe its characteristics and then relate those characteristics to chromaticity. Before discussing how distortion is corrected, we need to formalize terms as illustrated in Figures 7 and 8. Saturation of a color refers to how much pure spectral color it includes. The terms **shade** and saturation are sometimes used interchangably, but as shown in Figure 8, this description is technically incorrect.[1] Shade relates to hue (spectral color) and degree of black. Saturation is also called **chroma**.

To map the asymmetrical space of the chromaticity parabola into a symmetrical color space in which the regions of variability are comparable, three descriptors are used. These variables are hue, lightness, and chroma (illustrated in Figure 9):

- **Hue**: The color itself, such as red, blue, green. The hue corresponds to a color that would appear on a color wheel, in other words, the spectral colors that form the parabola of the chromaticity diagram.

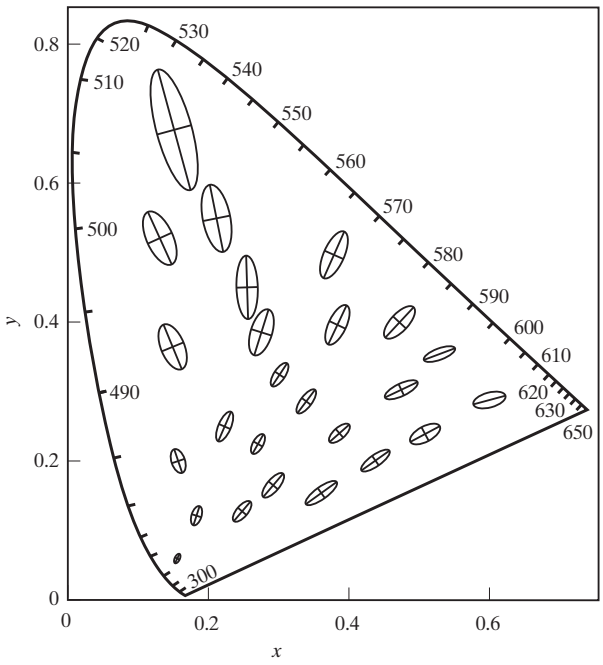


FIGURE 6 Regions of uncertainty are not uniform in the chromaticity diagram. Within any given ellipse, the color is indistinguishable. For greens, the uncertainty ellipse is large; for blues, small; and between the two extremes for the reds. The CIELAB mapping addresses this asymmetry.

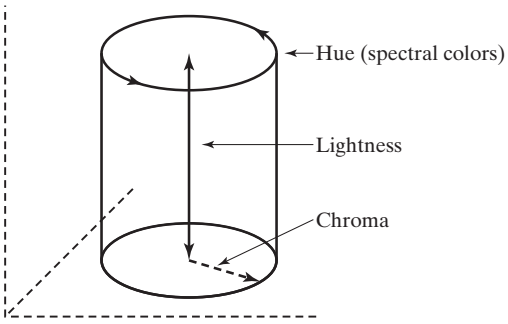


FIGURE 7 The hue-lightness-chroma color space (HSV) that underlies the CIELAB modification to tristimulus values and chromaticity coordinates.

- **Lightness**: The depth of the color, rated on a scale from darkest (black) to lightest (white). The term **value** is sometimes used in this context. A color that reflects more white light is brighter than one that reflects less.
- **Chroma**: The deviation of the color from gray. Pure spectral colors such as red and violet have high saturation. Saturation refers to the strength of the dominant wavelength or hue. Pink and red have the same hue but different saturation.

Distance between points in the **CIELAB**[†] color space (corresponding to a color difference) can be calculated with the use of a Euclidean distance. This technique uses the tristimulus values *X, Y,* and *Z* as inputs and converts these to the corresponding *L, a,* and *b* coordinates in the uniform color space. The distance between two points in the CIELAB system can be calculated as[‡]

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad \textbf{(10)}$$

[†]The terms CIEL*a*b*, CIEL *a*b and CIELAB are both used; the latter version is used in this text.

[‡]Chemists may find the use of *E* confusing in this context, because the same symbol is used to describe the energy gap in atoms and molecules. In turn, absorption by energy in the visible range occurs when the energy gap correlates with the color of the photon; thus, the two *E*'s are related to colors. Context is the best guide as to which interpretation of *E* applies.

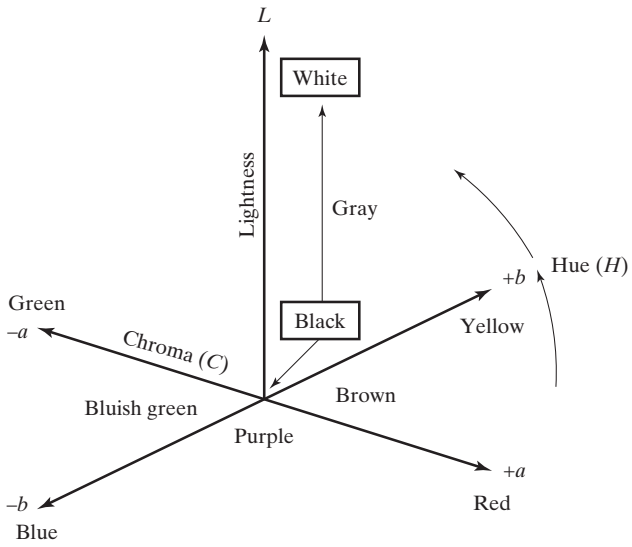


FIGURE 8 Limits and labels of the axes in CIELAB space. Color differences are expressed based on Euclidean distances. Another way to think of *a* is as measuring the "redness" of a color, with a negative value indicating less red and more green. An analogous interpretation for *b* is of "blueness."

Color difference:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

In some cases, only two of these criteria need be considered. For example, if the lightness of two colors A and B is the same, the ΔL term is zero and is not needed to calculate distances. This concept is illustrated in Figure 10.

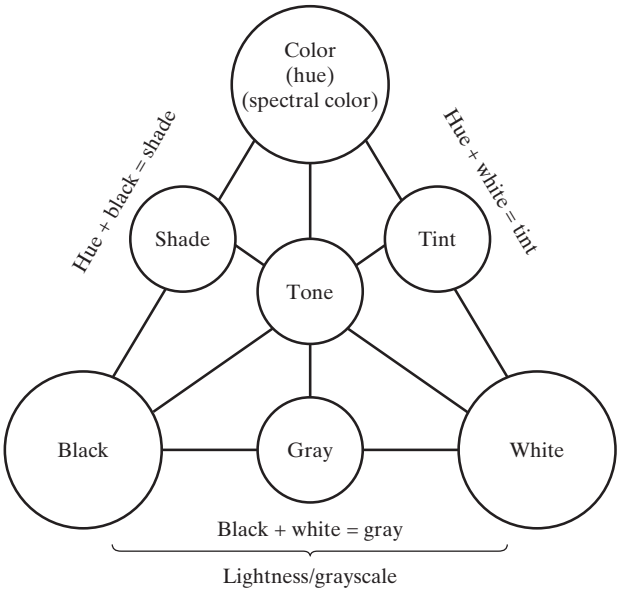


FIGURE 9 Terms used to describe color and their relationship.

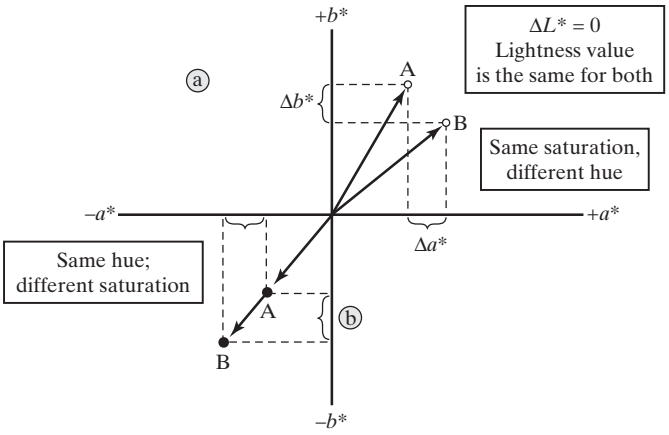


FIGURE 10 An example of two colors that have the same lightness coordinates but different values of hue (A) and saturation (B).

The transformations used to convert tristimulus values to the CIELAB equivalent are as follows:

$$L^* = 116\left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} - 16 \qquad \textbf{(11)}^{\dagger}$$

$$a^* = 500\left[\left(\frac{X}{X_n}\right)^{\frac{1}{3}} - \left(\frac{Y}{Y_n}\right)^{\frac{1}{3}}\right] \qquad \textbf{(12)}$$

$$b^* = 200\left[\left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} - \left(\frac{Z}{Z_n}\right)^{\frac{1}{3}}\right] \qquad \textbf{(13)}$$

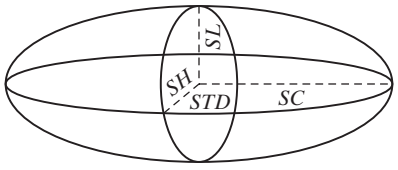


FIGURE 11 Elliptical region around a point plotted in CIELAB space. The region defines the uncertainty or tolerance; an observer would perceive no difference in color among spectra that plot within the ellipsoidal region.

The values X_n, Y_n, and Z_n are a reference white color and vary with the illumination selected. This color space also has some inherent distortion,[2,3] but far less than that of Figure 6. As in that figure, there is a zone around a point within which not color difference would be discerned; this is illustrated in Figure 11. Since the color space is truly three dimensional, the zone is an elliptical volume. The CIELAB system is widely used in forensic analyses to describe color and color differences.

One final and critically important consideration is uncertainty in color measurement, which here has a concrete and easily "visualized" meaning in the present context. Assume that three values are used to represent a color in the CIELAB system. The uncertainty associated with that color could be expressed as the magnitude of change in those coordinates that would cause an observer to perceive a difference in the color. Because color vision varies with the individual, there is no standard reference material that can solve this particular analytical challenge. However, it is possible to assign reasonable regions of uncertainty around a point; because the space is three dimensional, the uncertainty space is elliptical. The Colour Measurement Committee (CMC) of the Society of Dyers and Colourists developed a formula, also referred to as CMC, that constructs the elliptical area on the basis of a standard and taking into account relative differences in hue–saturation–lightness and the distortions in the color space. The calculations are beyond the scope of this discussion; however, many software packages exist, some built into spectrophotometers, that are capable of producing a variety of quantitative color measurements and outputs.

[†]Equation 12 applies to those cases where $Y/Y_n > \sim 0.01$. A slightly modified form is used for smaller values.

1.2 Munsell System

The **Munsell color system** is conceptually similar to the CIELAB system, but with some significant differences. The Munsell system was conceived by the American painter Albert H. Munsell in 1905 with subsequent revisions and variations.[4] The three variables used to describe colors in the system are hue, brightness (similar to lightness in CIELAB), and saturation (similar to chroma; also called value). As shown in Figure 12, the color space is cylindrical. The hue is divided into 100 equal spaces around the circle that forms the cross section of the cylinder, and the y-direction is the brightness, scaled from 0 to 18. The x-axis is the saturation, scaled from 10 to 18. Munsell charts and collections are used in the forensic analysis of paints and soils. Because books and samples of color are used for color comparison, the Munsell color space is sometimes referred to as a catalog system. An example application is in soil analysis in which soil particles can be seived, sorted, and grouped by their Munsell color.

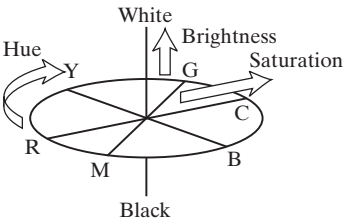
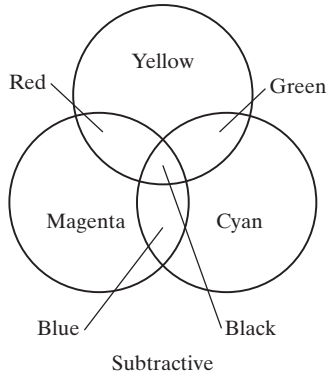


FIGURE 12 Munsell color space. The hue circle is divided into 100 equally spaced regions.

1.3 Other Systems and Conversions

Two other color systems encountered in forensic applications are the **CMY** (cyan–magenta–yellow) system used in printers and the RGB (red–green–blue) used in monitors. We have already discussed additive colors (Figure 14) in the context of the CIE calculations and combining spectral colors. Additive colors require projected or transmitted light. The CMY system is referred to as a **subtractive color system**, as illustrated in Figure 13. Subtractive colors are produced by reflection interactions. Consider a printing system such as a color computer printer that uses different inks to create a color on paper. The goal is to combine the different inks so that when the printed combination is viewed by an observer, the perceived color faithfully reproduces the desired color. Inks are combined in ratios such that the resulting mixture reflects the desired wavelengths to the observer. This approach can be thought of as subtractive in the sense that, by absorption, the mixture of ink subtracts wavelengths from the source of illumination so that the reflected light has the desired color. The CMY system is limited by its inability to faithfully reproduce black, so, in practice, a black ink is usually supplied in the printer, and the system is referred to as a **CMYK** system, with K representing the contribution of black. In contrast with the CMYK system, the RGB color system is additive and is used in applications where an image is projected, such as computer monitors and televisions.



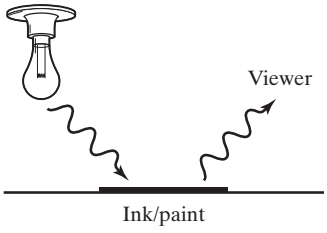


FIGURE 13 The CMY subtractive colors as used to create colors with inks, paints, and other coatings. The surface interacts with white light and subtracts wavelengths to create the perceived reflected color. See the color insert.

1.4 Color Conundrums

Pleochroism concerns a dichroic material appears to have a different color when viewed at different orientations owing to the crystal structure of the material. Similarly, different colorants, such as a dyed fabric, can appear to be a different color, depending on the illumination. This property is called **metamerism**, and often the effect is produced deliberately. It results from a change in illumination, one of the three components required to measure color. The color can also change with a change in observer, although to a lesser extent. Metamerism is not the same as dichroism, which depends on orientation, not illumination. It is also worth mentioning that the same perceived color can be produced in more than one way. For example, the color orange can be created by using a dye that reflects all orange light or by combining dyes that absorb combinations of yellow and red. The colors may be very difficult to distinguish by visual inspection but are easily separated by

colorimetry. Similarly, the perceived reflected color of black, such as used in the ink found on this page, can be created by many different combinations of colorants.

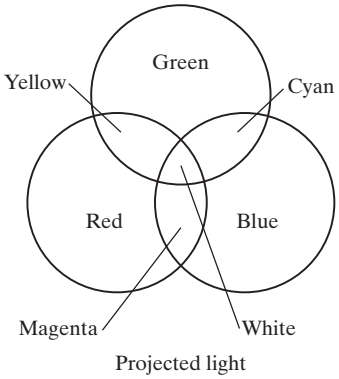


FIGURE 14 The RGB additive colors. See the color insert.

2 COLORANTS

The two colorants of forensic interest are **dyes** and **pigments**, both of which are encountered in many types of forensic evidence. Although these terms are sometimes used interchangeably, this usage is incorrect. Contrary to a common misconception, the difference does not have to do with organic versus inorganic constituents, nor is it based on a natural versus synthetic origin. The fundamental difference between dyes and pigments is *solubility*: dyes are soluble in the solvent they are carried in, whereas pigments are suspended particulates. Both dyes and pigments can be found in the same matrix, such as in an ink, but the distinction between them is a fundamental, important one and is illustrated in Figure 15.

EXAMPLE PROBLEM 2

Can a substance be a dye and a pigment?

Answer:

Yes. The difference between the two is solubility, a parameter that depends on the solute (pigment or dye) and the solvent. For example, "permanent" marker inks contain water-insoluble pigments that dissolve in pyridine.

Analytical chemists work routinely with dyes, even if they are unaware of them. Most acid–base indicators are dyes, as are a number of products of presumptive tests. Histological stains used in biological disciplines are also dyes. As mentioned, dyes are compounds that are soluble in the media in which they are contained (such as ink or paint). Dyes also have an affinity for the substrate on which they are placed

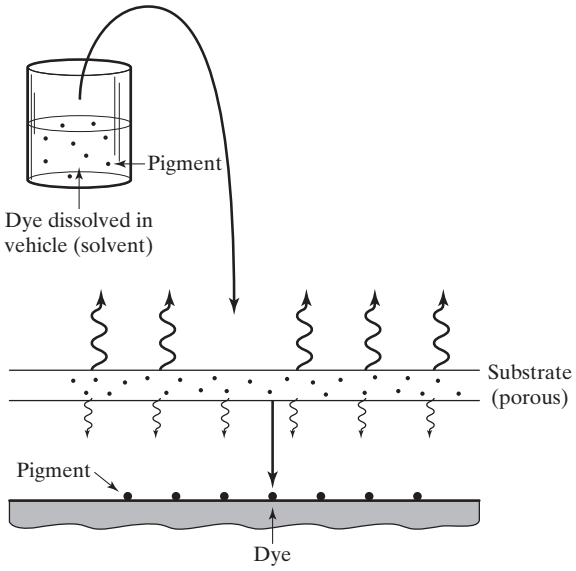


FIGURE 15 The feature that distinguishes a dye from a pigment is solubility. The dye penetrates with the solvent, whereas pigment particles remain on the surface.

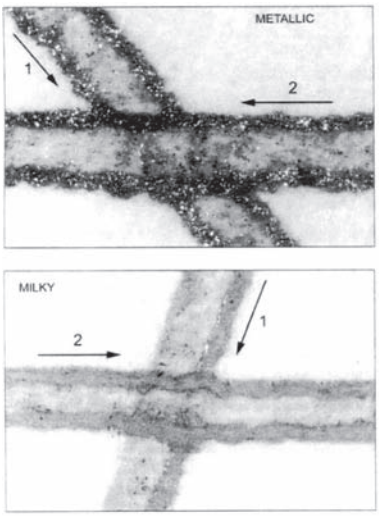


FIGURE 16 Intersecting lines of metallized ink; the order of drawing can be deduced from the layering. Figure reprinted with permission from Mazzella, W. D., and A. Khanmy-Vital, "A Study to Investigate the Evidential Value of Blue Gel Inks." *Journal of Forensic Sciences* 48 (2003): 419–24, Fig. 1. Copyright 2003, ASTM International.

(such as paper). Pigments, by contrast, are typically metallics or organometallics (an example is shown in Figure 16) that contain a transition metal, but organic pigments are becoming more common. Pigments are suspended or dispersed in a solvent but not dissolved in it. Unlike dyes, pigments do not have an affinity for their substrate. Thus, if a dye in an ink is applied to paper, the dye penetrates into the paper to the same extent as the solvent, whereas pigments in paint dry atop the paper. In other words, pigments must be affixed to the substrate. Dyes can be natural or synthetic, organic or inorganic. As a result of these various possibilities, there is a plethora of dye materials. Dyes were also one of the first modern industrial chemicals, as well as one of the earliest chemical commodities. The types of forensic evidence in which dyes and pigments are seen include questioned documents (inks and colorants), paints, and fibers among others.

Pigments may be simple inorganic compounds such as TiO_2 or complex organics such as the phthalocyanines. Dyes can be converted into pigments by chemical means such as making a salt from a cationic or anionic dye. An older term used to describe such a solid was "lake." Because pigments coat a surface, an observer's perception of the resulting color depends on chemical and physical properties of the pigment. It is also determined subtractively, as in the CMYK system shown in Figure 13. Because the pigment is a particulate, scattering also occurs. The more light that is scattered off the pigment, the better the hiding power of the paint. Scattering is optimal when the pigment particles are about half the size of the wavelength of incident light. When an ink or paint dries, the pigment is encased in a matrix of the binder, so the refractive indices of the binder and the pigment must also be considered when describing the apparent color. Optimal hiding power (maximum opacity) is favored when reflection and scattering are maximized. In turn, this situation is favored when the difference between the refractive index of the matrix and that of the pigment is large.[5] Thus, appearance and hiding power depend on the composition of the pigment, its strength, the size of the particulates, and their refractive indices compared with that of the binder.

EXHIBIT B

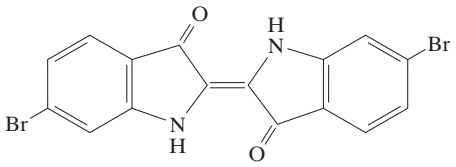
Why a Ruby Is Red

The gemstone called ruby is another manifestation of color due to transition metals, in this case chromium (Cr^{3+}). A ruby is composed of the mineral corundum, familiar to chemists as alumina (Al_2O_3) with bonds possessing ~60% ionic and ~40% covalent character. The resulting crystal structure is such that six oxygens are arranged in a distorted octahedron around any given aluminum ion. This packing arrangement generates an electrostatic field around the aluminum, since there is an excess of negative charge relative to the +3 on the aluminum. When the crystal lattice contains a small amount (~1%) of Cr_2O_3, the chromium atoms occupy the same crystal location as aluminum atoms. The distorted shape and electrostatic field splits the d orbitals in chromium and results in an energy gap corresponding to a photon in the visible range. A ruby absorbs light in the blue and yellow or green regions. The resulting transmission of red and some purple light gives the ruby its distinctive color.

Source: Nassau, K., "Color Caused by Transition Metals in a Ligand Field." Chap. 5 in *The Physics and Chemistry of Color,* 2d ed. New York: Wiley, 2001.

EXHIBIT C

The Color of Kings



Ancient Purple

Purple is a color (hue) often associated with royalty. The history of this association dates to the early Babylonians and the later Phoenicians and is related to one of the earliest commercial products, a natural dye extracted from mollusks and called Tyrian Purple, Royal Purple, Imperial Purple, or Ancient Purple (6,6-dibromoindigo). The name Tyrian refers to the city of Tyre in Lebanon, where production was centered. The preparation of the dye was labor intensive and required the extraction of tiny amounts of precursor chemicals from thousands of mollusks. Because of labor and material costs, the dye was literally worth more than gold; thus, kings were the only ones who could afford it.

Sources: Florence, D., "Spectral Comparison of Commercial and Synthesized Tyrian Purple," *Modern Microscopy*, November 18, 2003.
Zollinger, H., *Color: A Multidisciplinary Approach*. Zurich: Wiley Verlag, 1999.

EXAMPLE PROBLEM 3

The pigment particles in most white paints are between 0.2 and 0.4 μm in size. Why?

Answer:

This size corresponds to 200–400 nm. Scattering is maximized when the particle size is approximately half the wavelength of the incident light, so these particles will effectively scatter light in the 400- to 800-nm range, the range of visible wavelengths.

The crystal form of simple inorganic pigments is also important. For example, TiO_2, the most widely used white pigment, has three crystal forms:[6] rutile, anatase, and brookite.[7] The first two are tetragonal in shape and have high refractive indices, whereas brookite does not. Brookite is not used as a pigment. The rutile crystal form of TiO_2 has the best hiding power, but anatase is the whiter of the two. Until recently, inorganic pigments were based on transition-metal compounds that are also toxic, lead and cadmium compounds prominent among them. The use of these pigments has fallen as substitutes were found, both organic and inorganic. Compounds of titanium and zinc are notable in this regard.

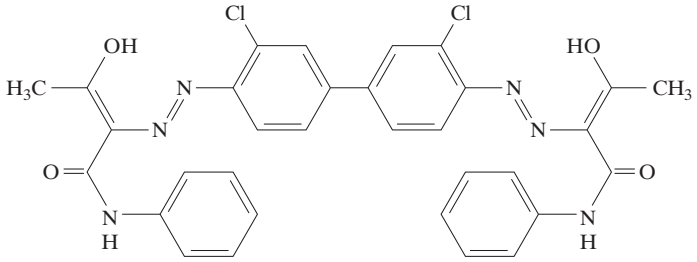
Other types of specialized inorganic pigments include those that produce specialized effects such as luminescence, pearlescence, and metallic sparkling (metal flakes). Luminescent pigments may fluoresce or phosphoresce and are used in paints and inks. Luminescent paint marks evacuation and escape routes in buildings, for example, whereas luminescent inks are useful in some printing and consumer inks. Pearlescent pigments mimic the appearance of pearls by utilizing internal reflection and the resulting interference patterns. An approach used to create pearlescence is to coat mica, a mineral with a relatively low refractive index, with TiO_2, which has a higher refractive

index. Incident light can undergo multiple internal reflections and produce the muted pearl appearance. Similar manipulations of coated metallic particles create sparkling pigments. Another group of pigments is used as **extenders** (fillers) in paints and inks. These compounds produce a coloring effect, but typically, they are present to reduce manufacturing costs by stretching the supply of more expensive pigments. Extender pigments include compounds such as $CaCO_3$, gypsum, talc, diatomaceous earth, quartz, mica, barium sulfate, silicates, and clay materials.

The oldest organic pigments are the azo type (—N═N—).[6] The copper phthalocyanines are now widely used in inks and paints. Numerous systems are utilized to classify and describe dyes and pigments; two are particularly useful in forensic contexts. The first is by application method or mode, and the second is by chromophore such as azo, nitroso, and carbonyl (Table 2). Regardless of how dyes are classified, all contain long conjugated bond systems. Many pigments do as well; exceptions are materials such as zinc oxide and TiO_2, widely used white pigments. Dyes bond or affiliate themselves with a substrate via the familiar mechanisms: ionic bonding, covalent bonding, and hydrogen bonding.[7] Dyes may be anionic, as in the case of acid dyes, or cationic, as in basic dyes. Reactive dyes form covalent bonds with the substrate, typically a fiber. In many cases, mixed bonding modes are seen.

Forensically, dyes are typically classified by their chemical structure or method of application (Table 3) or by their chemical dye class (Table 4). Quantitative description and classification of dye color is most commonly done with the Color Index (Colour Index) or CI number, although use of the CI number is limited by the application of the dye. The Color Index is compiled by joint efforts of the Society of Dyers and Colourists (SDC, www.sdc.org.uk) and the American Association of Textile Chemists and Colorists (AATCC, www.aatcc.org). The CI number system is similar to the CAS numbering system used for chemicals, in that it provides a common reference and numerical key that unambiguously identifies a dye or pigment regardless of other names applied to it. For example, indigo, a natural dye, has a CAS number of 482-89-3 and a CI constitution number of 73000. The constitution number contains five digits and refers to the time the dye was registered; the lower the number, the older is the registration. The first two to three digits code for the chemical class. The notation *dye* or *pigment* is included, and if appropriate, the salt form can be noted. For example, CI Pigment Red 48 (12070) is available as several salts, including the calcium (CI Pigment Red 48:2) and manganese (CI Red Pigment 48:4) salts. Many dyes and pigments also have common names, such as Tyrian Purple.

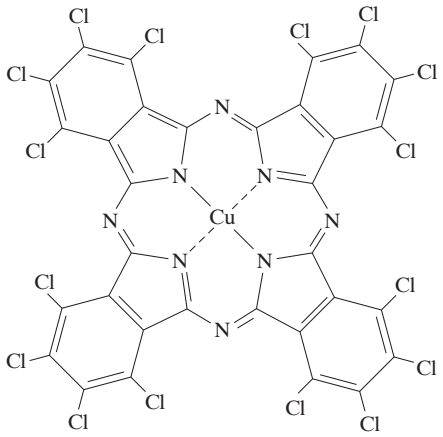
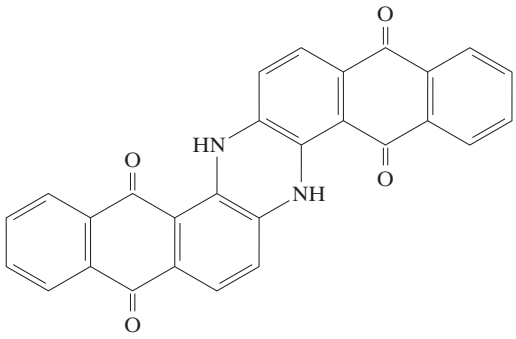
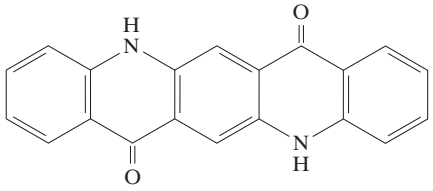
TABLE 2 Ink and Paint Pigments

Class	Pigment Group	Colors[a]	Example
Organic	Azo and metallicized azos	ROY	

Benzidine yellow

Pigment yellow 12

TABLE 2 Ink and Paint Pigments (continued)

Class	Pigment Group	Colors[a]	Example
	Phthalocyanines (copper)	BG	 Phthalocyanine green
	Anthraquinones	Many	 CI blue pigment 60 Indanthren blue
	Quinacridones	RV	 Pigment violet 19 Linear quinacridone
Inorganic	Transition-metal oxides	Many	TiO_2 (white)
	Graphite (carbon black)	Black	
	Metallic	Shine	Al, Cu, Zn, and alloys
	Transition-metal sulfides	Many	Cadmium sulfoselenide (R)
	Chromates	YO	$ZnCrO_4$ (Y)
	Ferrocyanides	BR	$Fe[Fe(CN)_6]$

[a]Spectral colors ROYGBIV (red–orange–yellow–green–blue–indigo–violet).

TABLE 3 Dyes and Pigments Categorized by Functional Behavior or Application Method[a]

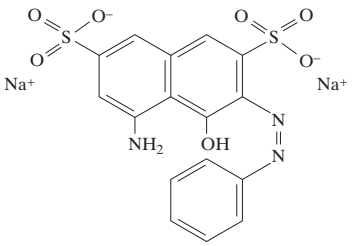
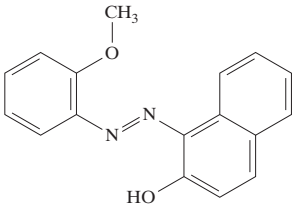
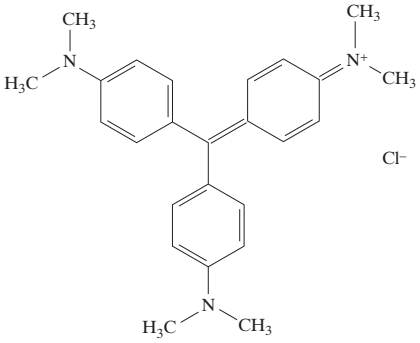
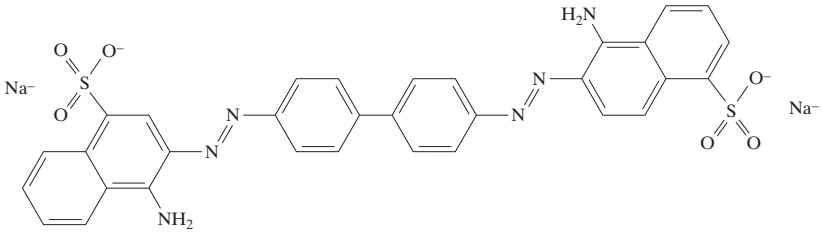
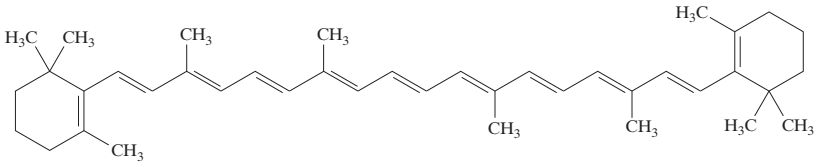
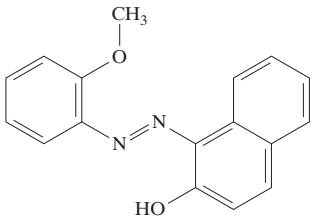
Class	Description	Example
Acid	Dyes used under acidic conditions; ionic interactions with substrate; anionic in solution; most are derivatives	 Acid red 33
Azo	Dyes created by coupling reactions to create diazonium salts or the like	 Solvent red 1
Basic (cationic)	Dyes that form cations in solution; tribenzene (trimethine) structural element is common	 Gentian violet/Basic violet 3
Direct	Dyes that are applied directly to cellulose (as in cotton fibers or cellulose component in paper); also called substantive dyes	 Direct red 28 (congo red)
Natural	As the name implies; Tyrian Purple is an example	 Natural yellow 26 (β carotene)

TABLE 3 Dyes and Pigments Categorized by Functional Behavior or Application Method[a] *(continued)*

Class	Description	Example
Solvent	Dyes that are molecular as opposed to ionic and that dissolve in nonpolar solvents	 Solvent red 1

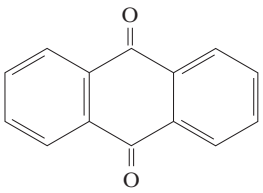
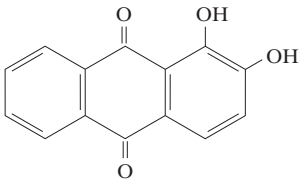
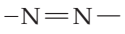
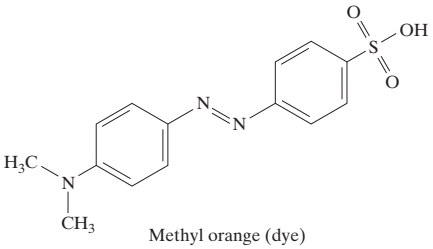
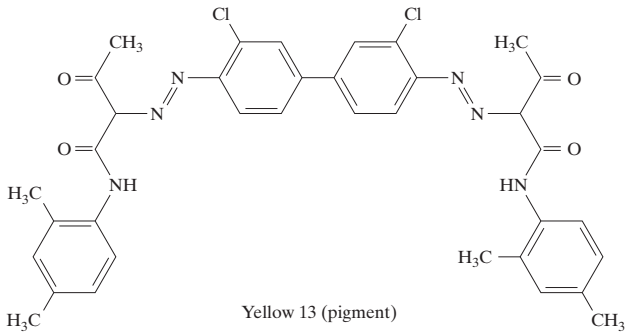
Sources: Eklund, D., and T. Linstrom, *Paper Chemistry: An Introduction*. Grankulla, Finland: DT Paper Science, 1991.

Hunger, K., ed., *Industrial Dyes: Chemistry, Properties, and Applications*. Kelkheim, Germany: Wiley-VCH, 2003.

Zollinger, H., ed., *Color Chemistry: Synthesis, Properties, and Applications of Organic Dyes and Pigments*. Zurich: VHCA/Wiley-VCH, 2003.

[a]Note that more than one classification can apply.

TABLE 4 Dyes and Pigments Grouped by Chromophore

Chromophore	Structure	Example
Anthraquinone		 Alizarin (dye)
Azo		 Methyl orange (dye)  Yellow 13 (pigment)

(continued)

TABLE 4 Dyes and Pigments Grouped by Chromophore *(continued)*

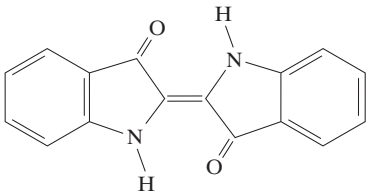
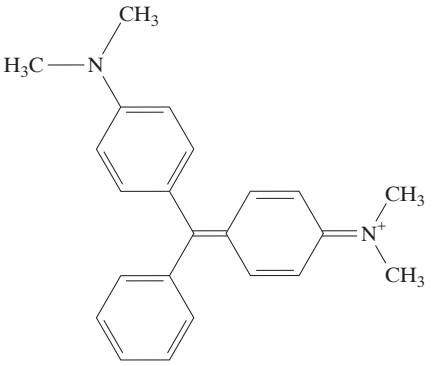
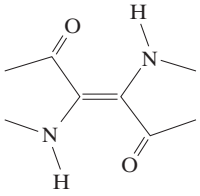
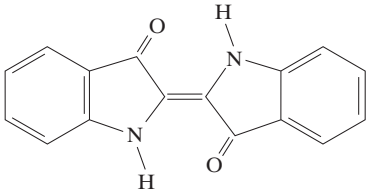
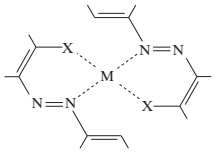
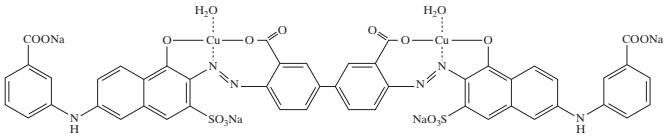
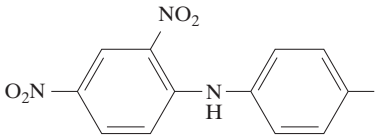
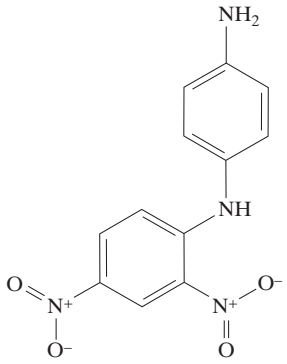
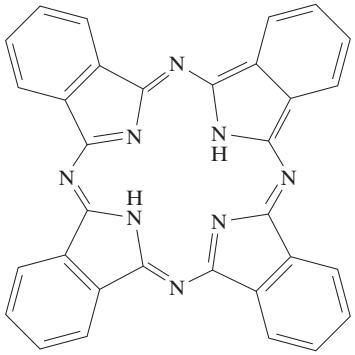
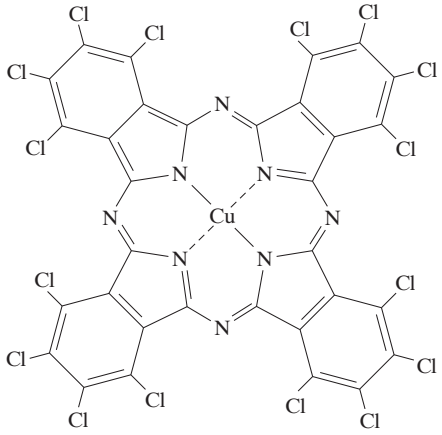
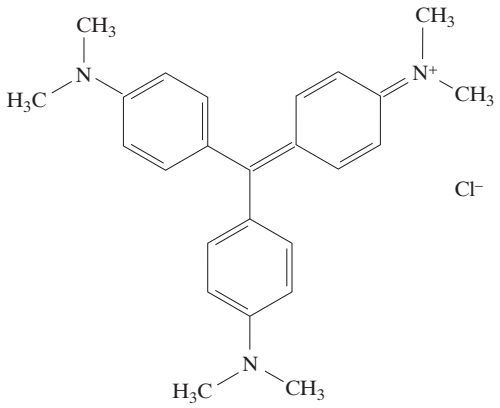
Chromophore	Structure	Example
Carbonyl	Conjugated C═O system	 Indigo
Cationic	R_4N^+	
Indigoid		 Indigo
Metal complexes	 M – Cu^{2+}, Ni^{2+}, CO^{2+} X – O, NH	 C.I. direct blue 93
Nitro and nitroso		 C.I. Dispersive yellow 9

TABLE 4 Dyes and Pigments Grouped by Chromophore *(continued)*

Chromophore	Structure	Example
Phthalocyanine		 Phthalocyanine green
Polymethine	Long chain of conjugated double bonds terminated by e^- donor and e^- acceptor	 Gentian violet

Sources: Hunger, K., ed., *Industrial Dyes: Chemistry, Properties, and Applications.* Kelkheim, Germany: Wiley-VCH, 2003.

Zollinger, H., ed., *Color Chemistry: Synthesis, Properties, and Applications of Organic Dyes and Pigments*. Zurich: VHCA/Wiley-VCH, 2003.

EXHIBIT D

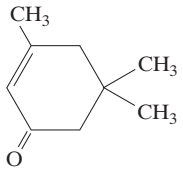
Drugs and Dyes

Drugs and dyes have historical as well as chemical properties in common. The dye industry began with the synthesis of mauve (aniline purple) by William Henry Perkins in 1856. Perkins, then an 18-year-old chemist, was attempting to make quinine to treat malaria. Bayer Pharmaceuticals, a German company famous for aspirin, was a dye-manufacturing company well into the twentieth century.

3 APPLICATION OF COLORANTS: INKS

Inks consist of a solvent called a **vehicle**, colorants (dyes, pigments, or both), and other additives to control the flow, thickness, and appearance of the ink when dry, as well as other qualities. The ingredients in an ink are optimized on considerations pertaining to the marketplace and the production facility. For example, ballpoint-pen ink must be pumped into a cartridge that is inserted into the pen. The ink must flow well, clean up easily, and not foam. Accordingly, the ink manufacturer might include a defoaming agent in the formulation to address the needs of the pen maker, not the desires of the consumer. This knowledge is important in interpreting analytical results. Each additional ingredient provides an additional potential target analyte (or analytical handle) that may be exploitable for characterization and differentiation. Ballpoint-pen inks are arguably the most frequently encountered ink evidence,[8] but the market changes constantly, and newer types, such as felt-tip pens with soft application surfaces and gel-ink pens, are now common. Inks are involved in questioned document cases such as forgery and counterfeiting. Chemical analysis is used to answer such forensic questions as, Was all the writing on this page done with the same writing instrument? When was the writing placed on the paper? and In what order was the writing done? The last question may be of importance in determining whether a pen stroke was added atop an existing stroke, but this is usually sorted out microscopically. See Table 5 for other relevant terms.

Inks and paint (to be discussed shortly) are similar in many respects. Both are designed to deliver a colorant to a substrate such that the resulting coloring is evenly distributed and has the desired characteristics once it is dry. As solutions, both consist of (water or organic) solvents and solutes. If a colorant is a dye, it is dissolved in the solvent; if the colorant is a pigment, it is suspended in the solvent. Other solutes include adhesives, finishing agents, viscosity control agents, antimicrobials, antifoaming agents, extenders, and a myriad of other materials. All ingredients in the complex ink solution can yield important analytical information to the forensic chemist. Ink formulations have changed dramatically since the 1970s as environmental concerns related to the manufacturing of ink have increased.[9] A similar development has occurred in the paint field. Solvents and binder compositions in particular have evolved significantly, moving toward more water-based products and ether solvents.



Isophorone

FIGURE 17

Solvents common in ink formulations (in addition to water) are methyl ethyl ketone (MEK), isophorone (Figure 17), and the glycol ethers such as ethylene glycol, a family of related compounds formed by the reaction of alcohols and ethylene oxide. As with alcohols, the longer the hydrocarbon chain, the less water soluble is the glycol. The terms **cellusolve** and **carbitol** are used to describe many of these compounds, which can be synthesized as shown in Figure 19.[10] The longer the alcohol chain, the less water soluble is the ether produced. However, having ether functionality increases the hydrogen-bonding capability of the ether. These solvents are also called alcohol–ethers because of their mixed functionality.

EXAMPLE PROBLEM 4

Two inks are submitted to a forensic chemist for comparison, one a dark blue and one a light blue. How would the chromaticity values be expected to differ?

Answer:

For the first part of the question, refer to the chromaticity space plot shown in the color insert. The darker the color, the farther the point will be toward the periphery of the parabola; the lighter the color, the closer to the center it will be. CIELAB values would be the best choice for expressing the color difference quantitatively.

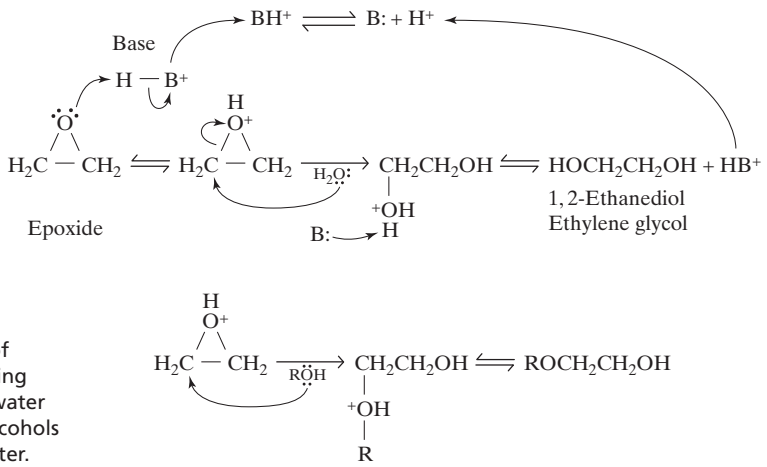


FIGURE 18 Synthesis of common ink solvents using epoxides. Epoxide and water form ethylene glycol. Alcohols react analogously to water.

The reaction mechanism for producing cellusolve and related ethers is illustrated in Figure 18 by the production of ethyl glycol, another important solvent in inks. Epoxides are extremely reactive. Once the epoxide is protonated, even relatively poor nucleophiles such as water (shown) or alcohols can open the ring and add to it. Altering the stoichiometric ratio of alcohol to epoxide dictates which ethers are produced.

APPLYING THE SCIENCE 1 The Vinland Map

The Vinland map has been controversial from the day it surfaced in 1965. The map appears to show the outline of Canada and North America—no great surprise, except that the parchment on which the map is drawn has been dated to the period 1411–1468. This dating, calculated from ^{14}C techniques, is not disputed. Columbus sailed to the New World in 1492, but Vikings had discovered and settled in North America hundreds of years earlier, another fact not contested. However, the authenticity of the map has been questioned on the basis of the ink and techniques used to create it. Modern forgeries created on old parchment are not uncommon, but it is difficult to forge the types of pigments that would have been used on an authentic map. This is the heart of the controversy.

The map was donated to Yale University in 1965, and at the request of the university, Dr. Walter McCrone, the famed microscopist and chemist, examined it. Using PLM and other techniques, he concluded that a clever forger had first drawn a yellowed outline on the paper with a yellowish anatase (TiO_2) pigment to simulate ink diffusion and fading. The forger then drew a darker line down the center of the yellowed region to complete the ruse. As evidence of this technique, McCrone maintained that the anatase he found was too finely ground and had other characteristics that only a modern anatase, produced after 1917, could have.

Dr. Jacqueline Olin, a retired chemist from the Smithsonian Institution, countered by creating a black ink starting from the mineral ilmenite ($FeTiO_3$). This ink, she argues, could explain the observed pattern. Another possibility is that the anatase could have come from kaolin, which is still used as an additive in colorant formulations. Instrumental analyses have produced conflicting results: a 1987 analysis using particle-induced X-ray emission lent support to those arguing for authenticity, whereas another study using Raman spectroscopy supported McCrone's assertions concerning the anatase. In addition to being an interesting historical mystery, the Vinland map case illustrates the need for deep knowledge of chemistry and formulations of inks as part of the investigative process.

Source: Graham, R., "Vinland: An Inky Controversy Lives," *Analytical Chemistry* "A" pages November (2004), 407A–12A.

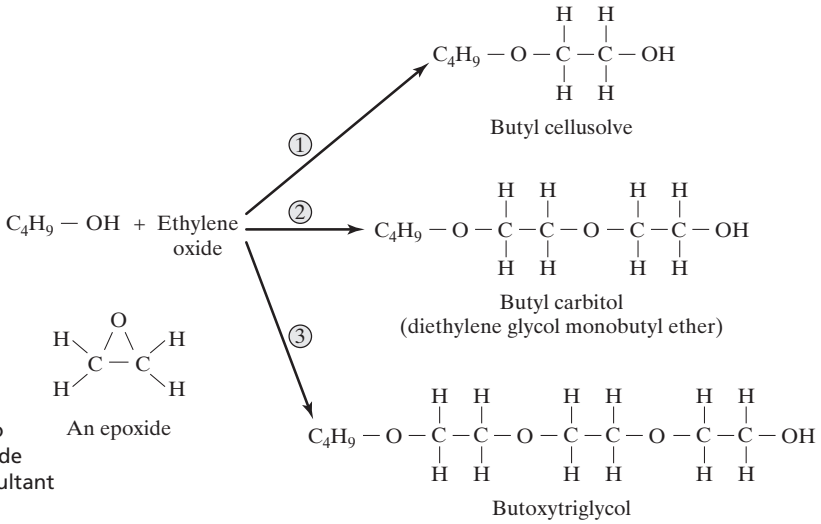


FIGURE 19 The stoichiometric ratio of alcohol to epoxide determines the resultant products.

Pigments in ink are not dissolved; accordingly, the particles have to be ground finely and dispersed as evenly as possible. Because many pigments are charged, the electrostatic interactions can be exploited to improve the dispersion of fine particulates. The solution is treated to prevent the formation and growth of particles.† Stabilization of the double layer (Figure 20) maintains electrostatic repulsion between pigment particles and prevents agglomeration of the fine dispersion into unevenly distributed globs. One approach to stabilization is to use a surfactant, as illustrated in Figure 21. In this example, the surfactant has an anionic end that associates with the particle. Here, it is important that the surfactant–particle complex remain soluble in the ink's vehicle, such as water. A similar approach is based not on stabilization of the double layer but on physically blocking the particle surface. This method is referred to as steric hindrance.[11]

In addition to pigments, many printing inks are metallized, such as metallized azo pigments.[11] Among the various applications of metallized pigments are printing on boxes and on currency. Some consumer ink formulations incorporate metal flakes, as is seen in Figure 16. Dyes are also used as colorants for inks, with the key criterion being solubility. Dyes penetrate the paper matrix to the degree the solvent does and may associate with the cellulose matrix on the basis of ion–dipole interactions. Dyes

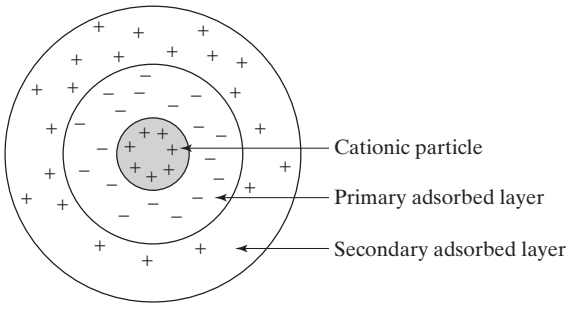


FIGURE 20 The double layer surrounding a charged particulate.

†Readers may recall performing gravimetric analysis as part of an analytical chemistry or quantitative analysis course. A successful gravimetric analysis requires that particle formation be encouraged and that the electrical double layer be disrupted and decreased in size. This is the exact opposite of what is desired for optimal dispersion. The particles are treated to ensure that they do not clump together in solution.

that would not otherwise adhere to cellulose can be modified to produce such association as desired. Other ingredients in inks include adhesives, **resins**, agents to control viscosity, and varnishes. Adhesives and varnishes are often used to bind pigment particles to the paper surface.

There are numerous types of inks, such as printing ink, ballpoint-pen ink, gel-pen ink, and inks used in printers and typing ribbons. Formulations vary with the application, and the differences are useful in chemical characterization and interpretation. One of the oldest inks, and one still in use, is carbon ink, also called India ink. The composition is as it sounds: ground carbon black suspended in glue or some other adhesive material to enable the particulates to adhere to the substrate. Modern uses of India ink are in fountain pens, printing inks, and drawing inks. Until the mid-1900s, fountain pens were widely used, with inks consisting of iron gallotannate formulations or mixtures of dyes. Interestingly, there are few true black dyes, and almost all black inks consist of mixtures of dyes that work in accordance with the subtractive color principle described earlier.

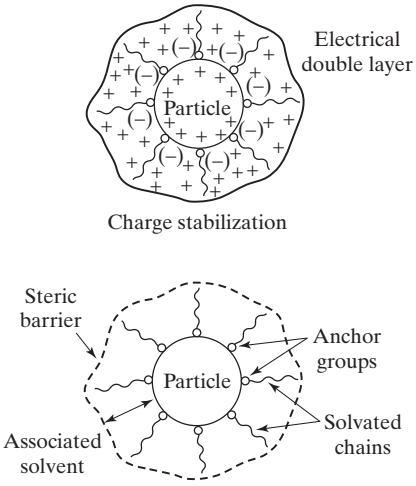


FIGURE 21 Methods of stabilizing the double layer using surfactants. The anionic group of the surfactant associates with the cationic particle and stabilizes the double layer, which in turn prevents particulates from aggregating and settling out of solution.

Iron gallotannate inks are made by suspending iron salts in solutions containing tannic acid (MW 1701), a polyphenol used to stain wood. Tannic acid functions as a **mordant**, or binding or bonding agent, for pigments and dyes. In this capacity, tannic acid is used to prepare cellulose fibers to receive dyes; cellulose is the primary ingredient in papers and in cotton fibers. The name originated from one of the earliest applications of tannic acid: the dyeing of leather, which is also called tanning. Iron gallotannate inks penetrate, bind to cellulose fibers, and can be removed only by physical means.[12] These inks are also interesting in that when first applied, they have little color, acquiring color with oxidation.[12]

The ballpoint pen, introduced in the United States around 1943, is based on the delivery of ink to a small metal ball that rolls over the paper's surface. The inks used are relatively thick and may contain dyes and pigments. Because the writing process involves mechanical actions, ballpoint-pen ink contains additives to lubricate the ball. Other additives range from adhesives to viscosity control agents, resins, and drying agents.[12] Common solvents include ethylene glycol, glycerin, and related compounds. Among the colorants currently favored are metallized phthalocyanines, such as those shown in Tables 2 and 4.

Another group of ink delivery devices consists of those that operate via capillary action. Fiber-tipped pens, also called "felt tips" deliver inks with solvent bases that are aqueous, glycol based, or xylene based.[12] The inks are generally less viscous than ballpoint inks. Gel pens are increasing in popularity and, as the name implies, deliver colorants via a gel rather than a comparatively thin solution. Colorants are primarily pigments, and once dry, the inks are virtually insoluble.[12] As a result, forensic analyses are more complex, but still feasible, as is discussed later in the chapter.

4 APPLICATIONS OF COLORANTS: MECHANICAL AND COMPUTER PRINTING

In addition to writing devices such as pens, a plethora of mechanical printing devices also deliver inks. Two decades ago, the term *mechanical printing* would have referred principally to one device: the typewriter. Computer technology has changed this situation, and now mechanical (as opposed to handwritten) documents have become much more important in society and consequently much more common as forms of physical evidence. For the forensic chemist, however, the common theme remains inks, dyes, and pigments. This section approaches mechanical printing from this perspective.

EXHIBIT E

Why Metals Are Shiny

The characteristic shine of metals is related to their ability to conduct electricity. In a simplified explanation, the bonding in a metal such as copper differs from that in covalent compounds such as H_2 in that the valence shell electrons are shared by all the metal atoms in a contiguous piece of the pure metal. The electrons are completely delocalized and move easily within the valence-bond bands that are available. When a photon of the appropriate wavelength is absorbed, an electron is easily promoted. The extinction coefficient for the transition is strong, and as a result, photons do not travel far into the metal. The absorption of a photon—a packet of electromagnetic energy—causes an electrical current to flow in the metal and emits a photon at the same wavelength. The collection of photons emitted is perceived by an observer as "shine" or luster.

Source: Nassau, K., "Color Caused by Transition Metals in a Ligand Field." Chap. 5 in *The Physics and Chemistry of Color,* 2d ed. New York: Wiley, 2001.

EXAMPLE PROBLEM 5

Two inks are to be compared, one extracted from a ransom note and one obtained from a pen recovered from a suspect. Both inks are a light-blue color. A reflectance spectrum of one reveals a single broad peak for one ink, whereas the spectrum of the other shows two peaks. Are the inks differentiated?

Answer:

Yes. The spectra indicate that, although they appear similar, the colors are produced by two different compositions. The first is likely a single colorant, the second a mixture. The inks are not from a common source.

TABLE 5 Paint and Coatings Terminology

Additives	Materials added to a paint or coating in small amounts and with varying purposes.
Binder	Substance that binds pigment particles to each other and the substrate; may also be the solvent or vehicle of a colorant solution.
Drying oils	Triglyceride compounds that form a coating via oxidation; also used as precursors to varnishes.
Extender/extender pigment	A pigment added to a colorant to alter its final appearance as well as reduce manufacturing costs.
Hiding power	The ability of a coating to cover what is below it.
Lacquer	A protective topcoat that dries by evaporation of the solvent. Lacquers do not polymerize, but they may be polymers.
Latex	Emulsion of colorant in an aqueous solution.
Modifier	A substance added in small amounts to alter physical or chemical characteristics of a coating.
Opacity	Degree of clearness of a coating. Topcoats should have minimal opacity and be nearly clear; colorant layers should be highly opaque to cover what is below.

Plasticizer	Substance added to a polymeric material to soften it and increase its flexibility.
Primer	The first coat placed on a surface; used to prepare the surface for receiving subsequent layers and binding them to the substrate.
Resin	Component that polymerizes from solution.
Sealant	Coating designed to seal a porous surface; can be used as a primer or topcoat.
Shellac	A varnishlike material derived from insects.
Stain	Colorant that penetrates a surface rather than drying upon it. Stains lack binder components and are similar to inks.
Varnish	A protective topcoat that may contain oils, resins, and solvents. Polyurethane varnish is an example of a varnish that polymerizes after it is delivered to the substrate.
Vehicle	Binder and solvent and colorants, usually a synthetic resin.

Sources: Ryland, S. G., "Infrared Microscopy of Paints," *Practical Spectroscopy* 19 (1995): 163–243.

"Standard Guide for Forensic Paint Analysis and Comparison," in *ASTM Standard E 1610-02* 14.02, ed. ASTM International, 2004.

Thornton, J., "Forensic Paint Examination." Chap. 8 in *Forensic Science Handbook,* 2d ed., vol. 2, R. Saferstein. Upper Saddle River, NJ: Prentice Hall, 2002.

4.1 Computer Printing

One of the first computer printers available, and one still used for printing some forms and checks, is the dot-matrix printer. Like a typewriter, a dot-matrix printer uses an impact method to create letters by striking paper through an inked ribbon. The letter is formed by a series of dots mounted on a printing head. Multiple printhead passes coupled to selective activation of the pin generate the letters. The printhead consists of a single row of pins, as shown in Figure 22. To make a capital E, all pins are active and strike the ribbon on the first pass, creating the "|" backbone. On the second, third, and fourth pass, pins 1, 6, and 10 are active. Any common letter, number, or symbol can be formed by a dot-matrix printer.

Laser printers, photocopiers, and ink jet printers employ nonimpact printing technologies. Ink jet printers work on much the same principle as dot-matrix printers except that ink is sprayed from the printhead onto the paper in tiny target areas. Laser printers and copiers work on a different principle, as shown in Figures 23 and 24. At the start of the print cycle, a rotating drum surface is charged electrostatically in a fine grid pattern. A laser selectively scans the grid, discharging any grid square that it strikes. This creates a pattern that corresponds to lines from the image on the drum, called the *latent image*. The next step is to apply color, either black and shades of gray, or color using toners. As shown in Figure 25, the difference between letters made by a laser printer and an ink jet printer can be distinguished microscopically.

Toner is a dry material that feels like powder but that contains waxy plastic in addition to the colorant. Finer grain sizes of toner allows for sharper lines and resolution in the printed image. The toner grains have a plastic consistency that melts much as wax when an image is fixed to paper in the fuser. Each color of toner is kept in a separate toner cartridge. Color copiers usually have three in addition to black: yellow, magenta (a light rose red), and cyan (a bluish purple), which is often abbreviated CMY, the subtractive color system. The abbreviation CMYK is sometimes seen if a separate black

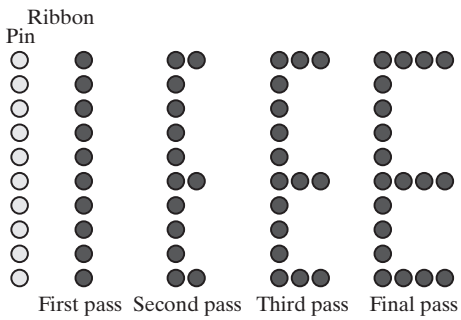


FIGURE 22 Mechanism of ink delivery in a dot-matrix printer.

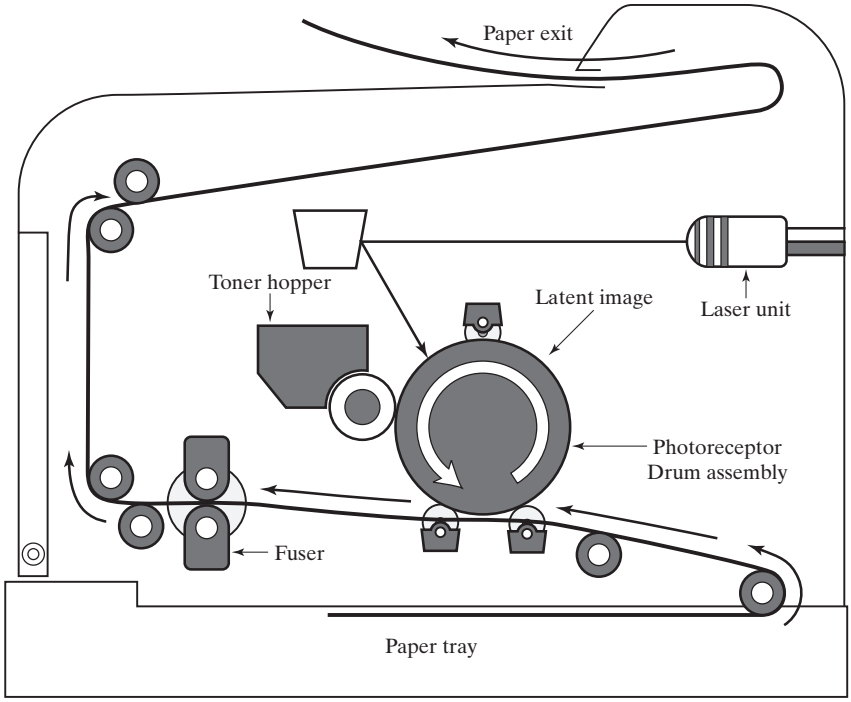


FIGURE 23 Operation of a typical laser printer or copier. More details are found in the text.

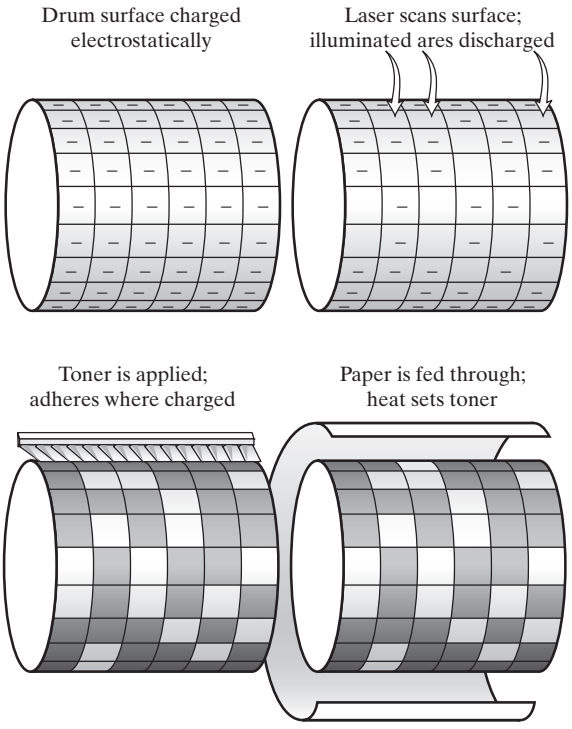
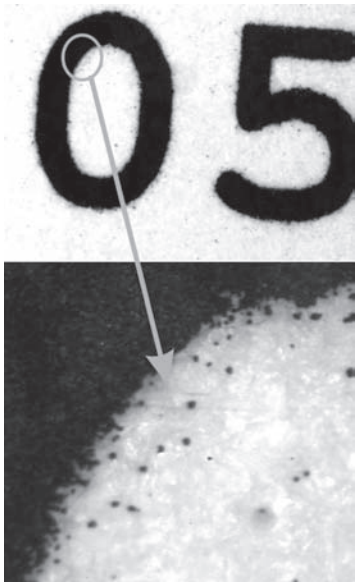


FIGURE 24 How toner is applied to the drum to form the latent image in a laser printer.



Amy Richmond and Jennifer Wiseman, WVU Department of Chemistry

FIGURE 25 On magnification, residual toner particles are visible and evidence of printing using a laser jet process. Image courtesy of Amy Richmond and Jennifer Wiseman, WVU Department of Chemistry.

toner cartridge is used in the printer. These colors can be combined to create thousands of other colors, as was discussed earlier in the chapter. Negatively charged toner is applied to the drum and adheres wherever the charge remains. Paper, also given an electrostatic charge, is rolled on the drum, transferring the toner from the drum to the surface of the paper. The toner is fused to the paper by heat, which melts the waxy toner so that it adheres to the paper. The final step in the process is to remove the latent image from the drum by means of a discharge lamp. The drum is then ready to receive the next image.

4.2 Mechanical Printing

Many types of documents are still printed mechanically. Of principal forensic interest are commonly forged documents, such as currency and stamps. Mechanical processes deliver ink to a substrate by mechanical pressure applied against an ink-coated metal plate. As an example, consider the process used by the U.S. Treasury Department's Bureau of Engraving and Printing (www.moneyfactory.com). The first step in making paper currency is to create an engraving called the *master die*. This is done by hand via a painstaking process, but the die can be used for years. For example, the portrait of Lincoln on the $5 bill was originally carved in 1869. The master die must next be duplicated exactly on a plate that produces 32 bills each time it is used. The complex process involves the creation of a cast of the die made in plastic and then combined with the other elements of the bill. The plastic relief, called an *alto*, is placed in an electrolytic bath that forms the foundation of the plate. Careful inspection follows. If the alto is deemed acceptable, it is used to make the final metal plate.

EXHIBIT F

Making Money (Literally)

"Money" can be described as a physical object of little intrinsic value that nonetheless represents wealth. The dollar bill is ink on paper and not worth much; it cannot be used for anything other than as a token to be exchanged for something else. Early human business interactions, such as exchanges of material or services, were based on bartering. The earliest forms of money were not coins or notes, but food such as cows, sheep, and crops. The most widely used form of early money, still seen today, is a small shell called a cowrie shell. The Chinese adopted it as early as 1200 B.C. Within a few hundred years, the Chinese were also using metal coins of copper and bronze. Many of these coins were made with holes in the middle to allow them to be strung together like beads, a widespread practice. Native Americans were using chains of clamshells on strings by the middle of the sixteenth century. These were called *wampum*. Coins of silver appeared in the West around 500 B.C. and took a form that is recognizable today: rounded and with figures and scenes stamped into them. These coins could also be made of gold and other precious metals, but many were made of the less expensive and less valued "base metals." Modern U.S. coins used in general circulation are made of base metals.

The Chinese also pioneered paper money, with the first examples appearing around A.D. 800. In a problem often repeated since, so much money was printed that its value fell and prices soared, a situation referred to as *inflation*. The problem became so severe that the Chinese abandoned paper currency in the fifteenth century, and it was only in the 1700s that paper money was seen on a large scale again. The first paper currency in the United States was issued in 1690 by the Massachusetts Bay Colony. After the Revolutionary war, states continued to issue their own currency into the 1800s. Anticounterfeiting features were incorporated from the beginning, including very fine engraving, signatures, and elaborate patterns that are difficult to re-create. The Bureau of Engraving and Printing was established in 1877 and continues to be responsible for printing paper currency. The Bureau also prints stamps for the U.S. Postal Service. Each day, the Bureau produces approximately 37 million bills.

For printing, the plate is coated with ink and wiped down, so that ink remains in the grooves in the plate. Special paper is rolled across the plates under extremely high pressure that forces the paper into the inked areas on the plates and transfers the ink to the paper surface. Because the paper is pressed into the grooves, the bill acquires texture such as fine ridges and grooves. This process of pressing is referred to as **intaglio printing**, and the texture created by pressing the currency cannot be easily re-created by counterfeiters using any other process. An example of intaglio printing is shown in the color insert. Because of the importance of deterring and detecting counterfeiting, the inks used in currency are unique and have features not found in typical consumer inks. This is an important consideration; most counterfeit bills are made with computer scanners and color printers. This aspect of ink analysis is discussed shortly.

5 APPLICATION OF COLORANTS: PAINT

Like inks, paints are solutions that deliver colorant to a substrate. However, the term "paint" has come to refer to the generic field of coatings. Most coatings contain three elements: a solvent (organic or water), a colorant (typically, a pigment), and a binding agent that polymerizes during a curing stage. The solvent ensures that the pigment suspension and polymer-forming molecule are evenly spread over a surface; once the solvent evaporates sufficiently, the polymerization begins. Paint may cure unassisted or be accelerated by heat or irradiation with UV or other ranges of electromagnetic energy. Ancient paints used materials such as beeswax and fats as the binding agent. Tempera paints, made from egg yolks, are used today as artists' and children's paint; they were among the most common types of paint from ancient times until the Renaissance. Around the sixteenth century, oil-based binders such as linseed oil, still widely used today in oil painting, became popular. They dissolved in turpentine or a similar liquid. The oils in these paints polymerize slowly by oxidation to form a clear, resilient matrix that binds the pigment particles to the surface and to each other.

Paints can be placed on porous surfaces, such as wood or paper, or on nonporous materials, such as metal. Paints are also frequently incorporated into a layered system, each layer representing a different type of coating and different forensic and analytical characteristics. As shown in Figure 26, a typical paint system consists of three generic layers: a layer that coats and prepares the substrate (the primer); the colorant layer or layers, in which the dyes and pigments are found; and a protective layer such as a varnish or clearcoat. Not all systems include all layers, but this model is a reasonable place to begin the discussion of paints. Table 5 summarizes the common terms used in such a discussion. When a coating is applied to a surface, a chemically complex process occurs. Consider the simple example of a paint containing pigment and a binder in a solvent (Figure 27). Once the paint is applied, two processes occur: solvent evaporates, and the binder polymerizes to bind pigment particles to each other and to the substrate. The time required may be referred to as the *curing time*, depending on the application. The colorants are usually chemically passive in this process, which depends on the actions and interactions among the solvents and binders in a given paint formulation.

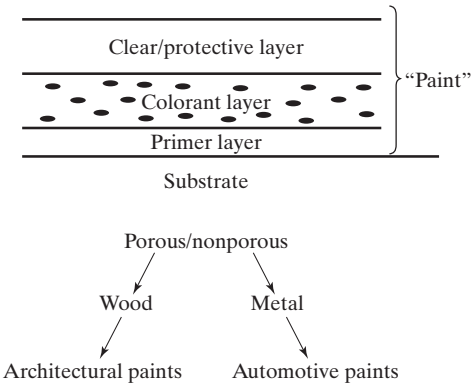


FIGURE 26 Paint is often found in layers. The primer prepares or seals the surface, the colorant layer delivers the pigment or dye, and the protective layer seals out moisture and protects from weathering.

5.1 Solvents

Solvents in paint function as do ink solvents. The solvent must ensure that the colorant is homogeneously suspended (in the case of pigments) and delivered evenly to the substrate. Once the colorant and the associated additives are in place, the solvent evaporates

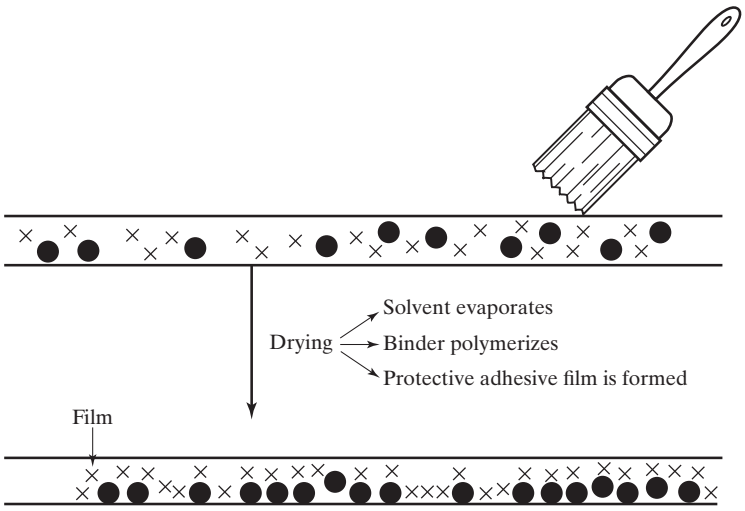


FIGURE 27 The drying of paint involves both evaporation of solvent and polymerization of the binder. This step is also called *film formation*.

and, in doing so, allows the curing and film-building polymerization process to begin. As with inks, environmental concerns have driven paint manufacturers to an increasing use of water as the solvent whenever feasible. Latex paints are water based and illustrate some of the challenges introduced when water is the solvent. Many of the binders and resins used in paints are not water soluble but can be made so by using micelles. As illustrated in Figure 28, the micelles surround and dissolve the monomers, which then polymerize. The resulting suspended aggregate is called a **latex particle** and remains suspended in an emulsion. Once a latex paint is applied to a substrate, the water evaporates and allows the polymerization to proceed to completion.

In addition to water, oxygenated hydrocarbon solvents are generally preferred whenever the formulation allows them, also owing to environmental considerations. Types of solvents used include alcohols, esters, ketones, and the alcohol ethers associated with inks. Some paints still use hydrocarbon solvents such as turpentine and kerosene, and chlorinated hydrocarbon solvents, such as trichloroethylene, are also sometimes used. Finally, solventless delivery systems such as electrodeposition are used in some applications such as automotive coatings.

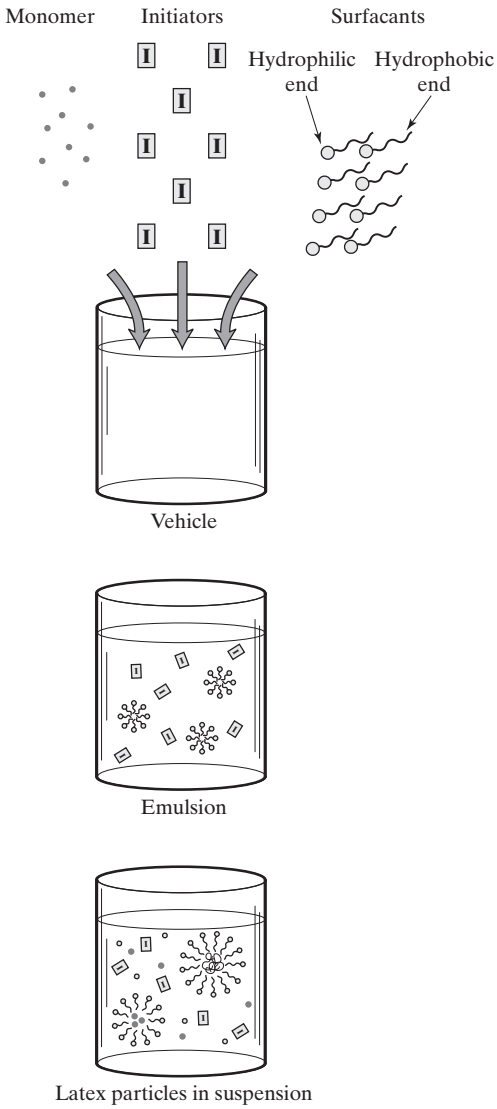
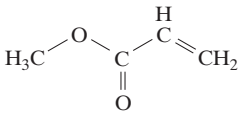


FIGURE 28 Formation of a latex emulsion. A micelle is created around the monomers, which partially polymerize within the micelle. The micelle remains suspended in the aqueous solution until the paint is delivered to the substrate.

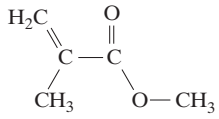
5.2 Binders or Resins

The role of the vehicle in paint is to form an adhesive film over the pigment granules that is optically clear, glossy (reflective) or matte (diffusely reflective) as desired, and protective. In this section, the term ***binder*** is used collectively to represent all similar compounds and components. Because of their function, binders may be referred to as **film-forming agents**, and they function in concert with the solvent system. Film formation, or curing, occurs by several mechanisms:[13,14]

- Polymerization, wherein resins polymerize as the result of heating or a catalyst.
- Oxidation, in which reactions between C═C sites in drying oil (discussed shortly) and oxygen lead to polymerization.



Methyl acrylate



Methyl methacrylate

Acrylic paint binders

FIGURE 29 The monomers used in acrylic paints.

- Solvent evaporation, in which evaporation allows film-forming agents to coalesce.
- **Coagulation**, wherein dispersed solutions of particulates coalesce into a networked structure. Latex paints are aqueous dispersions that cure by this mechanism.

Binders, vehicles, or resins may be of several types. Alkyd resins are among the most common and are composed of polymers of alcohols and acids—thus the term **alkyd** (*-alc* from alcohol, *-yd* standing for acid). Acrylic resins are based on polymers of methacrylate and methyl methacrylate (esters, Figure 29); similarly, vinyl resins are derived from vinyl chloride. Urethanes (polyurethanes) and some silicon-based resins are also encountered. Watercolor paints use gum arabic as a binder. This material is obtained from the sap of the acacia tree and, when dry, forms a clear, water-soluble polymer matrix.

Polyurethane resins are used as protective coatings, both as binders in paint or as separate protective clear coatings without colorant. Polyurethanes are made by linking existing polymer units possessing —OH end groups with a diisocyanate.[13] The process is outlined in Figure 30. The choice of the existing polymer and diisocyanate dictates the characteristics of the film that results. Some polyurethanes are used in two-part systems much like epoxy glues, wherein a separate hardener is added to initiate polymerization.[14] Thus, it is not surprising that there are epoxy binders as well, but they are not as common as the polyurethanes.

The polymerization of the binders may be speeded up by the addition of catalysts and drying oils and agents. The term *drying oil* is a misnomer, in the sense that the oils do not evaporate. Rather, they catalyze and speed film formation, which appears as drying. Perhaps the best-known drying oil is linseed oil, but fish and vegetable oils can be used. **Drying oils** are grouped on the basis of their drying characteristics; linseed oil is a drying oil, safflower oil is semidrying, and cottonseed oil is nondrying. All these oils are triglyceride oils with varying degrees of saturation. As saturation increases, the number of hydrogens bonded to the carbons in the hydrocarbon chain increases with a corresponding decrease in the number of C=C bonds. Lard, which contains highly saturated fats, is a waxy solid, whereas less saturated fats, such as vegetable and seed oils, are viscous liquids. The more saturated an oil is, the less active it is in drying.[14] This relationship can be understood in light of reactivity: A C=C bond is reactive toward oxygen, a key component in the drying process. A C—C bond is not reactive to any comparable extent, and C—C bonds dominate saturated systems, since a single C—H bond allows for more C—H bonds to exist.

The process that leads to the polymerization of oils is the same reaction that causes oils and foods containing high unsaturated fat content to go rancid. As shown

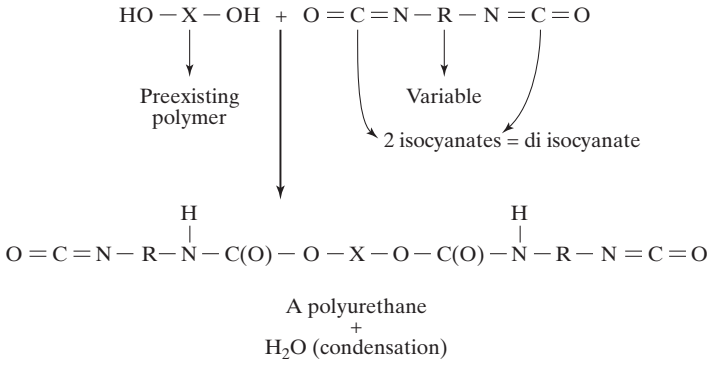


FIGURE 30 Formation of a polyurethane polymer. "X" and "R" can be varied to control the characteristics of the final polymer.

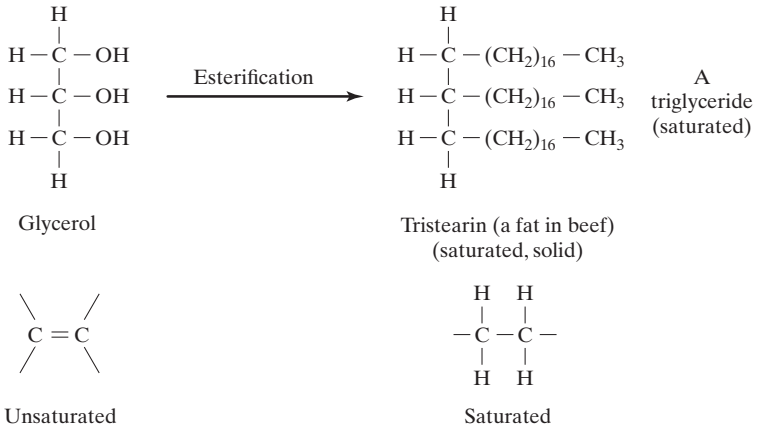


FIGURE 31 Esterification of the trialcohol glycerol yields a triglyceride. A saturated fat is maximally "saturated" with hydrogen and has no C═C reactive sites. Saturated triglycerides are waxy solid, whereas unsaturated triglycerides tend to be liquid oils.

in Figure 32, the double-bond sites can be oxidized by a free-radical mechanism that becomes a chain reaction that continues until polymerization is complete. In the case of paints, polymerization involves cross-linking to form a strong binding matrix for the pigments. The curing and polymerization process requires the loss of some solvent by evaporation: because the role of the solvent is to suspend and dissolve the colorants and polymerizing materials, some solvent must be removed to bring the polymerizing species into close enough proximity for the reaction to proceed. Additional drying agents, such as metals, may be added to speed the process. Examples include iron and cobalt, typically in the form of metal soaps[14] that form when the cation associates with the anionic end of the ionized fatty acid. This salt catalyzes both the formation of free radicals and the drying process.

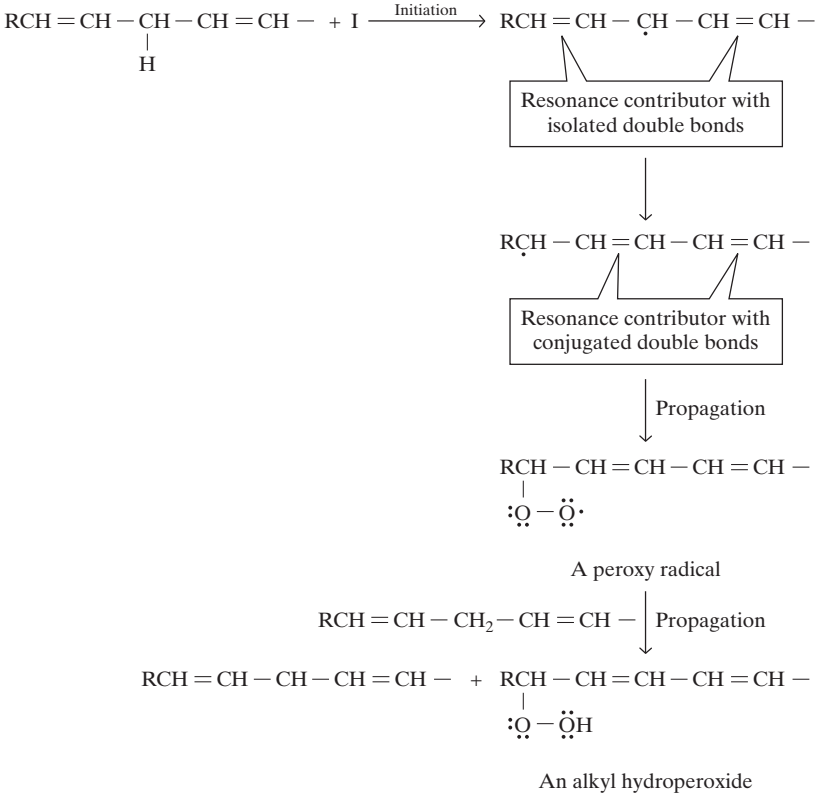


FIGURE 32 Polymerization of an unsaturated fat via a free-radical reaction.

5.3 Additives

Inks and paints contain small amounts of additives that may be of forensic interest. The drying agents already described are one example. Other additives, including plasticizers such as phthalates, are inserted into resins to impart flexibility to the polymerized mixture. These additives lessen brittleness and cracking. Antifoaming agents are also found in paints; recall the use of micelles to stabilize particulates in emulsions and in colloidal suspensions. Stabilization is frequently achieved by adding surfactants to paint (generically called **dispersing agents**). Surfactants, however, can cause foaming, which is undesirable because foam bubbles can form and mar the painted surface. Foaming is also undesirable from the manufacturing point of view, so antifoaming agents are used to control this tendency when it occurs. Finally, water-based paints such as latexes contain antimicrobial agents to prevent fungus, mildew, and other forms of microbial degradation.

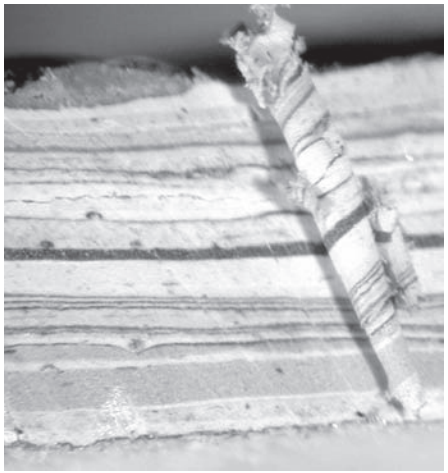
5.4 Automotive Paints

Among the most common types of paint evidence exhibits, automotive paint is worthy of brief mention. Cars are painted in a complex layering system that itself can be highly informative. Often, the layering pattern is sufficient to differentiate two automotive paint samples. Each layer has a specialized function and distinctive chemical characteristics. Exterior surfaces of cars are made of steel, plastics, and composite materials and are coated to protect against moisture and UV light. For steel, corrosion protection is required. The simple layering system presented in Figure 26 provides the starting point for more complex systems seen in the industry. That three-layer application is referred to generically as the clearcoat–base-coat system, which may have one

EXHIBIT G

Paint Stratigraphy

The discussion of paint in this chapter focuses on chemical aspects, but the layering is an important source of information that can be uncovered with microscopic examination. In some cases, the layer structure is so distinctive that further examinations are unnecessary. The architectural paint sample shown here is almost like geological layering of soils (stratigraphy), and within the layers may be decades of history unique to the house or other structure from which it came. Photo courtesy of William Schneck, Northwest Microvision.



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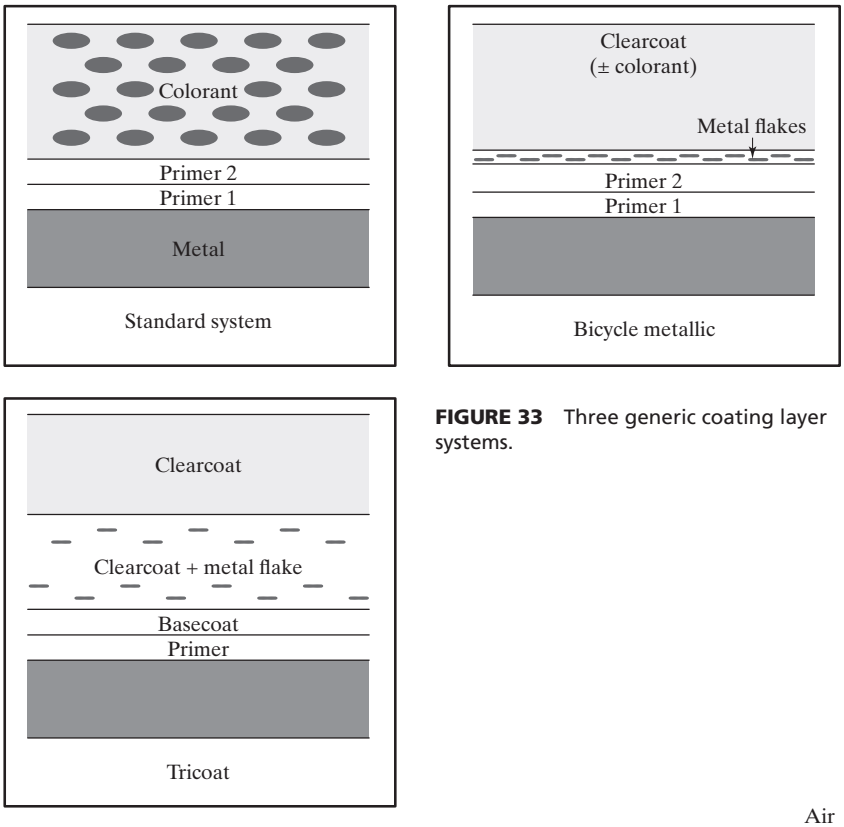

FIGURE 33 Three generic coating layer systems.

or two primer layers.[13] Examples of other systems are illustrated in Figure 33. The coatings on automobiles are designed to be smooth and glossy, another distinctive feature relative to household and other architectural paints.

Steel surfaces of a car are protected from corrosion by **galvanization** with zinc. Galvanization prevents corrosion of the iron in steel via anodic protection. Because of its standard half-cell potential, metallic zinc surrenders electrons and oxidizes preferentially to iron, becoming the anode in the corrosion redox reaction. The principle of anodic protection, illustrated in Figure 34, is of forensic interest because elemental analysis of galvanized surfaces will indicate that zinc is not associated with a pigment or other coating element. Applications of coatings are accomplished by spraying, dipping, baking, electrodeposition, and powder sprays, the last of which are increasingly used and are

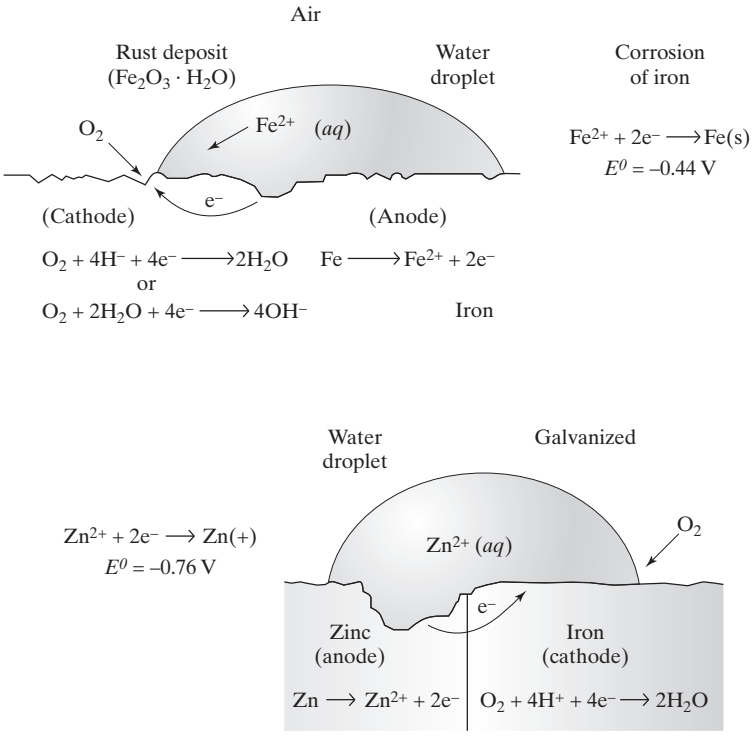

FIGURE 34 Use of zinc to prevent corrosion. In the absence of zinc, iron reacts with atmospheric oxygen and moisture to form rust. On a galvanized surface, the zinc acts as the anode and thus protects the iron from corrosion.

affixed by an imparted charge of static electricity to the part that is grounded. When a portion of the part is evenly covered, it no longer attracts the particulates, assuring a uniform layer. The coating is fixed in place with the use of heat, UV radiation, or other methods.[14] Powder spray application can be used for any layer.

Summary

This chapter completes our discussion of color fundamentals. We have covered which chemical structures create color, why they do so, and how they do it. Using color spaces, we have described how color, an inherently subjective property, can be described quantitatively. The next chapter will explore the principal types of evidence that involve colorants: inks and paints. The final chapter of the text will revisit colorants briefly in the context of fibers.

Key Terms and Concepts

Additive color system
Alkyd
Binder
Blackbody radiator
Carbitol
Cellusolve
Chroma
Chromaticity diagram
CIELAB
Coagulation
Color matching functions
Commission Internationale de l'Eclairage (CIE)
CMY
CMYK
Dispersing agent
Drying oils
Dyes
Extenders
Film-forming agent
Galvanization
Hue
Illuminant
Intaglio printing
Latex particle
Lightness
Metamerism
Mordant
Munsell color system
Pigments
Pleochroism
Resins
RGB
Saturation
Shade
Subtractive color system
Temperature
Toner
Tristimulus
Value
Vehicle

Problems

FROM THE CHAPTER

1. Methyl orange is a dye and acid–base indicator. Locate the structure and classify this dye on the basis of the systems presented in this chapter.
2. What is the difference between solvent evaporation, curing, and drying?
3. What is the core structure of a Rhodamine colorant?
4. Given the following data for a visible spectrum of an ink, derive the tristimulus values:

Wavelength	R (%)
400	10.0
420	12.0
440	8.0
460	12.0
480	19.0
500	65.0
520	75.0
540	65.0
560	65.0
580	22.0
600	12.0
620	10.0
640	5.0
660	4.0
680	6.0
700	2.0

What color is the ink? What dyes or pigments shown in the tables in this chapter might be responsible for the color, assuming that a single colorant is used?

5. A sample of writing by ballpoint pen is submitted for analysis. There is a suspicion that a suspect added two zeros to a stolen check, changing the amount payable from $10.00 to $1000.00. The writing is in blue ink that appears to be all the same color. You obtain two reflectance spectra—one from the "10" and one from the suspicious "00" in an attempt to see if the spectra can provide any additional information. You obtain the following data:

Wavelength (λ)	Ink sample 1	Ink sample 2
400	23.3	22.0
420	33.0	36.0
440	41.7	49.0
460	50.0	52.0
480	47.2	50.0
500	36.5	40.0
520	24.0	30.0
540	13.5	24.0
560	7.9	8.0
580	6.0	7.0
600	5.5	5.5
620	6.0	8.0
640	7.2	7.2
660	8.2	6.0
680	7.4	7.0
700	7.0	7.0

Plot both spectra on the same graph and calculate the chromaticity coordinates using the data provided in the chapter and offer an opinion, supported by the data available.

6. Review the structures of some of the dyes and pigments presented in this chapter. Are there any that would *not* show some absorption in the IR range?

INTEGRATIVE

1. Why are the orange and red dyes typically smaller molecules than the blue dyes? Relate your answer to the fundamental principles of color.
2. Drugs and dyes share at least two general similarities as a group of compounds. Discuss.
3. Locate the structure of chlorophyll and categorize it. Might this substance be found in items of physical evidence? Suggest an analytical scheme to identify it.
4. An older resin system used in automotive paints exploited cellulosic-based resins such as nitrocellulose. What are the components of this resin system? How does the film form?

FOOD FOR THOUGHT

1. Why are white boards (dry-erase boards) nonporous? For writing on such boards, what type of dyes or pigments and solvents would be best? Would special additives be needed? For what purpose? Justify your answer.

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The Chemistry of Polymers

OVERVIEW AND ORIENTATION

Polymers are a prominent form of physical evidence. Polymers are evidence in their own right, as in adhesive tapes, or as the substrate for colorants—for example, in paper and fibers. Thus, we can think of the previous chapter as describing the coatings and toppings placed on substrates described in the pages to follow. The common theme is polymers, biological and synthetic.

1 POLYMERS

Polymers can be classified in different ways. From the forensic perspective, a reasonable starting point is to divide polymers into biologically derived polymers (**biopolymers**) and synthetic organic polymers. Biopolymers are extracted from natural sources such as plants or animals. Even though proteins and DNA are biopolymers of unquestioned importance in forensic science, their analysis resides in the context of forensic biology. The biopolymer we will concentrate on is **cellulose**, the base material in paper and cotton fibers. Historically and chemically, semisynthetic polymers fall between naturally derived and synthetic polymers. **Rayon** and **cellophane** are made from regenerated cellulose and were among the first synthetics made, introduced in the late 1800s. Nitrocellulose is also known as guncotton, one of these regenerated materials. Semisynthetics bridge the gap between the naturally derived materials and the synthetic polymers that emerged in the 1930s.

Polymers are composed of linked **monomer** units. The monomers may be all the same or different (making **copolymers**) and with many different types of linkages, as shown in Figure 2. The monomers may be small and simple, as in PVC, or complex, as in nylon. Polymeric solids have characteristics that lie between the extremes of an ordered crystal (a crystalline substance) or an **amorphous** solid possessing little or

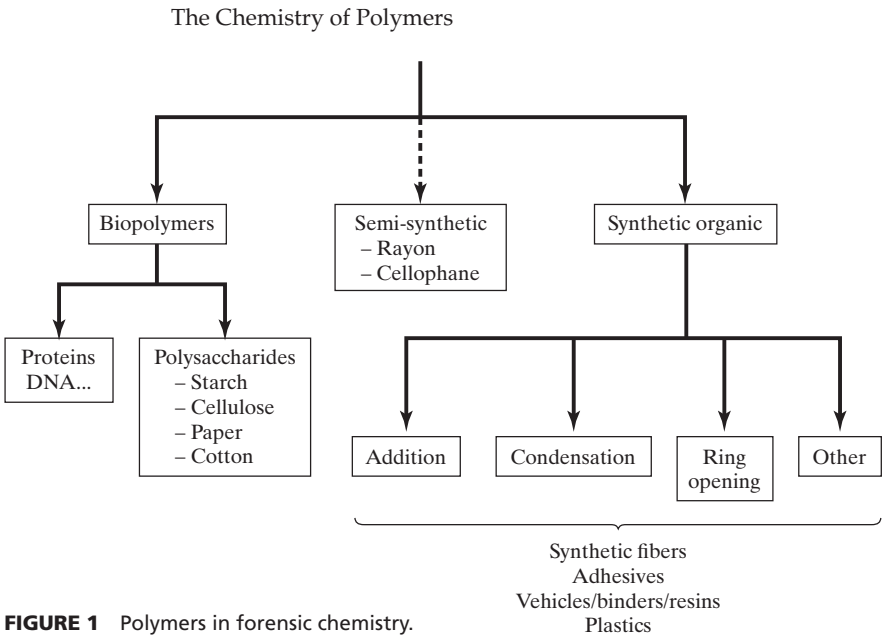


FIGURE 1 Polymers in forensic chemistry.

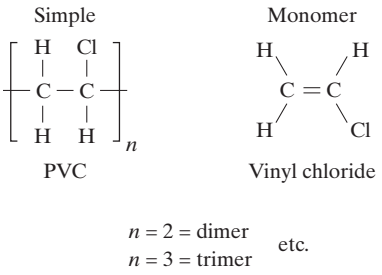
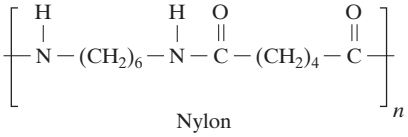


FIGURE 2 Polymers are composed of monomers in chains, with *n* indicating a number of repeating units. Vinyl chloride is an example of a simple monomer that polymerizes to form polyvinylchloride, or PVC. The monomer of nylon is more complex.



no organized structure (*a-morphus*, "without morphology"). Glass is an example of an amorphous solid that lacks internal organization. Polymers with repeating units and some degree of order are **pseudocrystalline**, a characteristic that is crucial to understanding the behavior of fibers and other similar materials studied by means of polarized light microscopy. Copolymers come in a variety of configurations, including random, block, and grafted types, as shown in Figure 3. Finally, polymer strands can be connected and interconnected in various ways, as illustrated in Figure 4. Descriptors can be combined, as in the case of a cross-linked random copolymer. The chemical characteristics of the monomers, together with their crystalline nature, linkages, and organization, give a polymer its chemical and physical properties.

For materials of forensic interest, such as coatings, paints, and fibers, the degree of crystallinity is important in determining the thermal response of the polymer

Random

X—Y—Y—Y—X—X—Y—X—Y...

Block

X—X—X—Y—Y—Y

Alternating

X—Y—X—Y

Graft

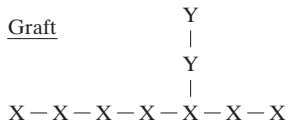


FIGURE 3 Possible arrangements in copolymers.

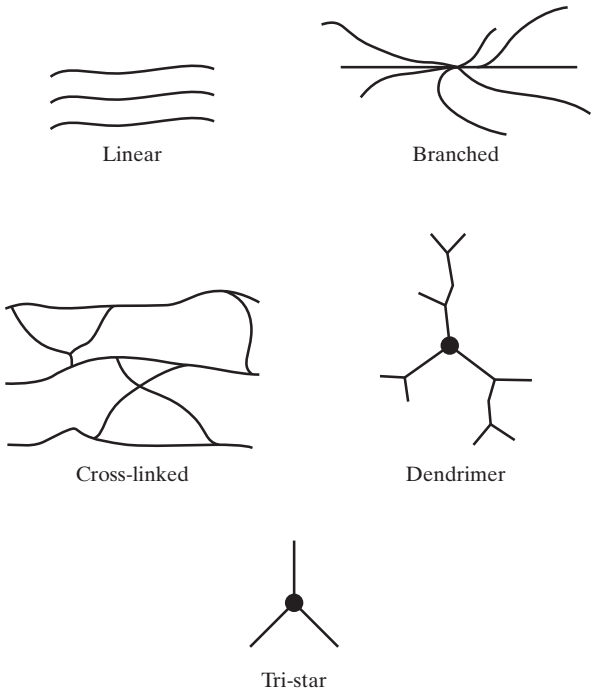


FIGURE 4 Modes of linkages in polymers.

(Figure 5). A polymer with a more amorphous character is a solid similar to glass at lower temperatures. A transition to a more rubbery solid occurs at the **glass transition temperature** (T_g). This temperature is not the melting point of the polymer but, rather, marks a temperature at which the tension in the polymer backbone lessens sufficiently to impart flexibility, but not flow.[1] The polymer's softness increases with temperature, with no further distinctive phase transitions. In a more crystalline polymer, a distinct melting point exists, as does a distinct liquid phase. Collectively, these changes with temperature are referred to as **thermoplastic behavior**.

Polymers frequently contain additives to alter their characteristics. One common additive is a **plasticizer**—a material that softens a plastic and reduces its rigidity.

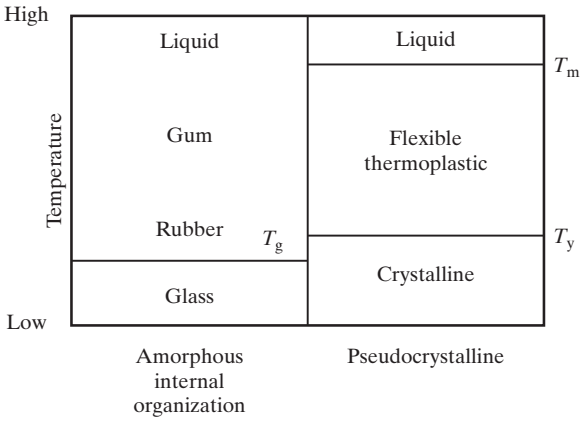
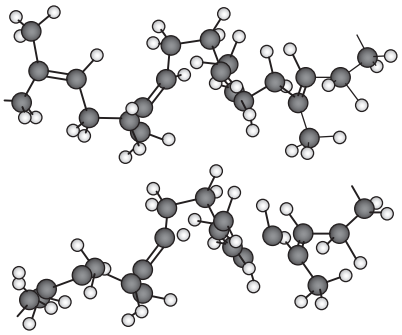


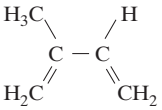
FIGURE 5 Response of polymers to temperature as a function of crystallinity. T_m is the actual melting point (solid → liquid).

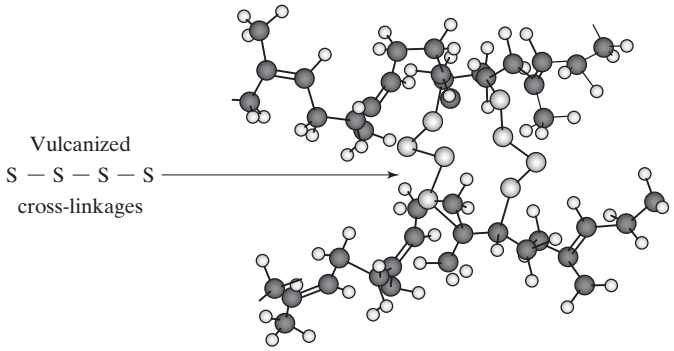
EXHIBIT A

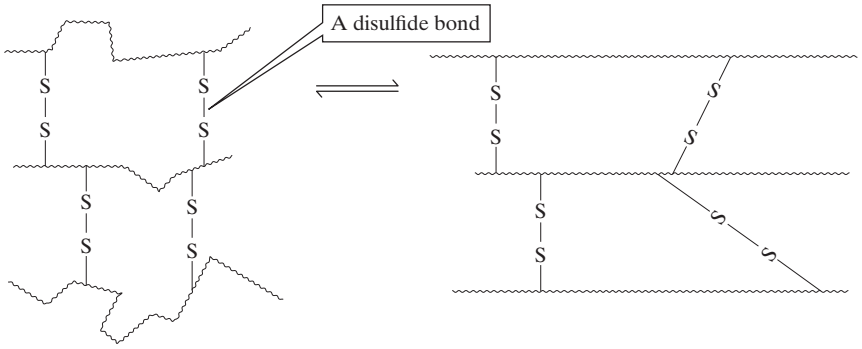
The Rubber Meets the Road



Rubber
(isoprene monomers)







The first useful polymerization reactions were conducted in the late 1840s. One important raw material in early work was rubber, which comes from the exudates of a tree. The vulcanization of rubber (a reaction with sulfur that produces a sulfurous volcano-like smell) was one of the first reactions studied. This reaction not only creates cross-linkages in rubber that improve its performance in materials such as raincoats but also imparts flexibility and memory so that stretched rubber (i.e., stretched within limits) will return to its original shape when the force is released. The discoverer of vulcanization was a man named Charles Goodyear, who was not a chemist but an inventor. Interestingly, he did not found the Goodyear Company, which is the largest maker of tires in the world and a leading manufacturer of polymeric materials such as resins and adhesives. Goodyear Tire and Rubber Company was founded in 1898, 38 years after Charles Goodyear died several thousand dollars in debt.

A tough coating is desirable to protect a layer of paint, but if the coating is too rigid, it will be prone to cracking. Consider the paint job on a car. The layers of coating sit atop metal that expands and contracts with temperature. If the paint layers cannot flex, they will crack. Similarly, polymers used in tubing, such as that from a Bunsen burner to a gas outlet, need to be flexible, whereas polymers used to hold carbonated beverages have to be rigid and strong enough to contain elevated pressures. Phthalates are a common group of plasticizers, and there are also antiplasticizers. However, the first polymeric group we will consider is one that initially has no additives: the biopolymers based on **polysaccharides** found in plants.

2 BIOPOLYMERS: POLYSACCHARIDES

Aside from proteins, another important class of biopolymers in forensic science are the polysaccharides. These polymers consist of carbohydrate monomers such as glucose. Sugars are classified as carbohydrates, and the formula of simple sugars such as glucose ($C_6H_{12}O_6$) can be expressed as "hydrated carbon," or $C_6(H_2O)_6$. The simplest polysaccharides are dimers (disaccharides), many of which are familiar. Table sugar is a disaccharide consisting of fructose linked to glucose. Saccharide monomers such as glucose exist in open-ring and closed-ring conformations, with the closed ring preferred. When glucose forms a ring (the hemiacetal form), carbon 1 (Figures 6 and 7) is converted to a chiral center yielding two diastereoisomers. This carbon is

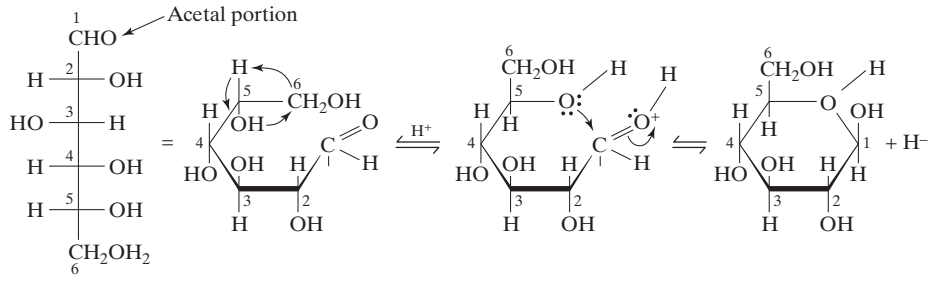


FIGURE 6 Different views and forms of glucose. The asterisk (*) indicates the anomeric carbon. The ring form is the monomer found in glucose polymers such as starch.

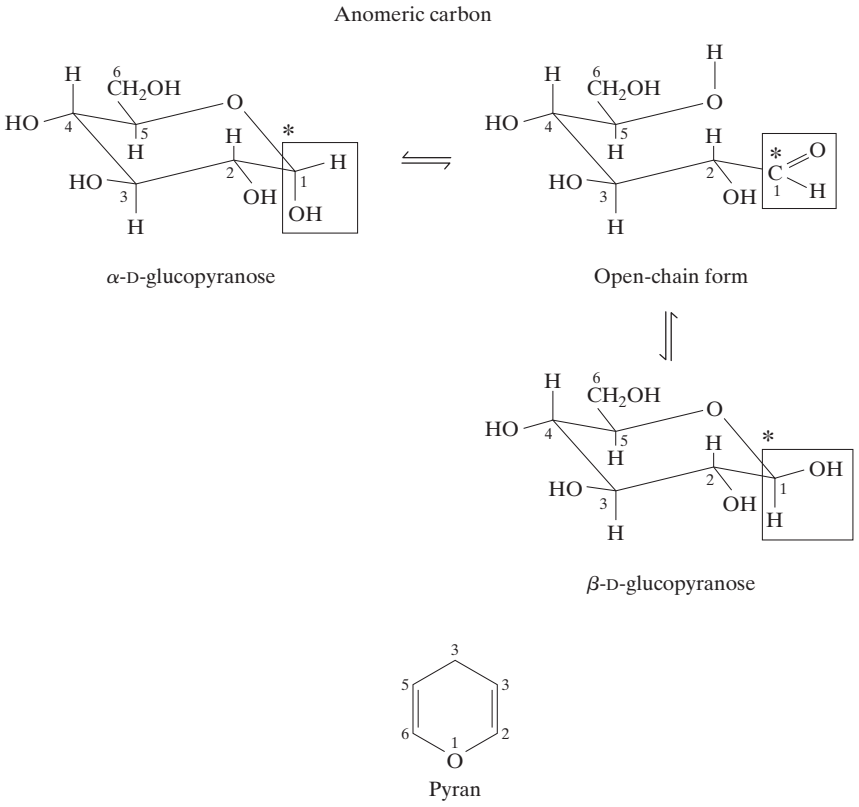


FIGURE 7 Formation of the ring converts C1 to an anomeric carbon, leading to a pair of diastereoisomers.

referred to as the **anomeric carbon**, and in the case of glucose, there are two anomers: the α-D-glucopyranose and the β-D-glucopyranose. The term **pyranose**, used to describe a six-membered ring structure, is nomenclature derived from the structure of pyran (Figure 7).

The existence of an anomeric carbon on a monosaccharide implies that dimers, trimers, and polymers linked through this carbon assume different geometries. Disaccharides form when two sugars link, and water is lost (Figure 8). The resulting structure depends on how the anomeric carbons are involved and how they interact. The three possibilities are shown in Figure 9. Sucrose results from a linkage formed between the anomeric carbon of glucose and the anomeric carbon of fructose.

There are four categories of saccharide-based biopolymers that are of interest to forensic chemists and forensic toxicologists, as shown in Figure 10. The first is **chitin**, the tough material found in the exoskeletons of insects and arthropods. The monomer is an amide formed from the replacement of a carbon in glucose with an amide group. Chitin (Figure 11) is the polymer that results from β-1,4′ linkages of the monomer. Because the carbonyl group forms strong hydrogen bonds with the N — H hydrogens, chitin is a strong but rigid polymer. Consequently, an insect must shed its exoskeleton periodically to grow. Forensic entomotoxicologists examine insects found associated with cadavers in an effort to identify characteristic metabolites of drugs or poisons. To do so, digestion of the chitins is required.

The remaining categories of biopolymers are based on glucose or are closely related to glucose structures. Glucose polymers are formed via different linkages, leading to a family of glucose-based polysaccharides, as shown in Figure 12. These polymers are also referred to as **vegetable fibers** or **anhydroglucose polymers**. **Starches** are glucose polymers used by organisms to store energy. Starches are differentiated from one another on the basis of how the chains connect and branch. Animals store excess glucose in the form of **glycogen**, a highly branched form of starch. To access a glucose unit, it has to be on the end of the polymer chain, so this design is an efficient way to store energy in a rapidly accessible form. The other components of starch are **amylose** and **amylopectin**. Amylose makes up about 20% of starch and is a linear glucose polymer. The monomers are linked by α-1,4′ bonds, which allow the chain to form a helix.[†] The other component of starch is insoluble amylopectin, which is also based on glucose monomers, but this time linked by both α-1,4′ and α-1,6′ bonds, as shown in Figures 13 and 14, respectively. The branching is found approximately every 6–12 monomer units, and the overall size of amylopectin may reach a million glucose monomers.

Cellulose is structurally similar to amylose and is a linear chain of glucose molecules. The difference is in the linkage. Cellulose is composed of β-1,4′ linkages, rather than the α-1,4′ type found in amylose. This seemingly small alteration results in significant differences in the chemical and physical properties of cellulose

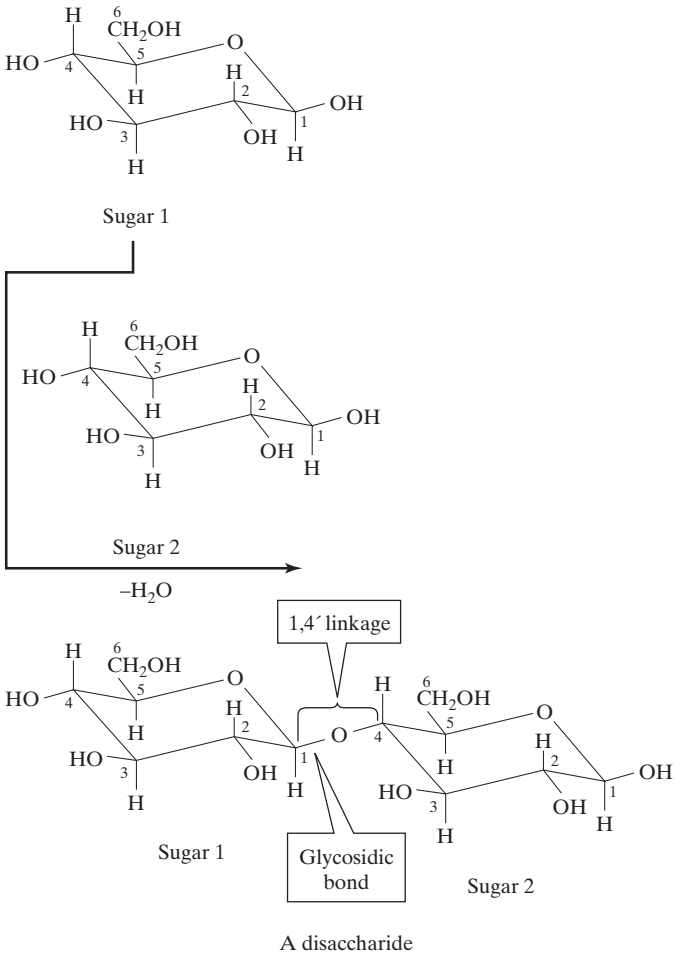


FIGURE 8 Formation of a disaccharide results in loss of water and the formation of a linkage. The type of linkage is important in determining the physical and chemical characteristics of the dimer.

EXHIBIT B

Sides and Tides

Polysaccharide is a generic term for a polymer composed of sugar monomers. This category is further broken down into disaccharides (two sugars), trisaccharides, and other polymer units consisting of 10 or fewer units. Collectively, these smaller polymers are called *oligosaccharides*. When the polymer chain exceeds 10 monomers in length, the term *polysaccharide* is used. In biochemistry, analogous nomenclature is used. A nucleotide unit consists of a ribose molecule linked to a phosphate group and base unit (adenine, guanine, etc.). DNA and RNA are polymers consisting of nucleotides. The term *oligonucleotide* is used to describe smaller nucleotide polymer units.

[†]The starch–iodine complex used as an indicator in titration reactions is formed when I_2 resides inside the helix.

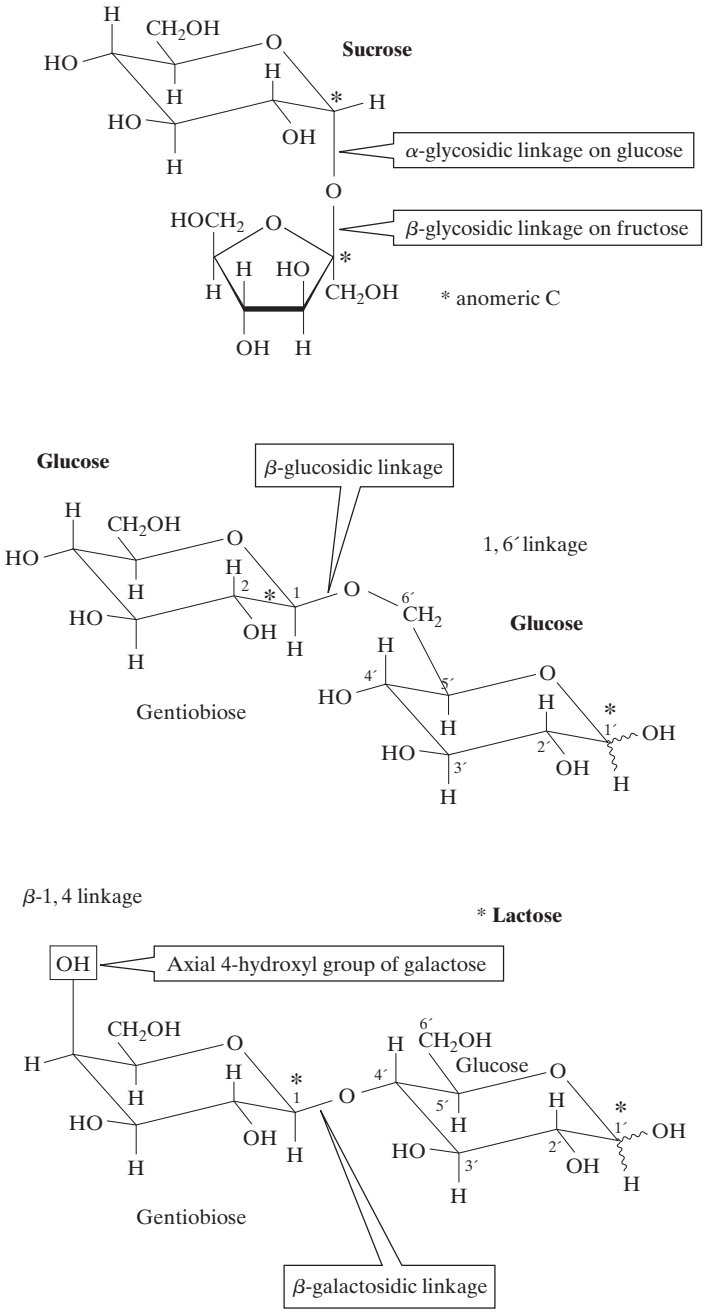


FIGURE 9 Different linkages possible in disaccharides and polysaccharides in general.

compared with amylose and is a common theme that will be seen throughout the discussion of polymers. The linkage in cellulose allows for hydrogen bonding, as shown in Figure 15, and imparts a strength and rigidity to cellulose that is lacking in amylose. Cellulose is insoluble in water and is used in plants as structural support in the form of microfibrils. Although cellulose is a glucose polymer, many animals, including humans, are not able to digest it because they lack the enzyme β-glucosidase necessary to break the linkages.

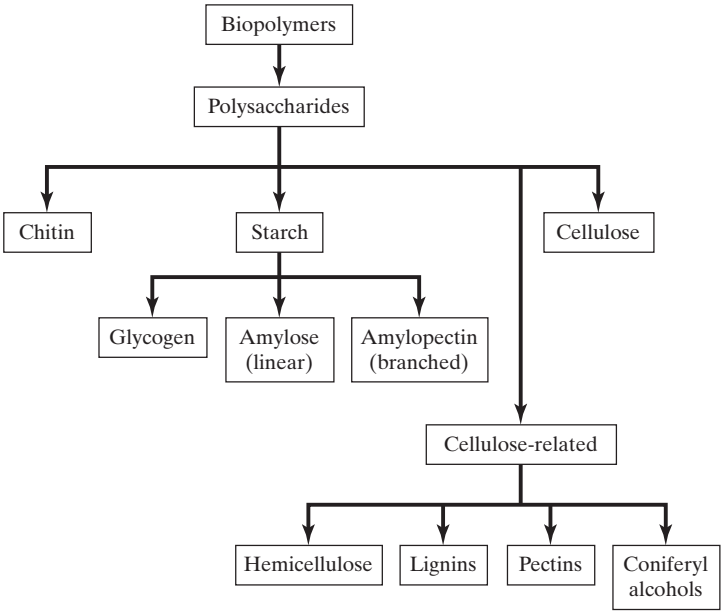


FIGURE 10 Biopolymers.

Cellulose is closely related to other biopolymers found in wood and plants. Together, these compounds are the raw materials used in a number of products encountered in forensic chemistry. For example, paper is made from wood chips derived from soft- or hardwoods, plants, or recycled paper stocks. Of interest in paper production are the polysaccharides found in wood—**lignin**, **hemicellulose**, and cellulose, all

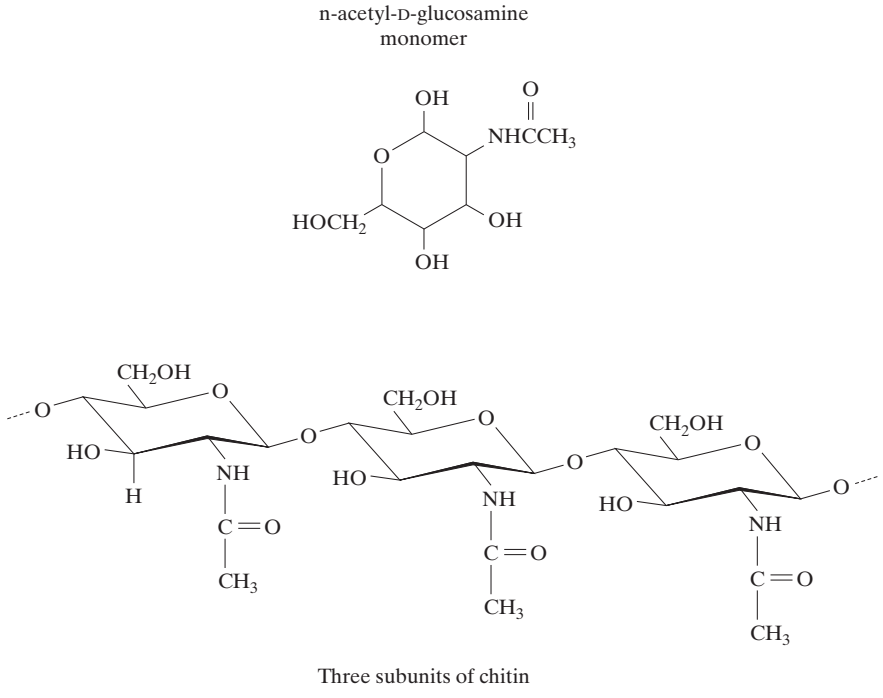


FIGURE 11 The monomer and structure of chitin.

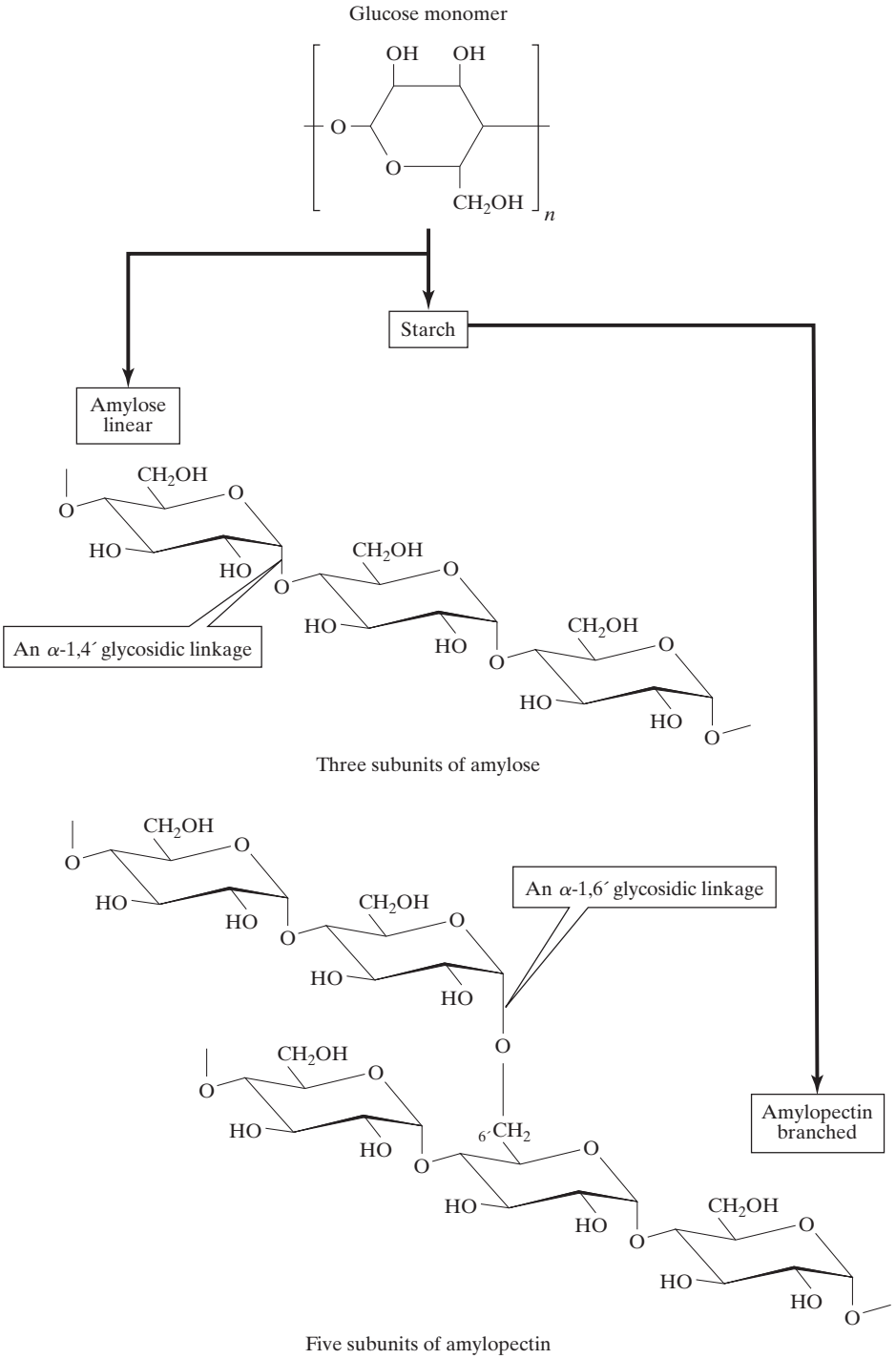


FIGURE 12 Starch is composed of two types of glucose polymers that differ only on the basis of their linkages.

classified as part of the **lignocellulose** complex found in biomass such as wood. The lignocellulose compounds impart strength and varying degrees of rigidity to plants. For example, a tree that stands several feet tall needs a different support framework than do mosses and grass, which are of little use in modern paper production. The organization of fibrils, structures in plants that contain lignocellulose compounds, is shown in Figure 16.

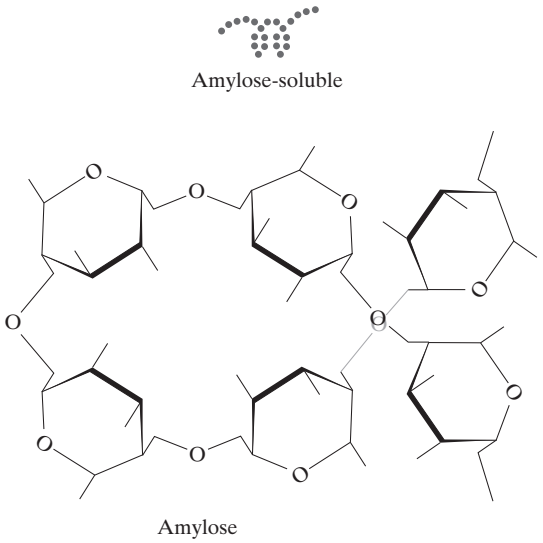


FIGURE 13 Amylose is linear and curls into a helix in water.

Typical wood samples contain approximately 40% cellulose,[2,3] and cotton is nearly 90%. Cellulose has a pseudocrystalline structure in the form of fibrils that are bonded together by lignin and hemicellulose.[4] Hemicellulose is also a saccharide-based biopolymer composed of subunits of glucose, galactose, mannose, and xylose, to name a few.[5] The most common subunit is xylans[5] as shown in Figure 17. Hemicelluloses constitute approximately 25% of wood solids.[2,3] Lignins are the most complex of the lignocellulose compounds and the least well characterized. When a plant decomposes, lignins produce humic and fulvic acids; lignins are also a precursor

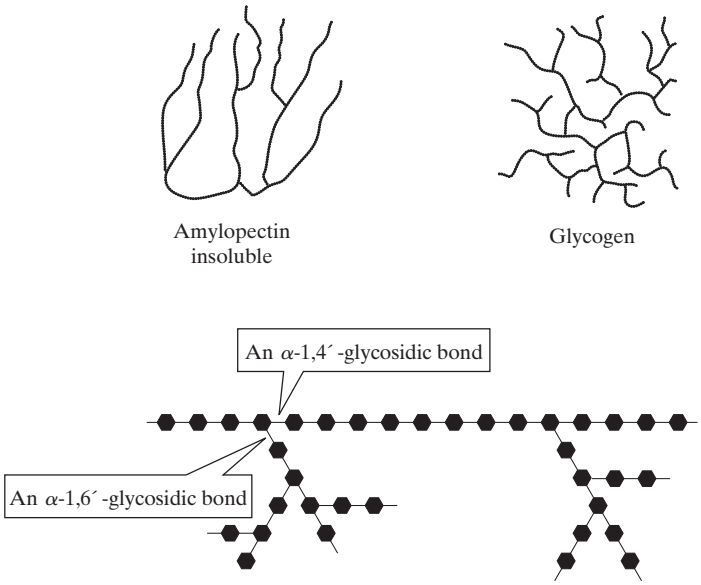


FIGURE 14 Amylopectin is branched, but not as branched as glycogen.

EXAMPLE PROBLEM 1

Forensic biologists make extensive use of starch gels to perform electrophoretic separations. Explain how starch forms a gel matrix.

Answer:

The helical structure affords the opportunity for strong intermolecular hydrogen bonds to form with water within the helix, causing it to swell and form a semirigid structure. When a starch gel dries, it forms a flimsy, thin film.

of peat and coal.[6] Lignins are a family of related biopolymers based on phenylpropyl units linked by strong C — C bonds that are resistant to many degradation pathways.[7] When extracted, lignins have a gluelike consistency—not surprising in light of their function.

2.1 Natural Fibers

Cotton fibers are the most prevalent of the natural fibers encountered in forensic laboratories. Other natural fibers include kapok, hemp, silk, and animal hairs, the last two of which will not be addressed here. Cotton is classified as a **seed fiber**. The cotton plant is a shrub that grows to a height of a few feet and produces pink flowers that fall off and leave behind a seed pod called a *boll*. The cellulose fibers grow inside until the boll breaks open when it is ready for harvesting. The raw cotton fibers are yellowish to

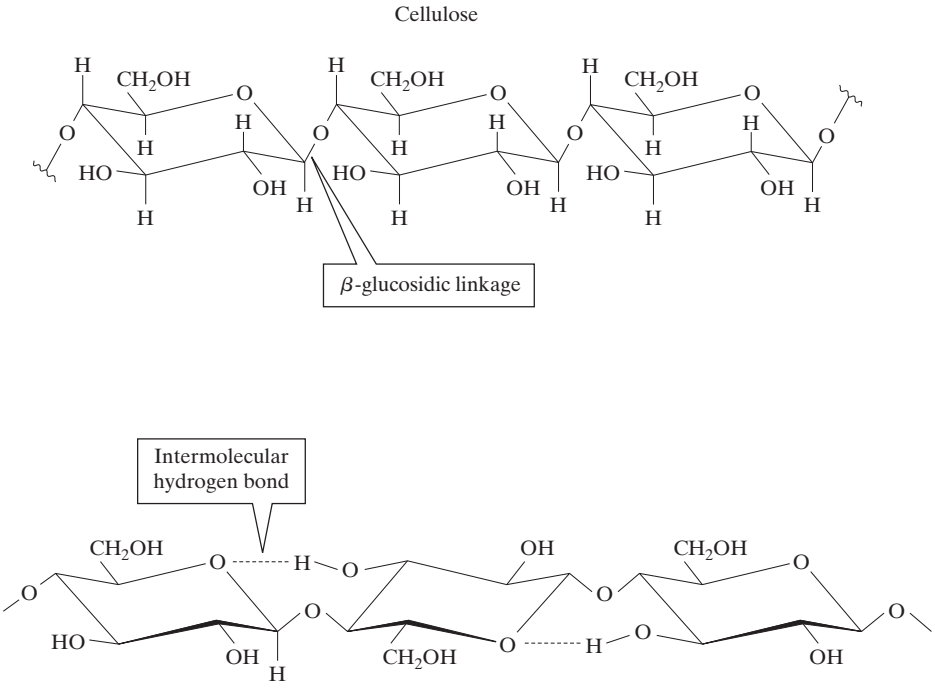
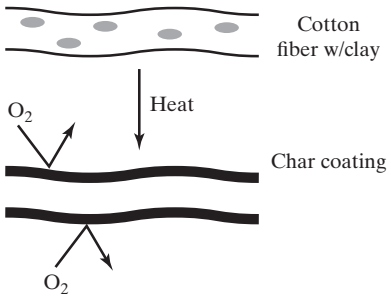


FIGURE 15 Cellulose forms linear chains strengthened by hydrogen bonds.

EXHIBIT C

Fireproofing Cotton

Cotton clothing is popular because it is comfortable to wear and easy to work with. However, it is highly flammable and thus is not recommended for use in applications such as children's sleepwear. One method of increasing the fire resistance of cotton apparel has recently been proposed. Researchers dissolved the cellulose from cotton and added montmorillonite clay particles before reconstituting the fibers. When heated, these treated fibers form a char layer on the surface that prevents oxygen from penetrating, thereby impeding combustion by depriving the process of the oxidant.



Source: Goho, A., "Textiles," *Science News* 165 (2004): 253.

white and up to 2″ long. The seeds are removed by a cotton gin, and the baled fibers are shipped for processing such as raking and spinning into yarns or fabrics.[8] Because cotton is so common, cotton fibers are typically not very useful as evidence,[9] although there are always exceptions.

Cotton fibers are easily identified by polarizing light microscopy and appear as thin, twisted ribbons. Although cotton lacks a uniform crystalline structure, cellulose has ordered regions that will interact with polarized light. However, these regions are randomly located, so cotton lacks distinctive extinctions or birefringence, also called *incomplete extinction*. Given the ease of identification with optical techniques, there is little reason for further instrumental analysis, unless other classifications are possible on the basis of dyes or other treatments. The —OH groups in cellulose are often targeted as sites for interaction, as illustrated in Figure 18.[10]

2.2 Regenerated and Reformulated Cellulose: Semisynthetics

The first manufactured fibers were regenerated forms of cellulose classified as semisynthetics. Cotton is a versatile fiber, but because the fibers are short, cotton yarns and fabrics consist of these shorter fibers spun together rather than single contiguous fibers; as a result, cotton is not a strong fiber. However, the —OH functionality of cellulose provides a chemical handle that is exploited for the generation of contiguous cellulose fibers. In the simplest process, raw materials such as wood are treated with a strong base,

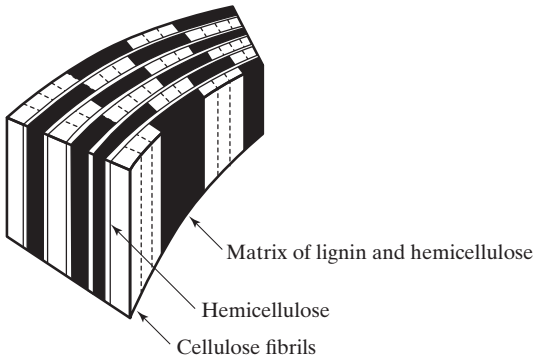
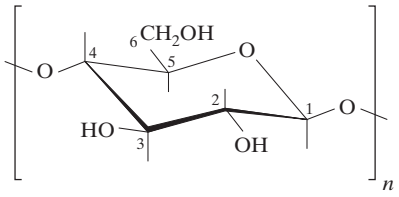


FIGURE 16 The structure of a fibril in plants.

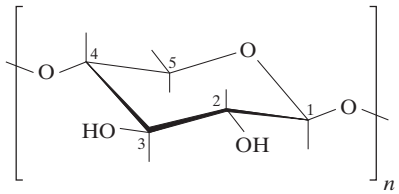
EXHIBIT D

Dyes, Drugs, and Now, Polymers

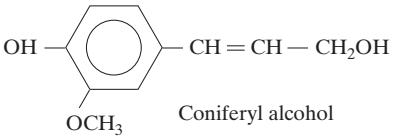
Previous Exhibit boxes have pointed out the linked history of drugs and dyes, which emerged in the late 1800s as bulk products. Polymers can now be added to that list. Rayon was first synthesized in 1865, but the first moldable polymer was introduced a decade earlier. Celluloid, a polymer made from nitrocellulose and camphor, was created as a substitute for ivory. Celluloid worked well and was used in applications such as billiard balls. Unfortunately, nitrocellulose (guncotton) is flammable and explosive, leading to predictable problems in pool halls. Celluloid was also used for making films, another inherently dangerous process, given film's proximity to hot projection bulbs. Eventually, cellulose acetate replaced celluloid in this role. Meanwhile, rayon was discovered by accident, as are so many breakthrough products. Frenchman Louis Chardonnet was working with nitrocellulose during an effort to find a replacement for silk. He spilled some nitrocellulose on a tabletop and when he wiped it up, noticed filaments forming. Cellulose acetates were pursued by two Swiss brothers, Henry and Camille Dreyfus, who went on to form the Celenase Chemical Company, named after their flagship product. The name was derived from the comfortable wearing characteristics of their cellulose acetate–based fabrics. The Dreyfus brothers began work in 1904 by making a synthetic dye but decided that the polymer industry had more promise. The company remains a primary supplier of many polymeric materials and precursors.



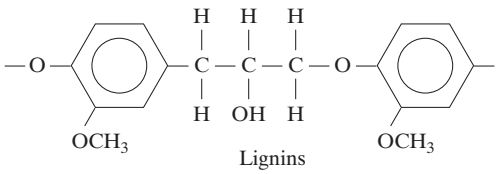
Cellulose



Xylan
(hemicellulose)



Coniferyl alcohol



Lignins

which promotes the partial oxidation of the cellulose and decreases the degree of polymerization by about a factor of three.[9] Carbon disulfide is then added to create cellulose xanthate, a thick, viscous solution that can be extruded through an orifice as a single long fiber. The extruded fiber enters a coagulating solution containing sulfuric acid and sulfate salts.[9] The outer surface forms first and then shrinks and wrinkles as the inner cellulose re-forms. The surface is much smoother than that of cotton fibers. The regenerated material is highly reflective and appears shiny if it is not treated with delustering agents. The characteristic sheen is the origin of the name *rayon*, referring to the shine as giving off rays of light. In addition to being made into fibers, the viscous solution can also be cast as a thin film, producing cellophane.

Both cotton and rayon are cellulose, but because of the extrusion process, their properties are significantly different. Even though rayon exists as a long fiber, the degree of polymerization of rayon is about ten times lower than that of cotton cellulose. Rayon has less of a crystalline nature than cotton, with predictable consequences based on Figure 5. Because of these properties and the silky sheen (luster) of rayon, it is used for undergarments and other delicate items. One variant of rayon is **hollow viscose**, made by adding Na_2CO_3 to the extruded solution. When hollow viscose is placed in the acidic coagulation bath, any Na_2CO_3 trapped inside the fibers is converted to CO_2,

FIGURE 17 Biopolymers related to starch. Hemicellulose is composed of xylan and other monomers. Coniferyl alcohols and lignins are important monomers in other structural polymers extracted from wood.

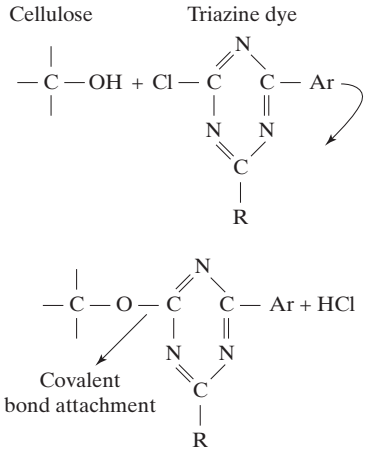


FIGURE 18 The covalent attachment of a dye molecule to cellulose via the —OH functional group. The dye molecule in this example has an active halogen site.

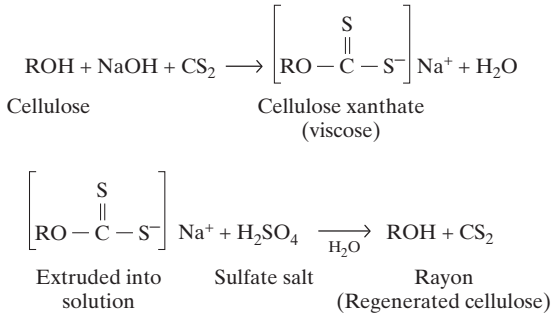
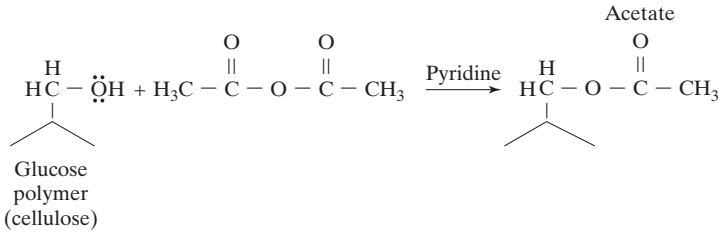


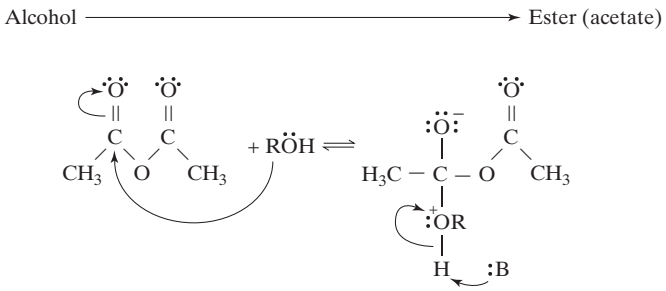
FIGURE 19 Generic process for regeneration of cellulose.

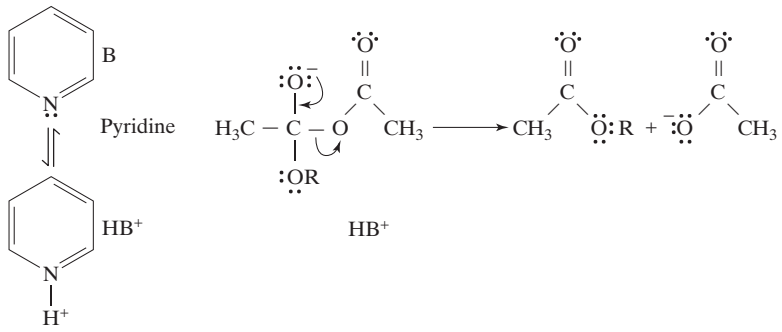
EXAMPLE PROBLEM 2

Cellulose acetate is another regenerated polymer based on cellulose. It is made by treating cellulose with acetic anhydride (seen previously in the conversion of morphine to heroin) and pyridine. Show this reaction.

Answer:







producing a void in the structure. There are a number of other production modifications in use that result in rayon with yet other chemical and physical properties.

The hydroxyl groups of cellulose can be esterified with the use of acetic anhydride to produce a group of semisynthetic **cellulose acetate** fibers. The chemical treatment of the raw wood pulp begins with soaking in acetic acid, followed by acetic anhydride. In any cellulose monomer unit (Figure 15), there are three —OH groups that are vulnerable to esterification, and when the reaction occurs, the product is a triacetate. Unlike rayon, the polymer is dried and ground up before being reconstituted in solvent and extruded.

2.3 Paper

Paper is made from wood pulp, consisting of fibers that are processed ("**beaten**") to incorporate water into the matrix. The generic steps are shown in Figure 20. Dewatering removes much of the moisture and allows hydrogen bonds to form between fibers, the interaction that gives paper mechanical strength. Chemical additives are introduced at several points in the process. Paper manufacturing is an example of mass production, and as a result, the batch-to-batch variation is small by design. From a forensic perspective, this small variation affords few opportunities to individualize paper, but assigning class characteristics is feasible.

The paper production cycle starts with wood chips or other ground-up starting material. Once reduced in size, the chips are moved to the pulping stage, where lignins are broken down and the fibrils are separated. Pulping is typically accomplished by treating raw pulp with NaOH and Na_2S at alkaline pH.[11] The process is referred to as the Kraft process or **Kraft pulping**, but there are many alternative names for it. Once the lignin is broken down, the pulp that remains is a brownish color that can be bleached

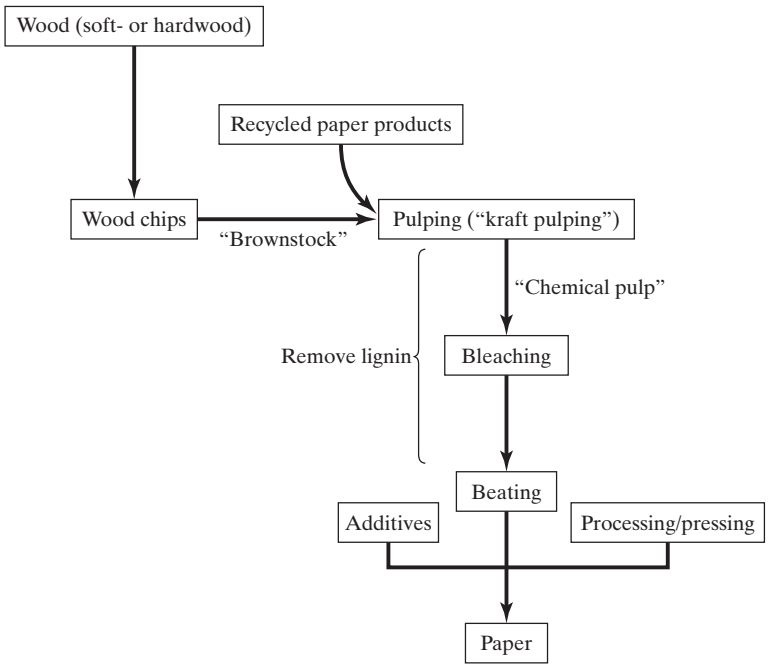


FIGURE 20 A generic outline of paper production.

by oxidizing agents to a whiter color and to remove additional lignin.[12] Chlorine gas was once the bleaching agent of choice, but environmental concerns have led to the development of alternatives such as ClO_2 and H_2O_2. One consequence of bleaching is the conversion of some of the end groups of cellulose to carboxylic or lactone forms.[2] The drug GHB, has a lactone form, GBL.

The beating stage serves several purposes. Large fiber structures are broken down mechanically, resulting in a roughened surface and internal changes in the fibers. These changes increase the potential for hydrogen bonding and create a matrix of fibers that can hold water and other materials. Fibers also become more flexible with beating. Approximately 35% of the lignin component is typically dissolved during beating. Hemicelluloses are also degraded to a comparable extent.[12] Although paper is dry to the touch, the fiber matrix is capable of holding significant quantities of water via ion–dipole and dipole–dipole interactions. The sites within the fiber matrix that can interact with water are shown in Figure 21; Figure 22 illustrates routes of water penetration into paper fibers.

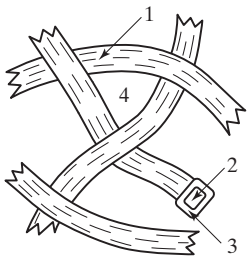


FIGURE 21 Places where water can interact with cellulose fibers: (1) on the surface; (2) internally; (3) within the wall of the fiber; (4) within spaces between fibers.

The beating stage is important for facilitating the associations of water and encouraging fibers to swell. Fillers can be added at this stage to fill voids in the fiber matrix and to give paper its desired physical characteristics and appearance. Additives include starches, binding agents, fibrous components, finishing agents, whiteners, cotton fibers, and colorants (dyes). Fillers range from clay materials such as kaolin (hydrated aluminum silicate) to $CaCO_3$ and TiO_2. Fillers have many functions, including altering the feel of paper, modifying its weight, and changing its optical properties such as reflectivity (gloss) and absorbance. Cotton is often an ingredient in paper because it is high in cellulose but low in lignin. In general, the higher the quality of the paper, the higher is the cotton or "rag" content. Fine writing papers are high in cotton content and have a thickness, strength, texture, and feel different from paper used for copiers, printers, and other mechanical printing applications. Some papers are treated with buffers to prevent gradual acidification of the medium; the cover page of this book indicates that the book is printed on acid-free paper. As a method of preservation, this technique ensures that the book will survive at least long enough for a bedraggled student to sell it back in reasonable shape at semester's end. The wet strength of paper—particularly products such as paper bags and cardboard—can be increased by the addition of binders and agents that increase the strength of the attractive forces between fibers.

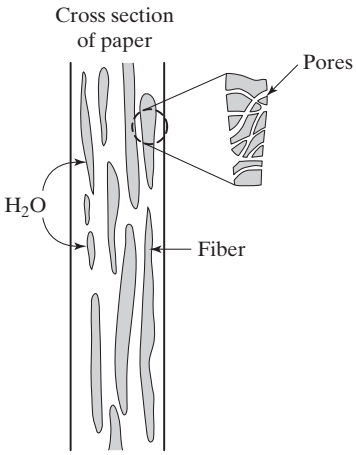


FIGURE 22 Ways for water to interact with, and be absorbed by, paper.

Once the pulp has been treated and lignin broken down, the preparation process involves combining the remaining ingredients, mixing them, and diluting the mixture into a thin slurry, which is poured over screens that capture the fibers as they are shaken. The fibers form a network held together by hydrogen bonds. Because excess water will disrupt hydrogen bonding between fibers, a heating and pressing stage follows, but not all of the water is removed. Coatings can also be added as required. A watermark may be placed in the paper at this stage. A watermark is a thinner area of the paper that is emblazoned with a manufacturer's characteristic mark that is visible when the paper is held to the light. Cutting is part of the process as well. Thus, in addition to chemical characteristics, striations and toolmarks may be useful in classifying papers. Figure 23 illustrates typical fiber matrix geometry in the final sheet of paper. The paper surface or the matrix itself can absorb water, which then moves through voids and fiber pores therein. Not surprisingly, excess water causes paper to lose strength because of the disruption of the hydrogen-bond matrix holding the

TABLE 1 Anionic Groups in Paper

Chemical group	Approximate pK_a
Phenolic	7–8
Carboxylic	4–5
Alcoholic	13.5–15
Hemiacetal*	12

Source: Turku, Finland: DT Paper Science, 1991. Used by permission.

*Recall from organic chemistry that a hemiacetal is formed when an alcohol is added to an aldehyde. For example, the addition of methanol to acetaldehyde results in formation of a compound with an — OH and an — OCH_3 group bound to what was the carbonyl carbon.

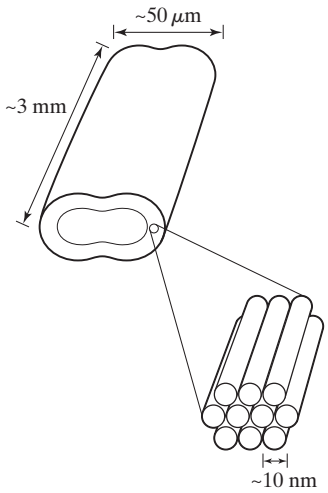


FIGURE 23 A processed cellulose fiber in paper, showing approximate sizes and the geometry of packed fibrils.

fibers together. Finally, a number of ionizable groups exist in paper and are important for processing and paper chemistry. All of these ionizable groups, summarized in Table 1, form anions.

Dyes and pigments can be incorporated into paper in many ways, including impregnation and surface treatments. Solubility is an important concern, as is the mechanism of binding. Other materials that are part of the papermaking process are foam- and slime-control agents, required to prevent problems with the machinery used to manufacture the paper.

3 SYNTHETIC POLYMERS

The cellulose fibers in paper are the starting material for regenerated fibers such as rayon and cellulose acetate which together form the historical bridge from biopolymers to completely synthetic fibers. Synthetic rubber was created in Germany in 1917, but from the forensic perspective, a much more important advance was the synthesis of **nylon** (specifically, nylon 6,6) in 1935. The discovery of nylon is credited to Dr. Wallace Carothers, who worked at DuPont Chemical Corp. Initially, his work was with esters and phenols, but he became interested in amides for possible use in the then-infant world of polymer science. What would become known as nylon was developed in 1935 and commercialized in 1939, initially for women's hosiery. World War II jump-started the polymer industry, and many advances quickly followed. The emphasis here will be on fibers, with later sections in the chapter examining other applications of synthetic polymers.

As a first-pass chemical classification, synthetic polymers can be categorized by how they are produced. The two major categories (Figure 24) are **chain growth** and addition, with **ring-opening polymers** sometimes placed in a separate category. Polydimethylsiloxane (PDMS) and related silanes utilized in chromatography are generated by ring-opening reactions. Addition polymers are also called **condensation polymers**, owing to the loss of chemical species such as a water molecule. Chain-growth polymers react and link via an active site. Regardless of their chemical mechanisms, polymerization reactions usually require the presence of an initiator, which can be a chemical or energy such as thermal or light energy. The initiator may also be a catalyst, but this is not always the case. Recall that a catalyst speeds a reaction by lowering its activation energy and that the catalyst is not destroyed in the process. It may be inert or regenerated, but it is not consumed. An initiator usually is consumed during polymerization, although a few initiators are also catalysts.

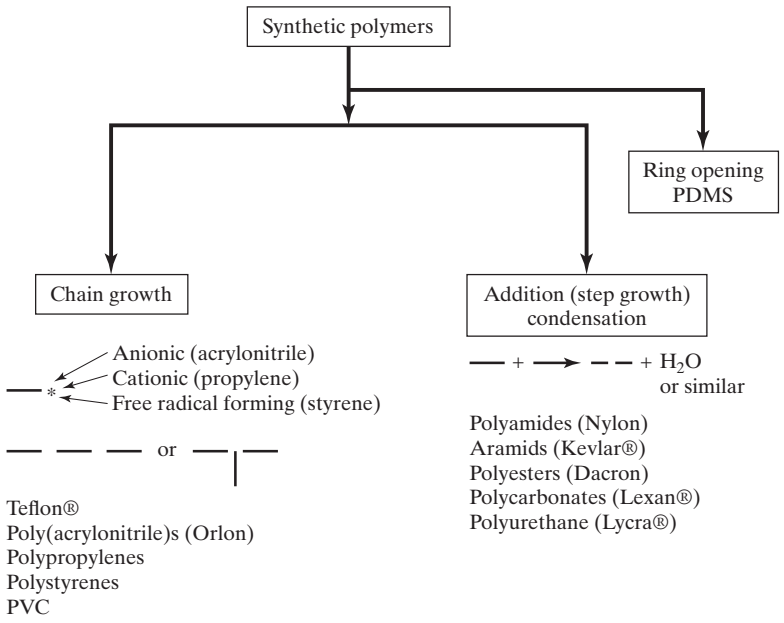


FIGURE 24 One approach to the categorization of synthetic polymers.

3.1 Chain-Growth Polymers

Chain-growth polymers grow by reactions occurring at an active site on the monomer or existing chain. Polymerization occurs via free radicals, anions, or cations generated by the initiator. Many monomers are capable of different mechanisms; styrene polymerizes by all three routes.

Radical reactions start with the formation of the radicals by initiators such as peroxides. As shown in Figure 25, radicals react with monomers to create more radicals that combine with monomers. The process continues until the chain reaction is stopped by one of three mechanisms, all of which involve the combination of two radicals. Monomers capable of free-radical polymerization include styrene, vinyl chloride, and methyl methacrylate. Chemical initiators include H_2O_2 and other peroxides and potassium persulfate. Most initiators have weak O—O bonds that cleave homolytically (i.e., an electron pair splits in half) to form radicals, as in peroxides.

EXHIBIT E

Teflon

In a repeat of a theme seen in many scientific discoveries, one of DuPont Chemical's most famous and profitable polymers was discovered by accident. Teflon, a fluoropolymer best known for use in cookware, is highly resistant to most solvents and acids and is used in analytical chemistry in applications such as soil and acid digestion. Teflon was accidentally made for the first time by DuPont chemist Roy Plunkett in 1938. Plunkett had been working with refrigerants based on chlorofluorocarbons when he returned to the lab one morning to find a waxy solid in a container where none should have been. Thus was Teflon born.

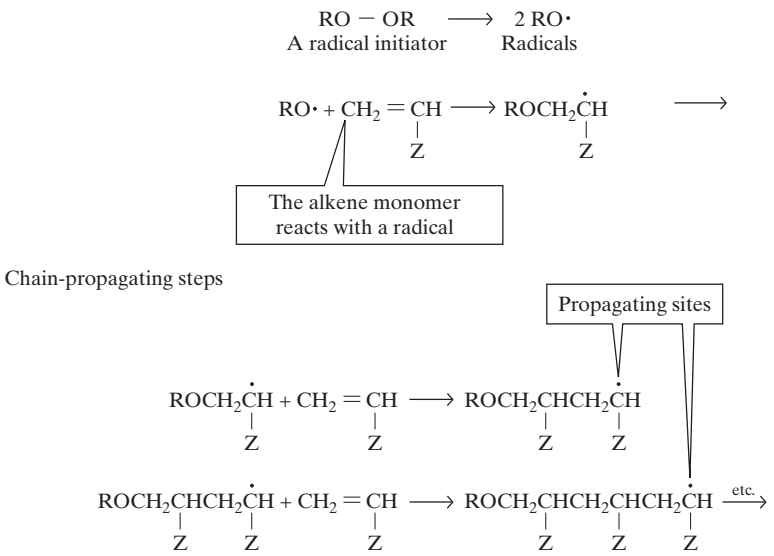


FIGURE 25 Polymerization via free radicals. Peroxides are common initiators, owing to their tendency to cleave homolytically to form radicals.

When radicals drive polymerization, branching can occur anywhere that a radical attacks hydrogen. The type and degree of the branching alter physical properties of the polymer. As shown in Figure 26, polyethylene is a chain-growth polymer that is formed by a free-radical mechanism. Reaction conditions can be controlled such that chains with numerous short branches result, yielding low-density polyethylene (LDPE, recycle symbol 4). The density is low because the branching is not conducive to tight packing, as are long strands. As a result, LDPE is a softer, pliable material with relatively low strength. It is used in grocery sacks and trash bags. When the branches are long, the chains pack more efficiently and produce high-density polyethylene (HDPE, recycle symbol 2), a rigid material that retains a molded shape. HDPE is used in food containers, shampoo bottles, and the like. Some of the polymers that are formed by chain growth are shown in Table 2.

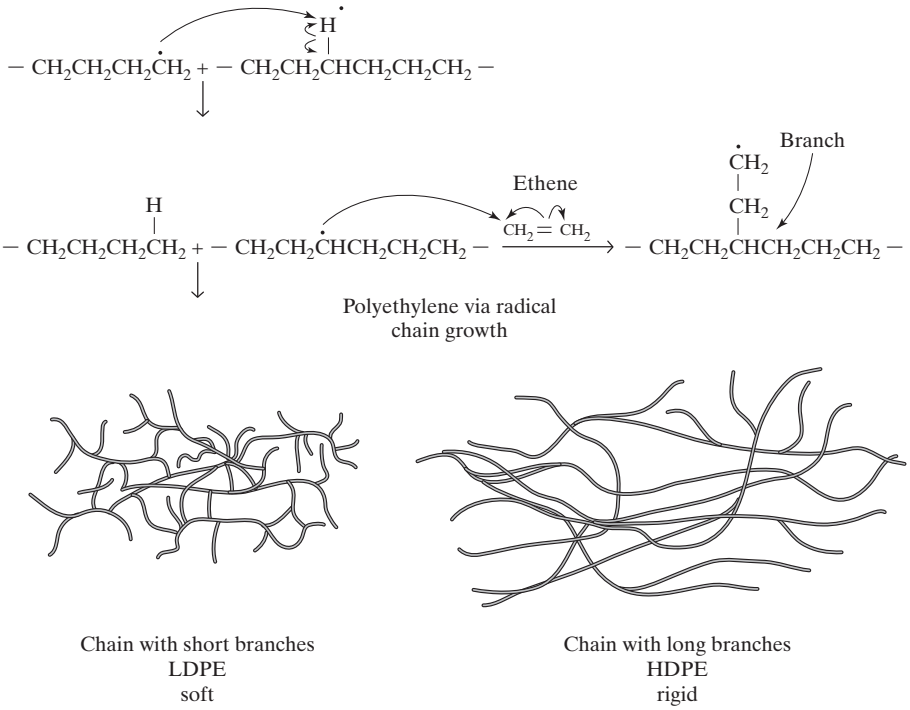


FIGURE 26 Branching and its effects on polyethylene.

Cationic polymerization begins when an electrophilic initiator attacks a nucleophilic (electron-rich) monomer, resulting in the formation of a cation such as a carbocation. The initiator is also called a catalyst and is usually a Lewis acid, such as BF_3.[†] The carbocation is attacked by another monomer, lengthening the chain and propagating the reaction, which terminates when the cation is neutralized. Three examples of cationic polymerization are shown in Figure 27. Anionic polymerization, illustrated in Figure 28, involves monomers with electron-withdrawing groups such as C═O, and the reaction mixtures are often highly colored due to conjugation.[13] The nucleophilic catalyst adds to the double bond in the monomer to form a carbanion that propagates the reaction until it terminates. Unlike the cationic case, in which proton loss and the formation of double bonds quenches the reaction, there is no such possibility here. Reactions with water or CO_2 are possible, as are reactions with materials purposely added to "kill" the reaction. Anionic polymerizations can also be **"living" polymerizations** for this reason. Unless a quenching agent is added, the carbanions will keep polymerizing until the monomer is gone.

Chain-growth polymers can be made from ringed monomers and ring-opening reactions. Epoxides, described briefly in the context of inks are common in ring-opening polymerizations, which proceed via cationic or anionic mechanisms, depending on the initiator (Figure 29). Monomers can also be activated by electromagnetic energy, such as the formation of radicals via photolytic and electrolytic methods.

3.2 Step-Growth Polymers

Monomers that polymerize by step-growth methods must have two active functional groups within their molecules. A familiar example is the combination of amino acids

[†]Recall that a Lewis acid accepts electrons, whereas a Lewis base donates them.

TABLE 2 Chain-Growth Polymers

Monomer	Repeating unit	Polymer name
$CH_2=CH_2$	$-CH_2-CH_2-$	Polyethylene
$CH_2=CH(Cl)$	$-CH_2-CH(Cl)-$	Poly(vinyl chloride)
$CH_2=CH-CH_3$	$-CH_2-CH(CH_3)-$	Polypropylene
$CH_2=CH$–C$_6$H$_5$	$-CH_2-CH(C_6H_5)-$	Polystyrene
$CF_2=CF_2$	$-CF_2-CF_2-$	Poly(tetrafluoroethylene) Teflon
$CH_2=CH(C\equiv N)$	$-CH_2-CH(C\equiv N)-$	Poly(acrylonitrile) Orlon®, Acrilan®
$H_2C=C(CH_3)COOCH_3$	$-CH_2-C(CH_3)(COOCH_3)-$	Poly(methyl methacrylate) Plexiglas®, Lucite®
$CH_2=CH(OCOCH_3)$	$-CH_2-CH(OCOCH_3)-$	Poly(vinyl acetate)
$nCH_2=C(CH_3)_2$	$\left(-CH_2=C(CH_3)_2-\right)_n$	Polyisobutylene (butyl rubber)
$nCH_2=CH-C(CH_3)=CH_2$	$\left(-CH_2-CH=C(CH_3)-CH_2-\right)_n$	*cis*-1,4-Polyisoprene (natural rubber)
$nCH_2=CH-CH=CH_2$	$\left(-CH_2-CH=CH-CH_2-\right)_n$	*trans*-1,4-Polybutadiene
$nCH_2=CH-C(Cl)=CH_2$	$\left(-CH_2-CH=C(Cl)-CH_2-\right)_n$	*trans*-1,4-Polychloroprene (Neoprene rubber)
$nCH_2=O$	$+CH_2-O+_n$	Polyformaldehyde (polyoxymethylene, Delrin)

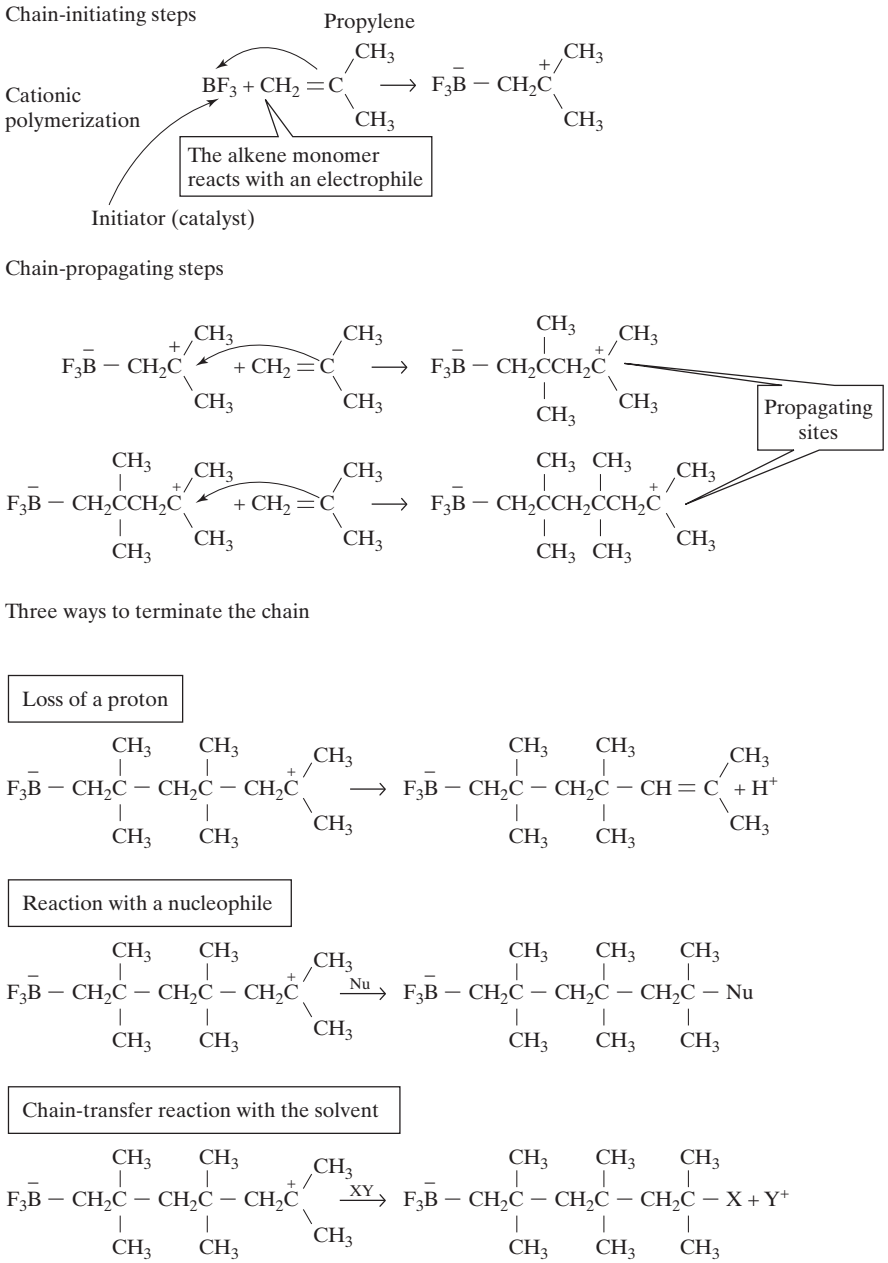


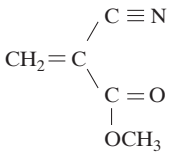
FIGURE 27 Cationic polymerization. The initiator is an electrophilic compound.

to form proteins (biopolymers) by the step-growth condensation mechanism shown in Figure 30. Amino acids have a carboxylic group and an amine group that is ionized, depending on the pH. This is the same pattern that was discussed relative to drug chemistry, wherein the same functionalities (amines and acid) are central. To form a dimer, the protonated amino group reacts with the protonated carboxylic acid group (acidic conditions), and water is lost—hence the term "condensation." There are no initiators in step-growth polymers, and there is no chain reaction.

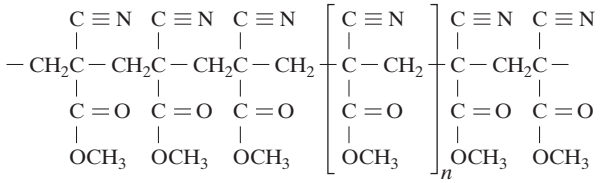
EXHIBIT F

Super Glue®

Methyl cyanoacrylate, the monomer used in Super Glue, polymerizes by an anionic mechanism. Its structure is typical of such monomers and consists of two electron-withdrawing groups that facilitate reactions with relatively weak nucleophiles such as the —OH groups found in cellulose. Through the formation of a strong polymer network, Super Glue will bind any two surfaces, such as paper, that contain initiator groups. Another common adhesive, epoxy, that works on a different principle is described near the end of the chapter.



Methyl α-cyanoacrylate



Super Glue

EXAMPLE PROBLEM 3

What types of monomers would be best suited for polymerization via a cationic process?

Answer:

Monomers that stabilize the positive charge. The most stable carbocations are tertiary and the least stable are primary, so a branched monomer or a monomer that contains stabilizing groups or one that can form stabilizing resonance structures is best suited to cationic polymerization.

The first completely synthetic polymer, nylon, is a polyamide that forms by step growth and condensation. Nylon 6 is a **homopolymer** made from 6-aminohexanoic acid, whereas nylon 6,6 is a copolymer. Kevlar® is closely related to nylon and is classified as an aramid because of the presence of aromatic rings. Kevlar's strength derives from a strong network of hydrogen bonds, as shown in Figure 32. Other condensation polymers are named on the basis of the type of linkages formed. Polyesters are held together by ester linkages, and polycarbonates are linked through carboxylate groups. Polyurethanes are interesting in that the step-growth process does not result in the loss of a small molecule. Some common step-growth polymers are shown in Table 3.

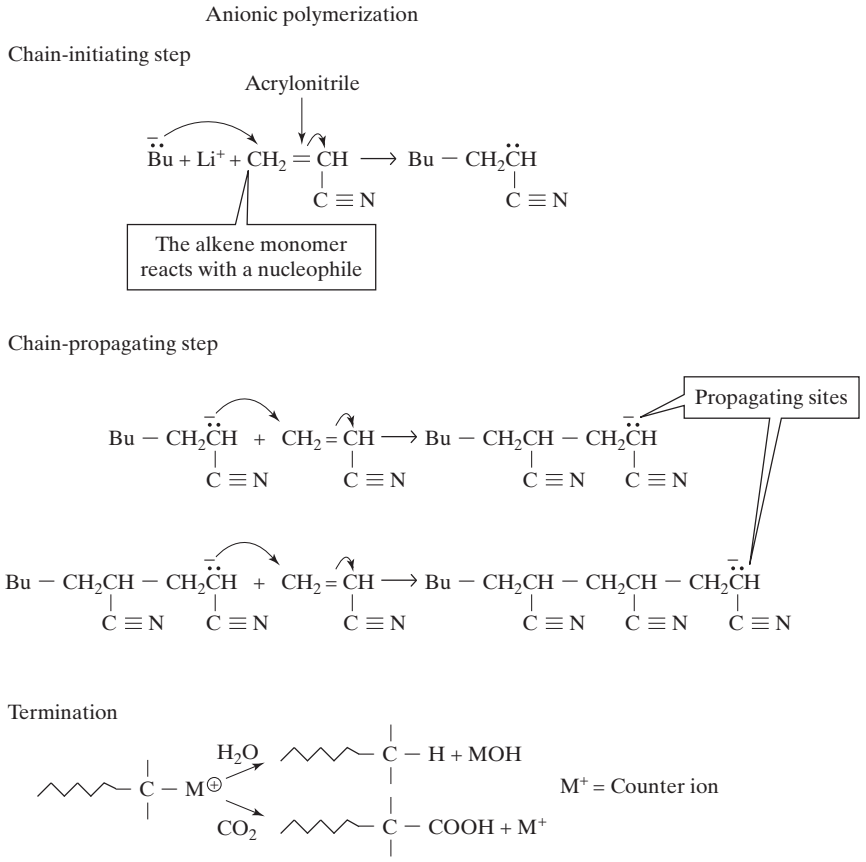


FIGURE 28 Anionic polymerization. The initiator is a nucleophilic compound such as the butyl lithium shown in this example.

EXAMPLE PROBLEM 4

Will a different pattern be observed for changes in the molecular weight of the polymer over time for chain-growth versus addition polymers?

Answer:

Yes. When an initiator is added to a chain-growth mixture, many monomers are activated, each becoming an active site to which other monomers will quickly add. Consequently, the molecular weight of the mixture jumps quickly. In contrast, step-growth polymers grow steadily, adding successively to the end of the chain. The molecular weight of the mixture increases gradually.

The last entry in Table 3, Melmac®, is representative of a large and forensically important class of formaldehyde-based polymers that includes phenol–formaldehyde, formaldehyde–melamine (triamino-*s*-triazine), and phenol–urea formulations. Melamine, shown in Figure 33, is one example. One of the earliest moldable polymers was Bakelite® and the associated group of "Bakelite" resins[14] synthesized in 1872. As shown in Figure 34, the initial reaction of phenol and formaldehyde generates prepolymers called resols that polymerize under heating and acidic conditions.[14] The form of the polymer depends in part on the temperature. Polymers made with urea and formaldehyde form

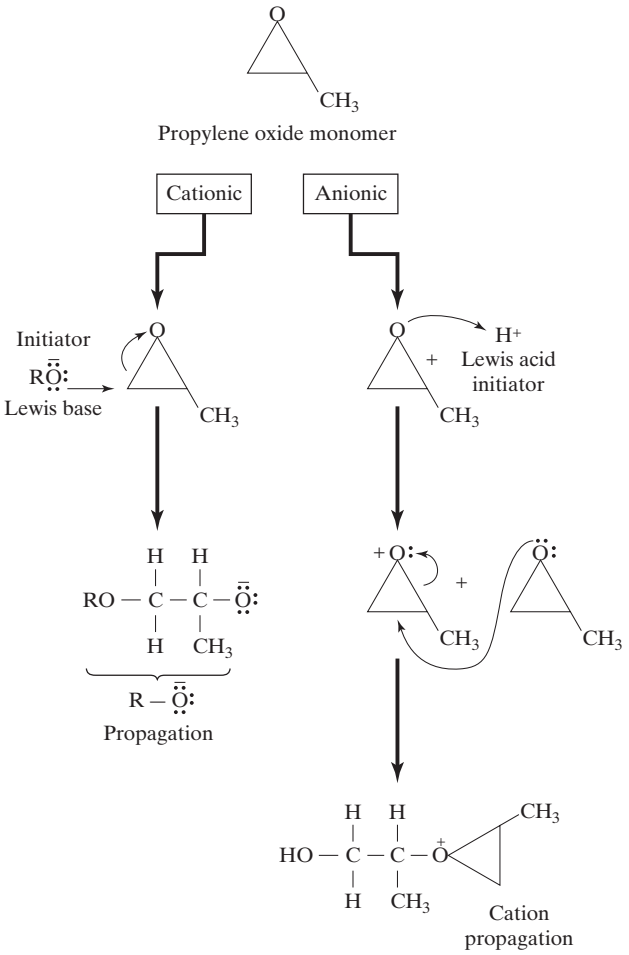


FIGURE 29 An example of polymerization based on ring opening.

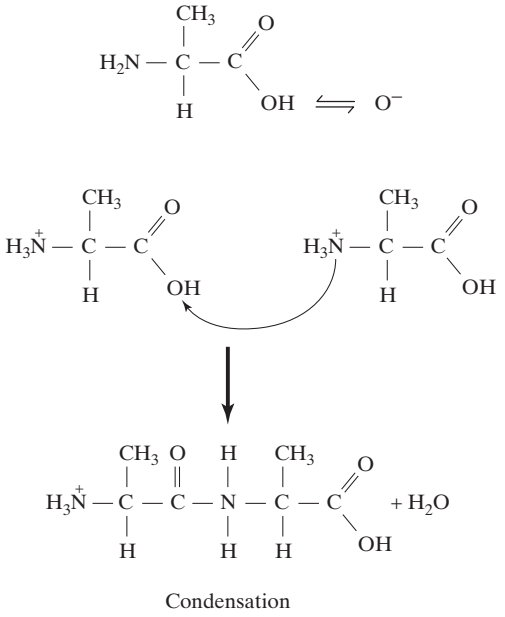


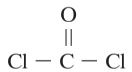
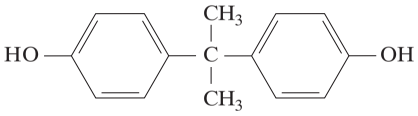
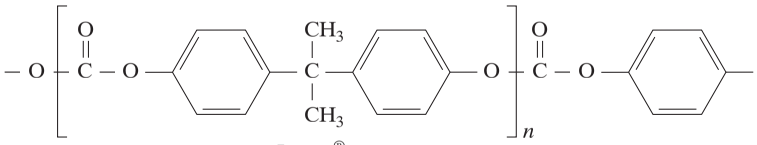
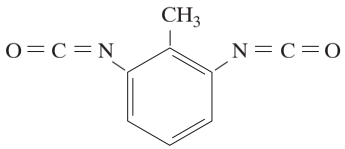
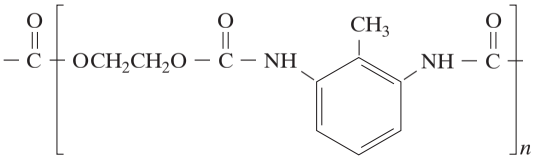
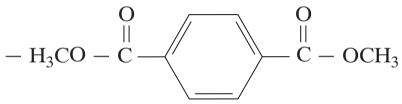
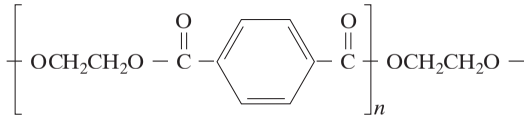
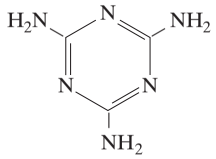
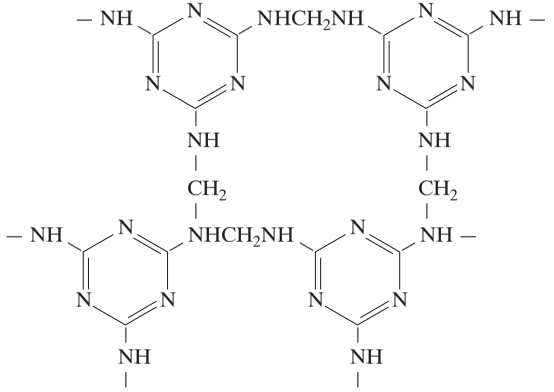
FIGURE 30 Amino acids such as alanine have dual functionality with pH-dependent activity. Most step-growth monomers are also bifunctional. A peptide bond forms by a condensation reaction between the amine group of one monomer and the carboxyl group of another.

tough, clear resins, as do melamine–formaldehyde combinations. Melamine resins are used as topcoats in automotive paint applications.

3.3 Physical and Chemical Properties

Before delving into the world of synthetic fibers, we need a few more generalizations and descriptions concerning polymers in general. As noted in the polyethylene example (Figure 26), the chemical structure and bonding within a polymer dictate its physical properties. Kevlar is strong enough to protect against bullet wounds because of cross-linkages of hydrogen bonds. Another way to engineer strength is to organize polymer chains such that all the strands are parallel in what is called an **oriented polymer**. Most polymers have some degree of order, owing either to chemical synthesis or physical processing such as drawing into strands. Figure 35 illustrates a polymer with ordered and random regions, a common occurrence. The more ordered a polymer, the denser it becomes, since chains can be lined up and packed tightly together. Increasing order also increases strength and heat resistance.

TABLE 3 Step-Growth Polymers

Monomer 1	Monomer 2	Lost	Polymer
 Phosgene	 Bisphenol A	HCl	 Lexan® (a polycarbonate)
 Toluene-2,6-diisocyanate	$HOCH_2CH_2OH$ Ethylene glycol	None	 A polyurethane
 Dimethyl terephthalate	$HOCH_2CH_2OH$ 1,2-ethanediol ethylene glycol	CH_3OH	 Poly(ethylene terephthalate) Dacron (a polyester)
 Melamine	$H_2C=O$ Formaldehyde	H_2O	 Melmac

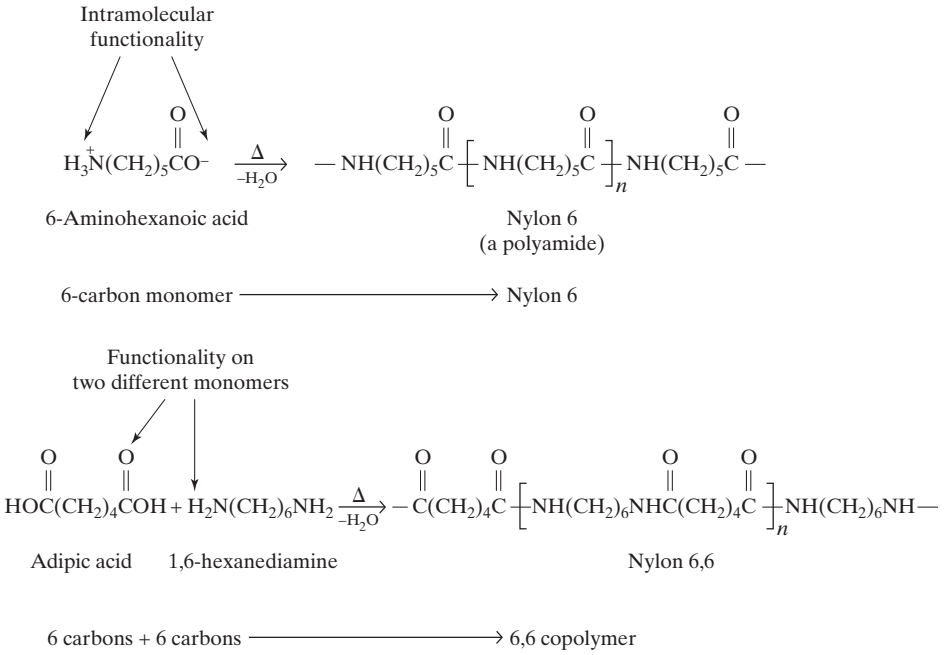
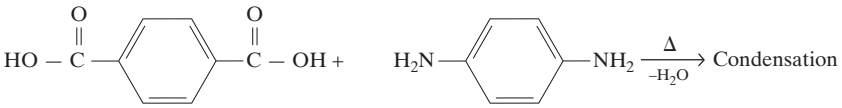


FIGURE 31 Nylon 6, a homopolymer made from a bifunctional monomer, and nylon 6,6, a copolymer made from monomers with different functionalities. The condensation reaction that forms nylon 6,6 has many similarities to the reaction whereby amino acids combine to form peptides, as shown in Figure 30.

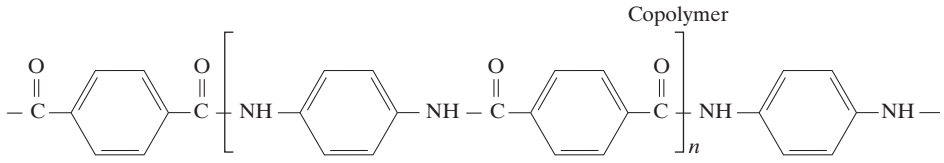
The behavior of polymers when heated is an important characteristic for manufacturing and applications. **Thermoplastics** are polymers with a mixture of ordered and random regions. At lower temperatures, the polymer is solid and holds its shape, but as it is warmed, the disordered regions facilitate motion and allow the polymer to be molded and shaped. Numerous consumer items are made of thermosetting plastic. **Thermosetting polymers** also respond to heat, but once they assume a shape, they cannot be heated and reshaped, because the heat promotes cross-linkages that are essentially irreversible. Thermosetting resins are used as topcoats in cars and form strong protective coats. However, the coatings lack flexibility and are prone to cracking and brittleness.

Elastomers are another large group of polymers classified by their ability to stretch (within limits) and return to their original shape. Rubber is an example of an elastomer. As described in Exhibit A, rubber was one of the first raw materials used in early polymer research. Because of its elasticity, untreated rubber flows when hot and becomes brittle when cold. It also will pull completely apart with little applied force. All of these properties are undesirable for applications such as rainwear and tires. When Goodyear vulcanized rubber, he introduced disulfide linkages into the polymer that stiffened the material to a certain extent but preserved some flexibility and moldability.

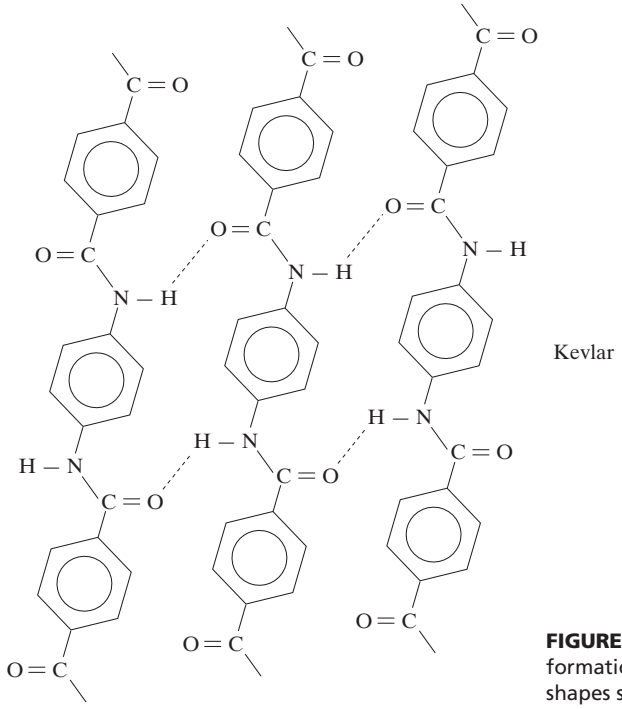
Finally, many polymers tend to be hard and brittle. When these properties are undesirable, plasticizers are added to increase softness and pliability. Most such plasticizers are phthalates, and with the advent of mass-produced plastics, they have become ubiquitous. "New-car smell" results from the volatilization of plasticizers from synthetic upholstery and similar materials; when enough of the plasticizer is gone, the material becomes brittle and can crack. Plasticizers have recently been identified for further study as a possible health concern for children, who tend to put soft plastic toys in their mouth.



1,4-Benzenedicarboxylic acid 1,4-Diaminobenzene

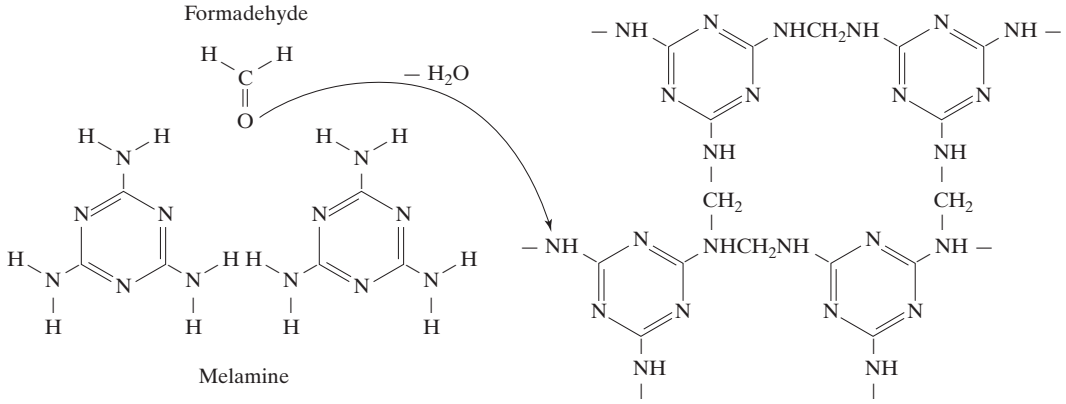


Kevlar
(an aramid)



Kevlar

FIGURE 32 The formation of Kevlar, similar to the formation of nylon 6,6. Kevlar can be molded into shapes such as helmets.



Melamine

FIGURE 33 Melamine, a formaldehyde-based condensation polymer.

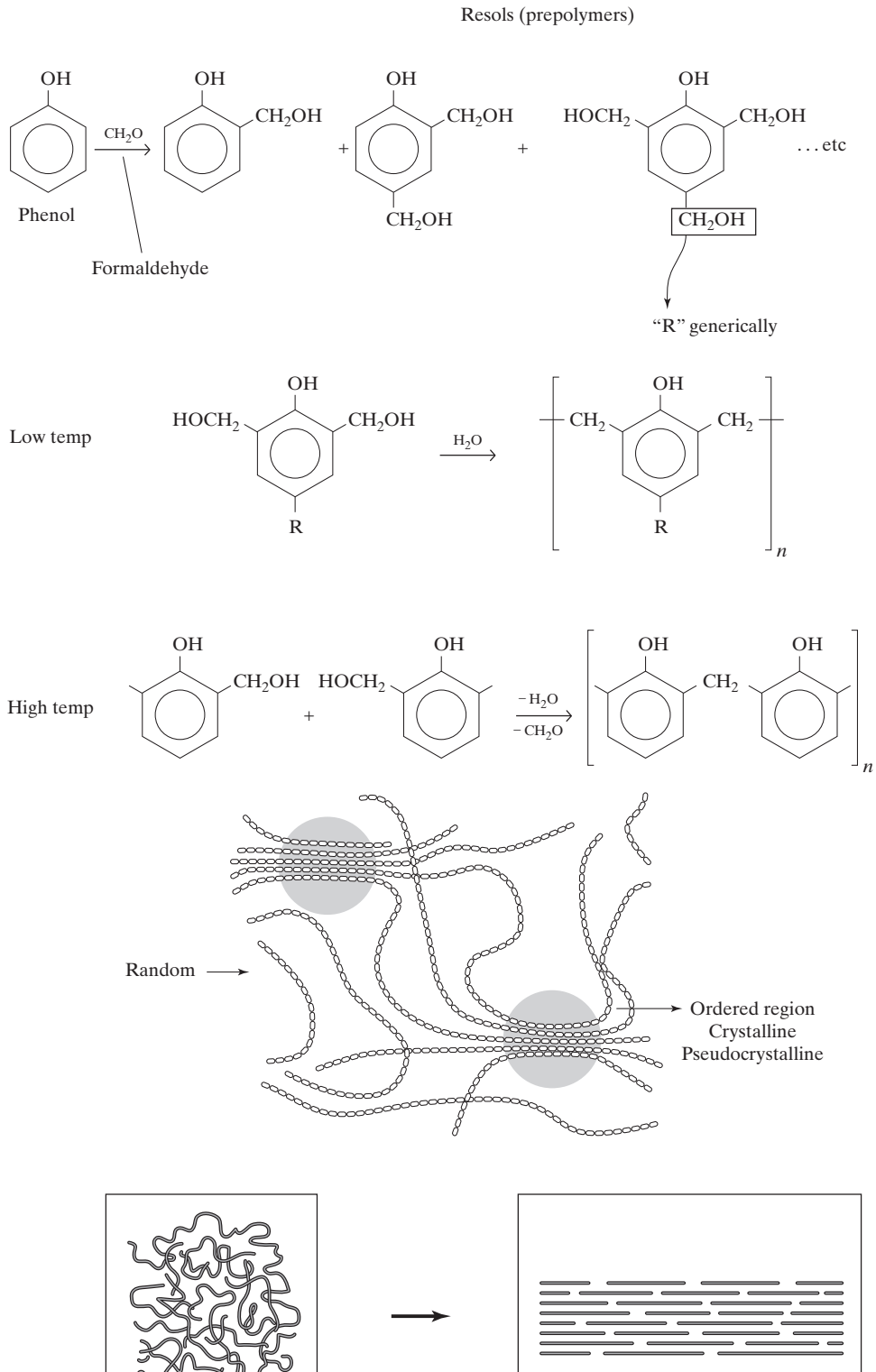


FIGURE 34 The formation of formaldehyde–phenol polymers.

FIGURE 35 Ordered regions and crystallinity in polymers.

3.4 Processing and Fabrication

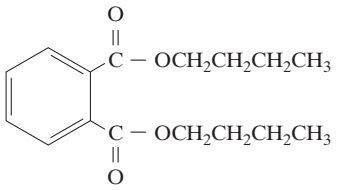


FIGURE 36 Dibutyl phthalate a plasticizer.

When a raw polymer is shaped, molded, or drawn into a configuration such as a film or fiber, properties and characteristics are added or altered. From the forensic perspective, these characteristics may play a central role in the study of polymer evidence above and beyond a purely chemical analysis. For example, plastic garbage bags are made from films of polymer cast on rollers, which leave distinctive striations in the bag. Suppose body parts are found in a plastic garbage bag dumped in a remote location and that a suspect is identified. The striation pattern on the bag containing the evidence may be sufficient to link the bag to a roll of garbage bags at the suspect's home. Such a physical match is simple, often definitive, and made on the basis of knowledge of how polymer products are produced. Three common methods of converting raw polymer to product are by film casting, drawing into fibers, and molding. The processes are illustrated in Figures 37 and 38.

Thin films are made by casting techniques. **Solution casting**, as the name implies: dissolving the polymer in a solvent system and pouring it onto a surface from which the dried or cured polymer sheet is removed. On an industrial scale, systems of rollers and

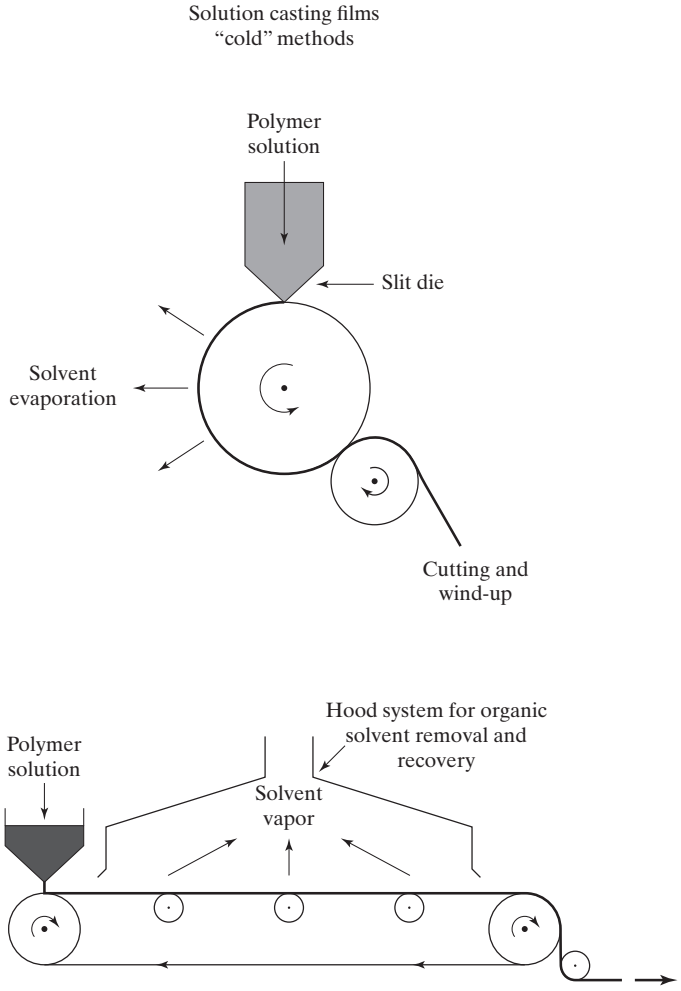


FIGURE 37 Methods of casting liquid polymer into a film. The polymer solidifies as the solvent evaporates.

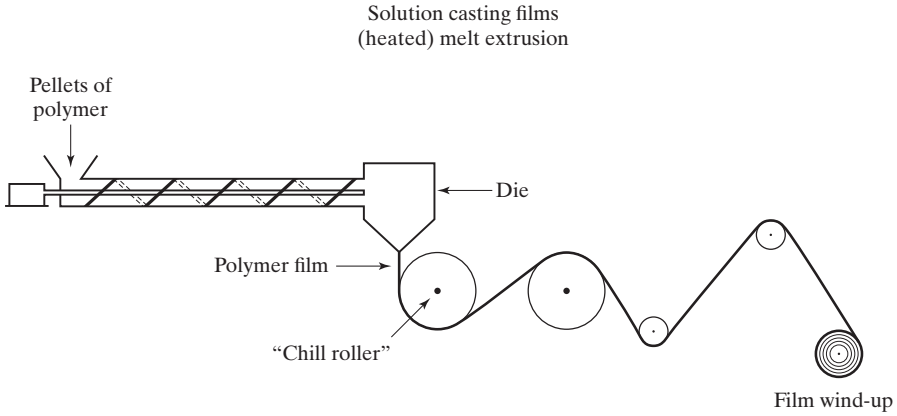


FIGURE 38 Melt extrusion film casting involves melting the polymer.

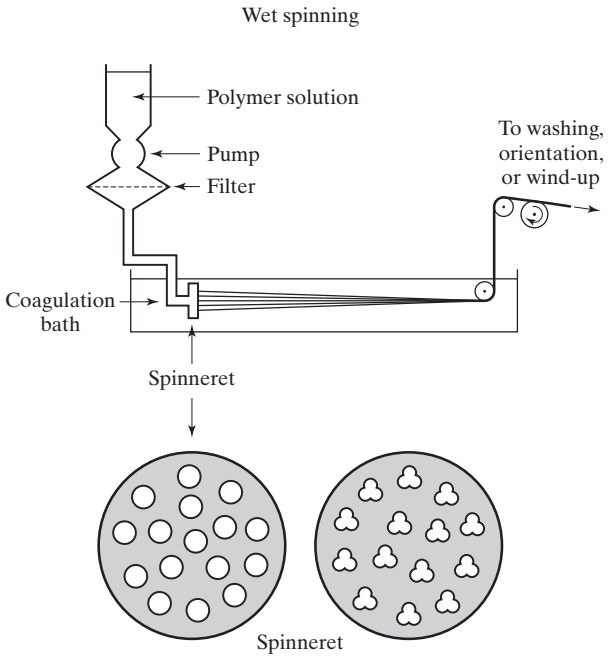


FIGURE 39 Wet spinning of fibers is similar to cold casting of films, except that the fibers are extruded through a spinneret, which dictates the cross-sectional shape of the strands.

presses are employed in methods analogous to those used to make paper. Depending on the nature of the polymer, the film is cast from solution (cold methods) or by heating, melting, and casting (hot, or **melt, extrusion** methods).

Hot and cold methods are also used to create synthetic fibers. A strand of fiber is a single filament that can be combined or spun with other filaments to create the final fiber product. The process from raw polymer to final product is called **spinning**, as illustrated in Figures 39 and 40. The cross-sectional shape of the strand is dictated by the shape of the orifice through which is it extruded. As shown in Figure 39, extruded fibers are spun together into a filament (bundle of fibers) that can be cut into smaller staple fibers with lengths on the order of centimeters or less that may be further processed into textile materials.[15]

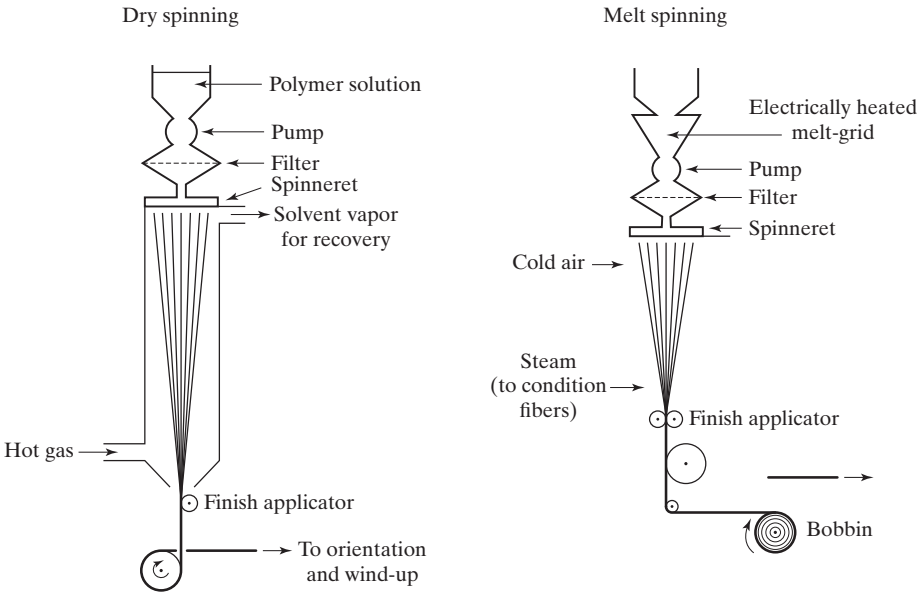


FIGURE 40 Dry and melt spinning methods of fiber extrusion.

A recent development in fiber processing is **bicomponent fibers** (Figure 41). As the name suggests, these are fibers that consist of two different polymer types within the filament itself. Bicomponent fibers are engineered for specific applications and take advantage of the characteristics of each. Two designs are used: side-by-side and sheath-core. The sheath-core designs can be quite exotic, with a letter or other branding identifier embedded down the length of the fiber. The polymers must be compatible for manufacturing, as are, for example, polypropylene and polyethylene.

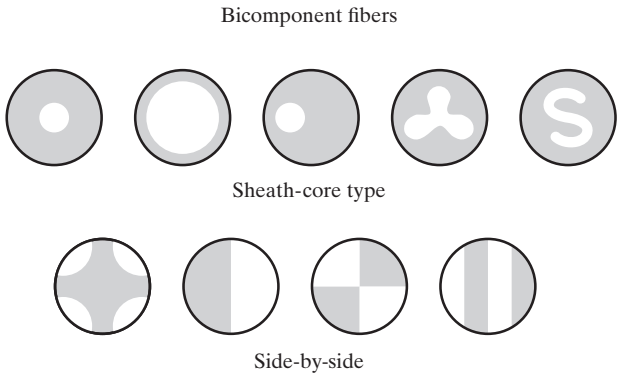


FIGURE 41 Examples of possible combinations in bicomponent fibers.

Summary

We now understand the basics of polymer composition and synthesis and are ready to move into the forensic analysis of such evidence, focusing on paper, fibers, and other polymeric materials. Cellulose makes up a large part of these materials as a component of papers, cotton, and regenerated fibers such as rayon. In addition, much of what we have discussed here helps round out our understanding of coatings. It should come as no surprise that our understanding of forensic chemistry is becoming more inclusive and integrative.

Key Terms and Concepts

Amorphous
Amylopectin
Amylose
Anhydroglucose polymers
Anomeric carbon
Beaten
Bicomponent fibers
Biopolymer
Cellophane

Cellulose
Cellulose acetate
Chain growth
Chitin
Condensation polymerization
Copolymer
Glass transition temperature
Glycogen
Hemicellulose
Hollow viscose
Homopolymer
Kraft pulping
Lignin
Lignocellulose
Living polymerization
Melt extrusion
Monomer
Nylon
Oriented polymer
Plasticizer
Polysaccharide
Pseudocrystalline
Pyranose
Rayon
Ring-opening polymers
Seed fiber
Solution casting
Spinning
Starches
Thermoplastic behavior
Thermoplastic polymers
Thermosetting polymers
Vegetable fibers

Problems

FROM THE CHAPTER

1. Why is the term *anhydroglucose* used to describe glucose polymers? In which classification of polymer does this place cellulose?
2. Provide the chemical explanation for the expectation that lignin would contribute to the acidity of paper. What other groups are involved, and how does the bound water in paper (Figures 21 and 22) contribute?
3. Most papers lose their strength when wet. What is the chemical explanation for this phenomenon?
4. Based on the characteristics of cellulose, what group(s) of dyes would be appropriate for dyeing cotton and paper, aside from those already discussed? Explain on the basis of intermolecular forces such as ion–ion and ion–dipole forces.
5. In 2002, Cargill Dow introduced a new synthetic fiber labeled PLA made from lactic acid monomers. What are the characteristics of this fiber? How is it made? How is it similar to cellulose?
6. Kevlar is relatively easy to identify. Why?

INTEGRATIVE

1. An older, color-based presumptive test for saliva is based on the presence of the enzyme amylase. This test employs starch gel and iodine in an interesting way. Explain how the test is performed, and discuss its chemical basis and its limitations. How is this test related to material presented in the chapter?
2. Describe and discuss similarities and differences between the helical conformation of amylose and the helical structure of DNA. What fundamental chemical principles and intermolecular forces lead to the respective conformation and structure?
3. Locate a reference that describes sample preparation in entomotoxicology. What approach is used to break down chitin?
4. From your knowledge of the structure of glucose and cellulose, identify which groups are infrared absorbers. Where in the spectrum would most IR spectral features be expected to appear? Locate or obtain an IR spectrum of cellulose, and correlate absorptions to chemical functionality.
5. A question from a previous chapter dealt with the indicator phenolphthalein, which is also used in a common presumptive test for blood. If a typical sheet of office paper is slightly wetted, and a drop of phenolphthalein placed on the surface, what color will the indicator likely assume and why? From the perspective of using this indicator for a presumptive test for blood, is there a possibility of interference?
6. Could the cation exchange capacity of paper be used to characterize the paper? Why or why not? What variables would have to be controlled?
7. A simple TLC experiment can be performed in which water-soluble inks are applied to filter paper and the chromatogram is developed with the use of water as the solvent. Explain what interactions in the paper allow for separation to occur. Examine the various classes of dyes, and predict how each would be expected to behave under these chromatographic conditions.
8. How is the process of dyeing a fiber similar to the processes that occur in chromatography? How is it similar to the movement of ions in solution to the surface of an electrode?

FOOD FOR THOUGHT

1. Why would a bicomponent fiber be considered to have strong evidentiary value?

Further Reading

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Website: Scientific Working Group for Materials Analysis: http://www.swgmat.org/fiber.htm. Last accessed March 2011; lots of good resources regarding forensic fiber analysis and related materials analysis topics.

Website: www.tappi.org; Technical Association of Paper and Pulping Industry. This website has a wealth of information on paper and pulping.

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Forensic Analysis of Inks and Paints

OVERVIEW AND ORIENTATION

The two types of evidence we will focus on in this chapter are inks and paints, which have much in common. Both contain dyes or pigments dissolved or suspended in a complex solvent system that when delivered to a substrate, undergoes predictable chemical changes as the mixtures dry and cure. Often, this process incorporates polymerization, and the polymeric components can be used as part of the forensic analysis. In most forensic analysis of materials, the primary goal is not an identification per se but rather analytical characterization that can be used to compare, classify, and differentiate. For example, suppose you are tasked with comparing a paint chip recovered from the body of a hit-and-run victim with a paint sample collected from a suspect's car. Paint is a mixture of materials and in the case of car paint, a layered material. If you analyze this using microspectrophotometry (MSP), you will likely not obtain a spectrum of a single component that can be submitted to a library search and identified. Rather, you will obtain a spectrum of a mixture. This does not mean the data are useless—far from it—but it does mean you must approach the analysis from the forensic perspective. Here, you would try to determine whether the spectra are similar or dissimilar and what those results mean. You could also submit the spectra to a database, but not the type you are probably used to dealing with. These databases are becoming central in forensic analysis of materials.

Ink analysis is generally within the purview of questioned document examination. Chemical analysis is used to help answer forensic questions such as Are these two writings made in the same ink? Were these writings made at the same time, and How old is this writing? In the context of counterfeit investigations, the forensic question may relate to the authenticity of currency. Much of this investigation relies on specific characteristics of different types of inks. For paints, the forensic question usually relates

to comparison and determining the likelihood that a questioned and known (*Q* and *K*) have a common source. Analytical data are essential for addressing all these questions, so we will start with a discussion of general methodologies, where we discussed instrumentation.

1 ANALYTICAL METHODS

Inks and paints offer the forensic analyst many characteristics to work with. Aside from their invaluable physical characteristics, such as layer structure and markings, inks and paints are amenable to all three general analytical techniques (optical, organic, and inorganic) that are useful for the analysis of colorants. Older wet chemical and destructive tests have given way to microscopic and instrumental analysis in most cases. Because comparison is at the heart of most forensic chemical analysis of paints and inks, libraries, databases, and other collections are vitally important for the analysis of colorants. The Royal Canadian Mounted Police maintains the **PDQ (Paint Data Query) database** of infrared spectra of automobile paints. To date, this collection includes data from more than 13,000 vehicles and has a library of more than 50,000 paint layers (http://www.rcmp-grc.gc.ca). The United States Secret Service maintains an ink library containing more than 9000 inks, toners, and inkjet inks.[1–3] A Munsell color chart for paints is useful, as are reference spectra of dyes and pigments obtained by using a variety of instrumental techniques. For microscopy, a reference set of pigments is indispensable.

There are several ASTM standards dealing with paint and ink analysis. The two current standards regarding ink analysis are ASTM 1789-04 (Standard Guide for Writing Ink Identification) and 1422-05 (Standard Guide for Test Methods for Forensic Writing Ink Comparison). For paint, the current standard is E1610-02(2008) Standard Guide for Forensic Paint Analysis and Comparison. These guidelines do not dictate forensic

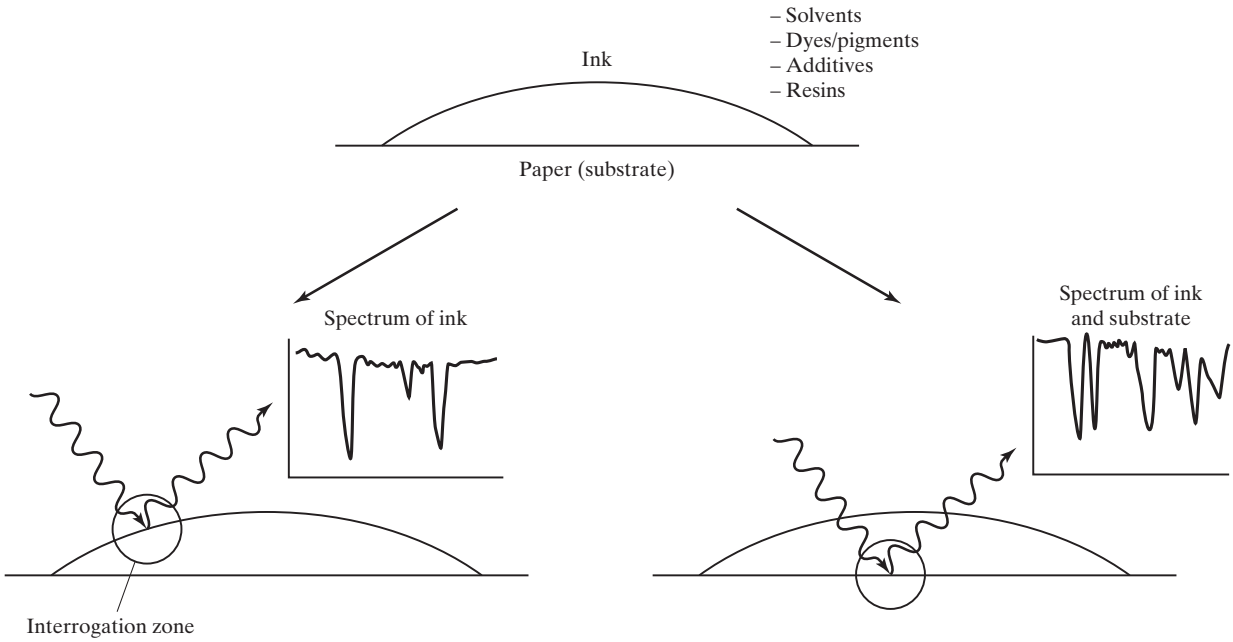


FIGURE 1 Ink on paper is a complex chemical system that changes over time. Any spectral data obtained from this sample will reflect characteristics of the mixture as well as the depth of penetration of the energy.

practice but do provide valuable guidance and, as the names imply, standardization for many of the analytical practices used.

It is important to remember that inks and paints, unlike drugs, are not single analytes but rather are mixtures that change over time. Thus, the analytical "targets" and scheme differ from what we discussed in the context of drug analysis and are somewhat similar to what we discussed regarding arson evidence and its analysis. Frequently, the goal is to obtain what is called, for lack of creativity and a better term, a "**chemical fingerprint**." For example, if you have a pure cocaine sample obtained from a reliable reference material, the IR spectrum of that powder will be unequivocal and easily matched to a standard in a library. If, on the other hand, you obtain an IR spectrum of an ink sample on paper, the resulting spectrum will contain information regarding all components of the ink and substrate that interact with IR energy. In other words, the spectrum is that of a mixture. The concept is shown in Figure 1. You cannot match this spectrum to a compound in a traditional compound-based library, but you can compare it to reference spectra from a collection of ink spectra through a pattern matching process. You can also examine the spectrum and glean some information about what is in it based on peak absorptions. This spectrum is an example of a chemical fingerprint. As with a fingerprint, it can be compared with a spectrum of known origin to assist in the determination of a possible common source. However, the analogy should not be taken too far—the spectrum in this example is not unique in the sense that a human fingerprint is. Therefore, it is wise to always take such informal descriptors with a grain of salt.

Ink (and paint) is a mixture that may contain complex combinations of colorants (dyes and pigments), solvents and vehicles, resins, additives, and film-forming agents. Ballpoint-pen ink typically comprises about 50% solvent, 25% colorants, and 25% additive resins.[4] When the ink is still in the cartridge, the term **closed system** is used to describe it. When the ink is applied to paper, the term **open system** is used.[5] The distinction is necessary given that the mixture begins to change the moment it is placed on a substrate. These changes are both physical and chemical. As shown in Figure 2, the ink will spread and diffuse to some extent, depending on the nature of the substrate as well as the characteristics of the ink. In the short term (typically hours), solvents will evaporate, and film-forming agents will begin to polymerize and harden. Over longer periods of time, further hardening occurs, and the dyes and pigments may degrade. Understanding of these processes provides the foundation for aging and dating of inks, although as we will see, this is a challenging task.

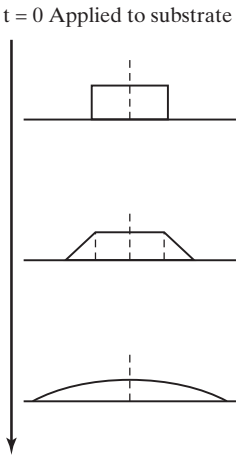


FIGURE 2 Diffusion of ink or paint after application. The depiction is exaggerated to illustrate the point. The material spreads on the surface and into it. The degree of spread depends on the characteristics of the substrate. Over time, components of the ink may move a significant distance from the position of original deposition.

There are a variety of analytical methods applied to inks and paints, and an overview of these is provided in Table 1. You should refresh your understanding of each of these methods as needed using the text cross-references provided. For ink analysis, thin layer chromatography (TLC) remains a mainstay for comparison of colorants, and the ASTM method cited earlier lists recommended solvent systems. The notation high performance thin layer chromatography is also used (HPTLC) in the literature, and the improvements over traditional TLC are analogous to those with liquid chromatography versus HPLC and UPLC. These include smaller particle sizes, optimized solid-phase compositions, thinner layers, and improved sample applications methodologies. We will use the term TLC to refer to both.

A few other techniques and instruments are worth special mention in the context of ink analysis. Pyrolysis is the thermal decomposition of a sample prior to its introduction into a gas chromatograph. The process involves rapid heating of a very small sample to several hundred degrees and directing the gases that are formed during decomposition into the instrument. The technique is useful for polymers, fibers, and here, for paints.[6–8] Other forms of mass spectrometry are also being applied to ink analysis. Inductively coupled plasma mass spectrometry (ICP-MS) has been used

TABLE 1 Common Analytical Methods and Instruments Used for Paint and Ink

Method	Destructive?[a]	Application	Text Reference Chapter.Section
Visible and polarizing light microscopy (PLM)	No	Macroscopic examination Order of placement Characterization of colorants	6.1
Thin-layer chromatography (TLC/HPTLC)	Yes	Comparison of dye and pigments	5.6
Infrared spectroscopy and microspectrophotometry (MSP)	No	Comparison of samples General characterization of colorants General characterization of polymer	6.2.5 6.5.2
Raman spectroscopy and MSP	No	Comparison of samples General characterization of colorants General characterization of polymer	6.2.6 6.5.2
UV/VIS spectroscopy, colorimetry, and MSP	No		6.2.4 6.5.1
X-ray methods	No	Characterization of inorganic pigments	6.4.1 6.5.3
Mass spectrometry (organic)	Depends on application and ionization mode	Characterization of organic components	6.3 6.3.3
Mass spectrometry (inorganic) ICP-MS – bulk	Destructive	Characterization of pigments	6.3.2
ICP-MS laser ablation	No/minimally	Characterization of pigments	6.3.2
Pyrolysis GC/GC-MS	Destructive	Characterization of resins	6.7

[a]As typically applied.

recently for the characterization of inks, targeting trace elements.[9, 10] For this type of analysis the laser ablation method of sample introduction is used. This methodology has also been applied to paints.[11] Aside from pyrolysis GC-MS, little work has been done with organic mass spectrometry, but as new ionization interfaces are developed, this could change. We will see one example of organic MS analysis using a MALDI interface later in the chapter.

1.1 Optical Microscopy

When a colorant is a pigment and thus particulate, optical microscopy and PLM are a critical part of any analytical scheme.[12, 13] Microscopy can include microchemistry and solubility tests, as well as direct observations of layers (**paint stratigraphy**) and pigment particles. In the case of inks, microscopic examination will reveal the presence of metallic particulates and other solids that are part of the formulation. Skilled microscopists may be able to identify pigments on the basis of their optical properties and crystalline form. Microscopy of documents is useful for differentiating printing methods. As was seen earlier, toner particles from a laser printer are visible under low magnification and differentiate toner-based printing from ink-jet-type printing. Fluorescence microscopy, although somewhat specialized, is also useful for screening and analyzing paints and inks. In such applications, an excitation wavelength in the UV range is used to stimulate emission in the visible range and to create a fluorescence spectrum.

1.2 Alternative Illumination

UV and IR illumination is a common nondestructive tool used in the analysis of questioned documents. The principle behind most of these techniques is fluorescence. Many inks fluoresce in the visible region or infrared region of the spectrum when exposed to ultraviolet or visible light, respectively (see Figure 3). In addition, many printing inks, such as those used in currency, have fluorescent security features that are not easily duplicated by counterfeiters. Collectively, these techniques are called *luminescent techniques*, and they avail themselves of both absorbance and fluorescence characteristics. To observe infrared fluorescence, the analyst illuminates the document with visible radiation, and the induced fluorescence is detected by means of filters and IR-sensitive film or video equipment. The effect is to convert IR emission to a visual equivalent. The excitation of visible fluorescence by UV light is analogous, except that the visible emissions can be observed without conversion. Currency notes often incorporate single fibers with exact fluorescent properties as an added security measure. UV and, particularly, IR characteristics are useful in differentiating inks with similar colors, as well as in examining charred documents and obliterations by using another ink or correction fluids.

1.3 Thin-Layer Chromatography and HPTLC

If a colorant can be solubilized, it can be evaluated using TLC. This technique is commonly employed for ink analysis but not for paints. A 1982 report described the use of TLC in isolating pigments in house paint, followed by IR identification of separated spots,[14] but this is one of few such reports, and TLC is infrequently used for this purpose. However, for inks, TLC is an accepted and widely used tool that is applicable to a variety of inks and toners.[15–21] The ASTM guide for ink analysis (E1422) recommends pyridine as a solvent for glycol-based ballpoint inks and an ethanol–water (1:1) combination for nonballpoint inks. The stationary phase is typically silica gel, and the

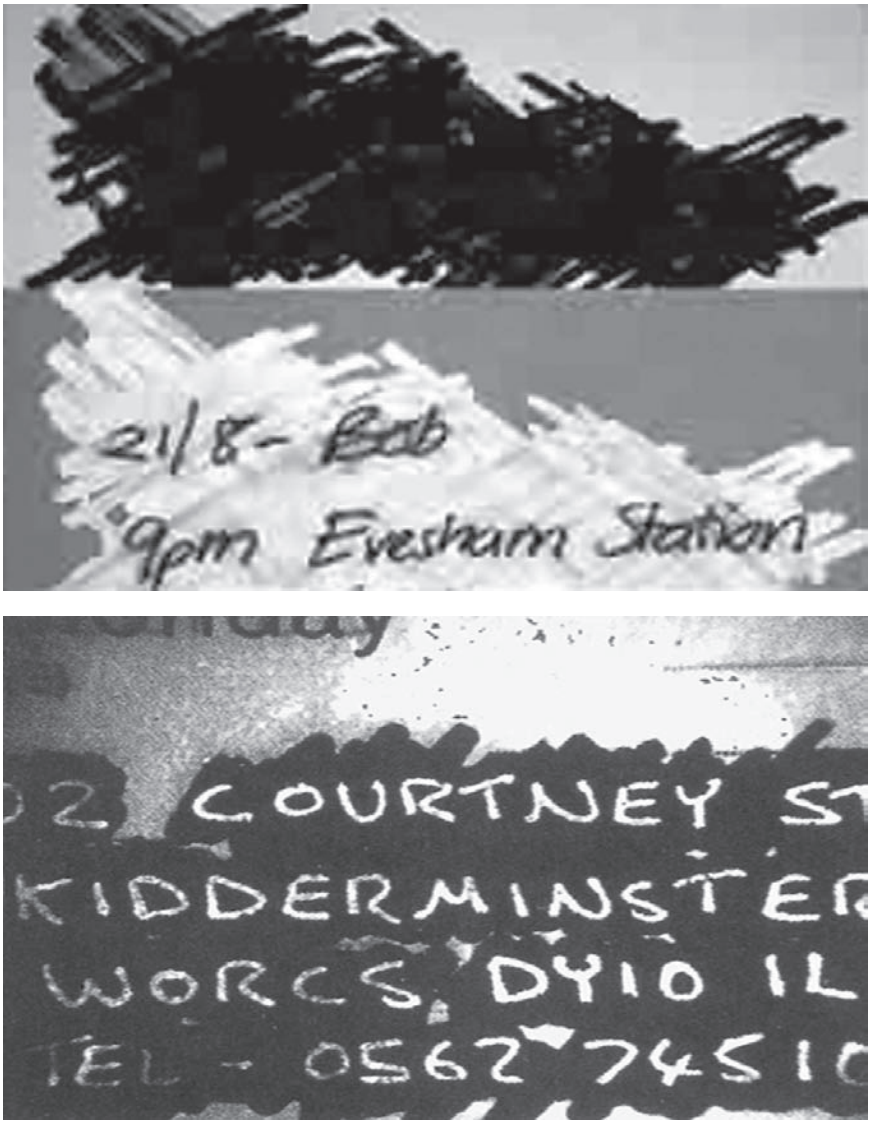


FIGURE 3 Two examples of obliterated writing made visible using near- and mid-IR illumination and imaging. Images courtesy of Foster & Freeman USA.

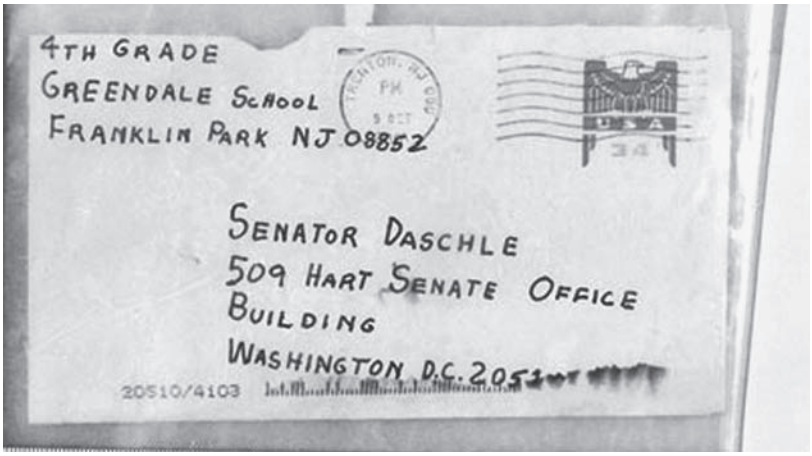
solvent systems are based on ethanol and water. An example of an ink separation performed using TLC is shown in the color insert. There are still many inks in which the colorants can be separated with nothing more than water and filter paper. Dyes in textiles can be similarly examined.[17]

During the last few years, TLC and HPTLC have been combined with various types of spectroscopy. In these cases, the ink components are first separated on the TLC plate and then the individual spots are analyzed using the detection system. As examples, TLC has been combined with image analysis and extraction of the RGB color channels[22] and Raman spectroscopy.[23]

EXHIBIT A

Examination of Questioned Documents in the Terrorist Age

In October 2001, envelopes containing anthrax were sent through the United States Postal Service, resulting in seven deaths, thousands of exposures, and major disruptions. The anthrax powder was contained in an envelope along with a handwritten letter. The envelope and letter were invaluable physical evidence, but analysis had to wait months while methods were developed to open and sterilize the evidence before traditional forensic examinations such as ink analysis could even begin.



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Applying the Science 1 Dyes in Drugs

The dyes used in the preparation of illicit pills can be incorporated into drug analysis. In one study, researchers used capillary electrophoresis to evaluate 14 dyes as part of a drug-profiling application that included Bayesian statistical analysis. The study, conducted in Europe, focused on dyes developed for use in foodstuffs. Many of these dyes, which are acidic and water soluble, are available in the United States and Canada. In the study, LOQs were in the range of ~0.008–0.06 ppm, and LOD was reported at ~0.008 ppm. Calibration curves (an internal standard method) had about two orders of magnitude of linearity. Detection was achieved with UV at 255 nm, although different wavelengths were used in some cases to optimize the response for a particular dye.

The analysis consisted of three stages, beginning with SPE of the dyes from pills, using a polyamide solid phase. Thin-layer chromatography was utilized to screen the dyes via two solvent systems and solid supports (silica gel and cellulose), followed by confirmation by CE coupled to a diode array detector. The authors reported that the 14 dyes could be unambiguously identified with these parameters.

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1.4 Spectroscopy and Microspectrophotometry

Spectroscopy and, particularly, microspectrophotometry are the most versatile tools for the analysis of colorants, inks, and paints. Visible spectroscopy and colorimetry are used to define color, assign color space coordinates (usually with CIELAB), and differentiate

among colorants with similar appearance but different visible spectra. IR spectroscopy is useful in characterizing colorants, binders, and coatings, whereas Raman spectroscopy is used for inks and paints. Microspectrophotometry has significantly reduced the need for large sample sizes, lessened destruction of samples, and made it much easier to examine layers without physically separating them. Extensive and growing forensic databases are continually increasing the utility of FTIR in colorant analysis.

Microspectrophotometry in the visible range is used to compare pigments and dyes, to evaluate pigment mixtures, and to compare samples with similar color. Visible spectra are information-poor relative to IR spectra and are not amenable to library searches. However, differences in spectra are useful in classifying and discriminating similarly perceived color and in identifying mixtures of colorants.[24] Homogeneity and preparation of the sample are paramount to interpretation. Aging and weathering, addressed in Section 2, are also important considerations in comparing visible spectra of paint samples. As an example, a recent review reported that within the same sample layer, distances between replicate spectra plotted in CIELAB space ranged from 1.3 units for a 10-year-old green paint to 7.2 units in a 10-year-old yellow paint.[24] Differences between cars using the same finishing colors were in this same range.

FTIR is particularly valuable in classifying the binders used in paints as shown in Figure 4.[12, 25] Flowcharts are useful in interpreting absorption band patterns and relating them to types of resin. Binders and pigments are also amenable to flowchart analysis. Because paints are mixtures, the entire absorption spectrum is of interest, not just the fingerprint region, which is the spectral region of interest when evaluating pure compounds such as drugs. The analysis of pigments in paint by FTIR and MSP has been studied in detail, and a large amount of information is available in the literature. Early studies used diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) techniques,[26] but they were supplanted by MSP in the 1990s. Starting in 1996,[27–33] Suzuki and Marshall published a comprehensive series of articles addressing pigments and

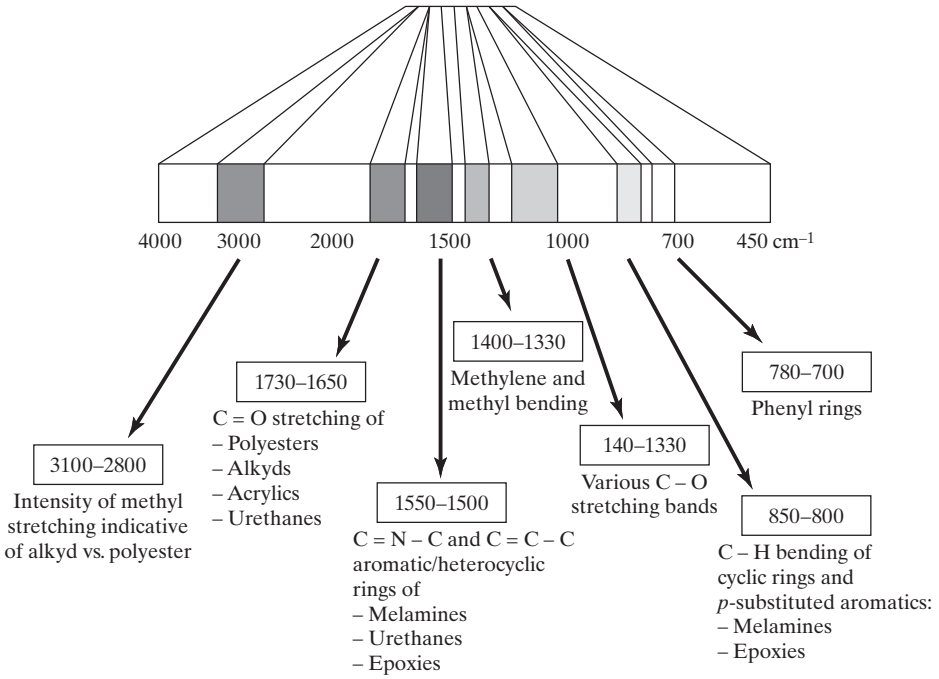


FIGURE 4 IR absorption bands that correlate to functional groups common in resins, binders, and vehicles.

binders in automobile paints. The organic characterizations were supplemented with elemental analysis using XRF. An interlaboratory report published in 2001 demonstrated that by that year, practices and procedures using FTIR and micro-FTIR were reproducible and intercomparable among the testing laboratories.[34] The samples analyzed were a black base-coat and clearcoat layer system selected because the pigment was carbon black, an IR-inactive compound. This experimental design highlighted the use of IR applied to binders, without the complication of pigments adding to the signal. The binders were acrylics with other components present. As illustrated in Figure 5, three of the samples showed consistent and essentially indistinguishable IR spectra; however, pyrolysis GC successfully differentiated them (Figure 6). A few of the peaks that distinguish the three samples (Figures 5 a–c and 6 a–c) are highlighted in Figure 5.

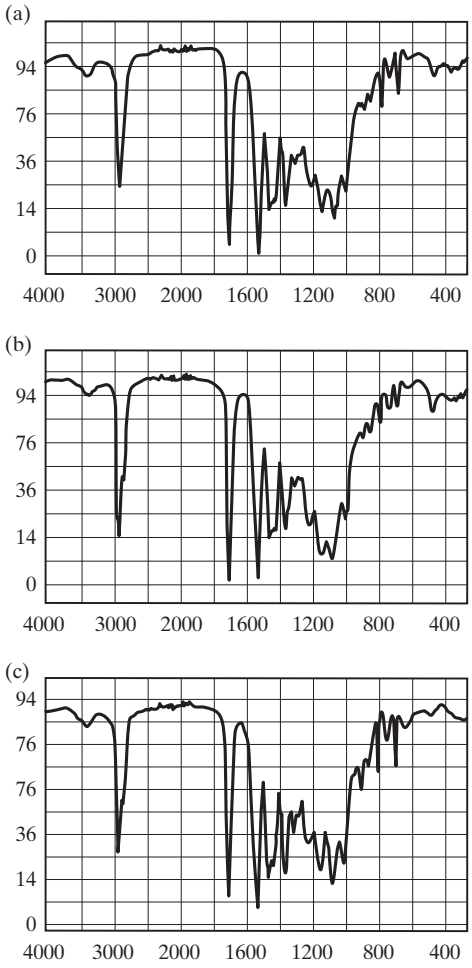


FIGURE 5 IR absorption spectra of three similar clearcoat resins. These spectra are indistinguishable, but addition of a second analytical technique, as shown in the next figure, can facilitate discrimination. Reproduced with permission from Ryland, S. G., et al., "Discrimination of the 1990s Original Automobile Paint Systems: A Collaborative Study of Black Nonmetallic Base Coat/Clear Coat Finishes Using Infrared Spectroscopy." *Journal of Forensic Sciences* 46 (2001): 31–45. Copyright 2001 ASTM International.

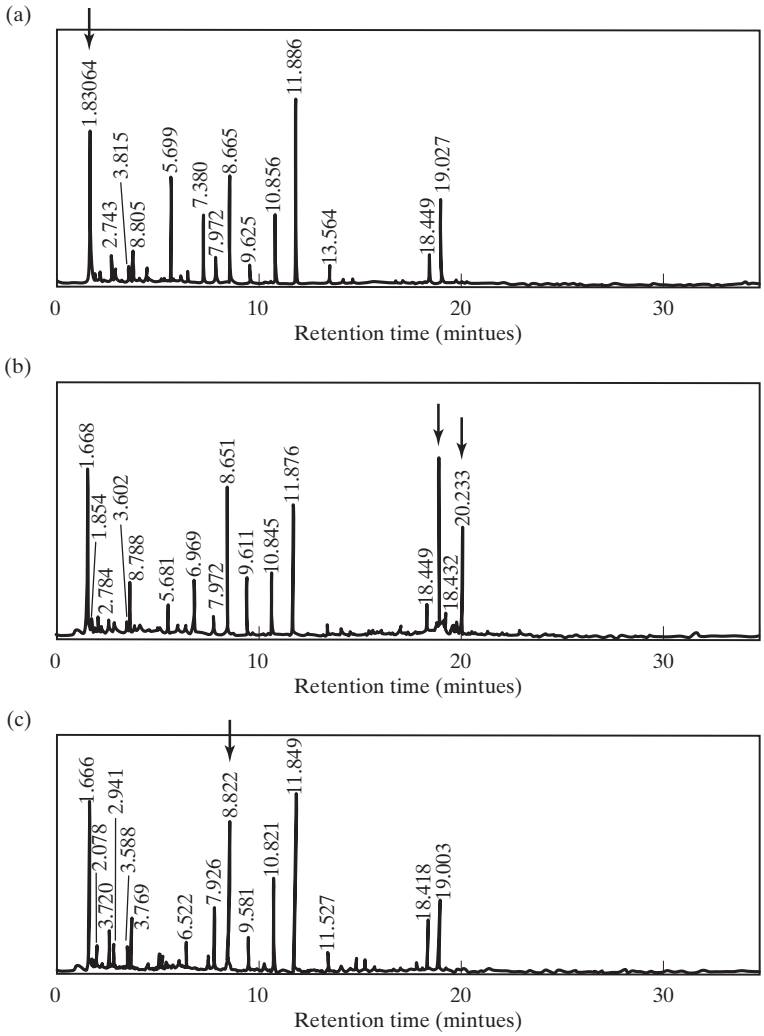


FIGURE 6 Pyrolysis GC analysis of the three resins shown in Figure 5. Reproduced with permission from Ryland, S. G., et al., "Discrimination of the 1990s Original Automobile Paint Systems: A Collaborative Study of of Black Nonmetallic Base Coat/Clear Coat Finishes Using Infrared Spectroscopy." *Journal of Forensic Sciences* 46 (2001): 31–45. Copyright 2001 ASTM International.

Note that it is the difference in the patterns that is critical in this type of analysis and comparison. This example illustrates the value (often, the necessity) of multiple analytical techniques in paint and ink analysis.

Raman spectroscopy and microspectroscopy are also used to characterize colorants and paints. With paints, one significant advantage of Raman spectroscopy is that binders and resins contribute minimal interference, allowing for characterization of the pigment.[29] Many spectral features are sharper in Raman spectra than in IR spectroscopy,[29] and the combination of the two kinds of spectroscopy provides significantly more information than does either one alone. In general, IR spectra of inorganic pigments such as TiO_2 have broad peaks, whereas the corresponding Raman spectra have narrow peaks.[29] Figure 7 illustrates the value of the combined technique for characterizing a white finish using TiO_2, which, as noted previously, has two useful crystal forms. The Raman spectra differentiate these two forms, whereas the IR spectra do not, owing to their broad absorption peaks.

Ink analysis and related analyses of questioned documents have come to rely heavily on IR spectroscopy, principally MSP.[35–38] Inks are analyzed in situ or on sampling substrates such as KBr plates. Like paints, inks produce IR spectra representative of mixtures; unlike paints, inks rarely have a layered structure. Regardless, spectra of inks do contain information that is useful for classification. For example, a study of 108 inks demonstrated that distinctive absorption bands could be rationalized on the basis of ink composition and then employed to divide the test population into two groups.[38] As shown in Figure 8, one group contained C=C stretching characteristic of an epoxy resin and features associated with triarylmethane dyes. A second group of inks had spectral features consistent with an alkyd resin. Note that this is the same general approach described for paint, and similar considerations apply to the IR analysis of toners used in laser printers and copiers.[35, 39–45]

One of the interesting aspects of toner particulates is the difficulty in sample preparation, since toner is a waxy solid before it is bonded to a paper substrate. Heat affixing to a metallic substrate such as foil has been used, along with KBr salt plates, an older form of sample preparation used for IR prior to the widespread adaptation of ATR methods and instruments. Using heat to affix toner to paper was found not to be an issue, except in isolated cases.[41,43] As with inks, toners could be grouped on the basis of the polymer resins they contain,[45] such as acrylates, methacrylates, polystyrene, and epoxides and corresponding spectral features.

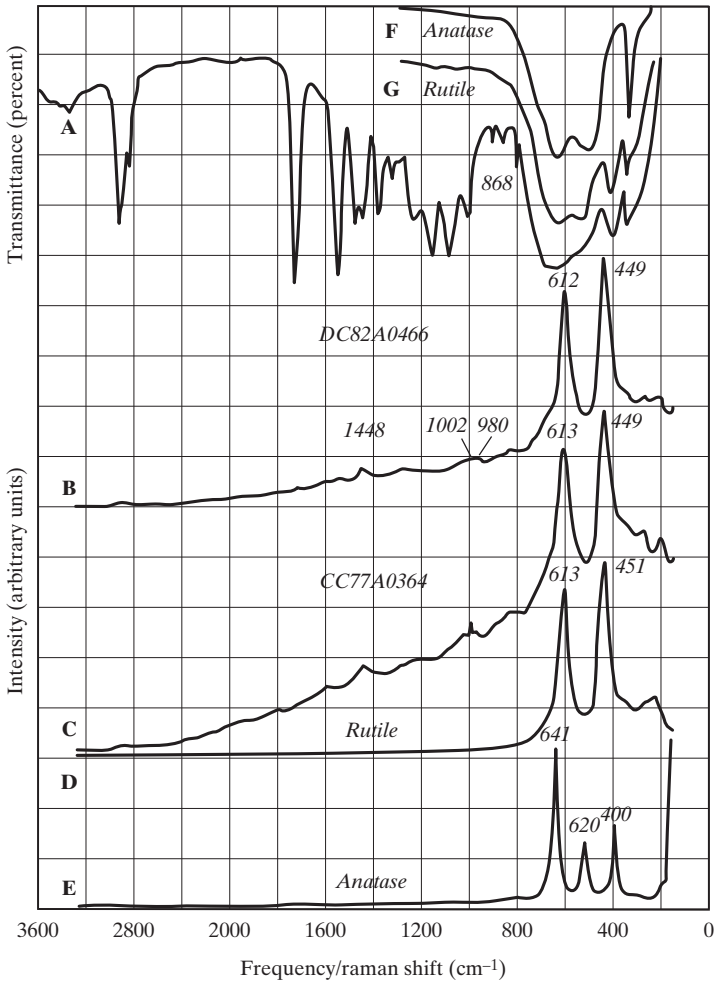


FIGURE 7 IR and Raman spectra of a white coating. Note that the Raman spectra differentiate the rutile and anatase crystal forms, whereas IR data cannot. Reprinted, with permission, from Journal of Forensic Sciences, copyright ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

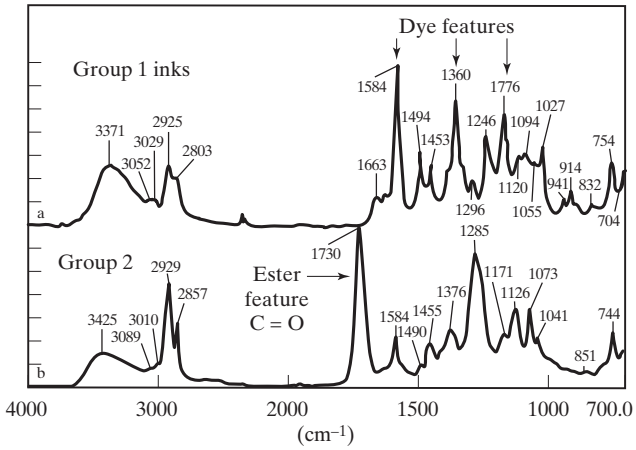


FIGURE 8 IR spectra illustrating features used to divide sample inks into two populations. Reproduced with permission from Wang, F., et al., "Systematic Analysis of Bulk Blue Ballpoint Pen Ink by FTIR Spectrometry." *Journal of Forensic Sciences* 46 (2001): 1093–97. Copyright 2001 ASTM International.

1.5 Mass Spectrometry, GC-MS, and HPLC

Mass spectrometry can be applied to inks and paints as instrumentation allows. Routine GC-MS, as described for drug analysis and toxicology, is limited to volatile and chromatographically suitable components of inks and paints such as solvents. In addition, volatile components dissipate over time as they dry and cure. Many dye and pigment molecules have such high molecular weights that typical solvent extraction GC-MS is impractical or only of limited use.[43] With gel-pen ink, GC-MS has been reported to be useful in identifying solvents and other volatiles in samples up to six months old.[46] Volatiles identified included glycerin (the largest single component), triethylene glycol, pentaethylene glycol, and triethanolamine, compounds extracted from the substrate through the use of ethanol.

Mass spectrometry using alternative ionization and sample preparation methods are employed in ink and paint analysis. The oldest of these techniques is based on pyrolysis of the sample (typically, a paint) prior to its introduction into the GC. Detectors for pyrolysis GC are MS and FID. Pyrolysis patterns can be examined in the same way accelerant patterns are, but increasingly, GC-MS is preferred over FID. Pyrolysis is, by definition, destructive, but the sample size is reasonably small, and recently a micropyrolysis GC-MS has been developed and applied to photocopier toners and paint.[47] A laser is focused on the sample through a microscope, and the pyrolysis vapor product is directed into the GC-MS system. The pattern of the pyrolyzates and chemical composition allowed for delineation among paint and toner samples. One significant advantage of the system was its ability to directly sample toner applied on paper with only one pyrolysis product—chlorobenzene—attributed to the paper matrix. The mass range scanned went up to 550 amu. Because pyrolysis does not directly reveal chemical components of the mixtures (i.e., it reveals pyrolysis products), potential disadvantages and limitations include sampling homogeneity, standardization, and reproducibility of heating profiles and the resulting patterns.

Laser ionization without pyrolysis has been applied to paints and inks.[48–50] Briefly, in desorption–ionization methods, a laser is used to volatilize and ionize a sample prior to its direct introduction into an MS. The latter is usually a magnetic-sector or TOF detector. Several ionization variants are included in this category, including matrix–assisted laser desorption and ionization, or **MALDI**. As shown in Figure 9, the sample is embedded in a matrix consisting of inorganic crystals. These compounds absorb the laser energy and ionize, and transfer charge to the sample, yielding positive and negative ions. Fast-atom bombardment (FAB) achieves desorption and ionization. The advantage of laser desorption and ionization is that inks and paints can be analyzed as

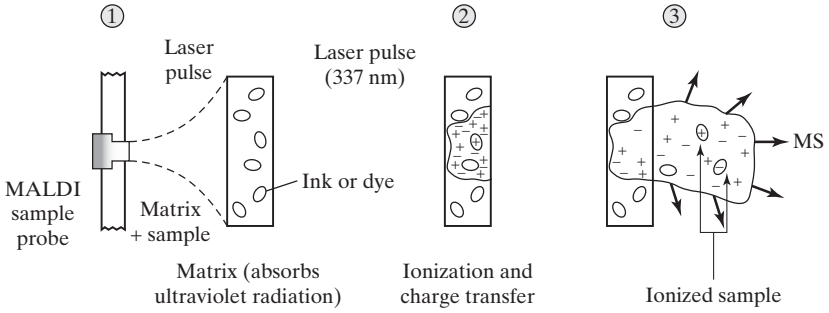


FIGURE 9 Formation of ions in matrix-assisted laser desorption and ionization (MALDI).

discrete samples or in situ. The disadvantage is that all desorbed and ionized species are introduced into the mass spectrometer simultaneously, rather than sequentially as is the case with GC-MS or LC-MS. Some discrimination is inherent in the method, since pigments and dyes are composed of larger molecules than solvents, but the mass spectrum remains a composite of multiple components.

A study by Balko and Allison addressed the characterization of security dyes—compounds used to stain currency and persons who take it in events such as bank robberies.[47] Figure 10 illustrates the identification of two cationic red dyes on currency and cotton fabric and shows no interference from dyes and pigments found in the currency. The cationic structure was deduced from the observation that no negative ions were produced when the dyes ionized. In Figure 11, the mass spectrum of a green pigment in currency (Pigment Green 7) is shown and illustrates the high mass range of this technique, as well as the ability to obtain natural-isotope ratios which can be elucidated by comparing the larger peak to the smaller in the peak group. The compound in question could not be analyzed with traditional solvent extraction GC-MS, given its size, poor chromatographic performance, and low volatility.

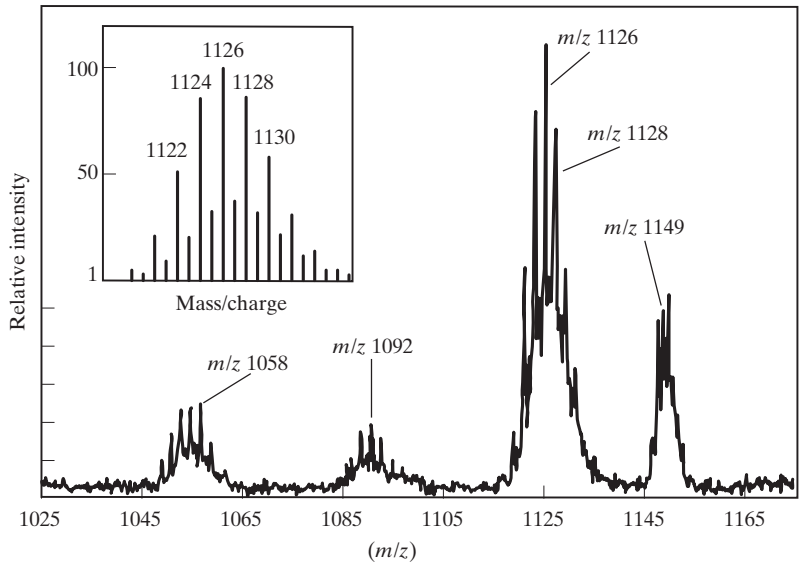


FIGURE 10 LCMS spectrum. Reproduced with permission from Balko, L., and J. Allison, "The Direct Detection and Identification of Staining Dyes from Security Inks in the Presence of Other Colorants, on Currency and Fabrics, by Laser Desorption Mass Spectrometry." *Journal of Forensic Sciences* 48 (2003). Copyright 2003 ASTM International.

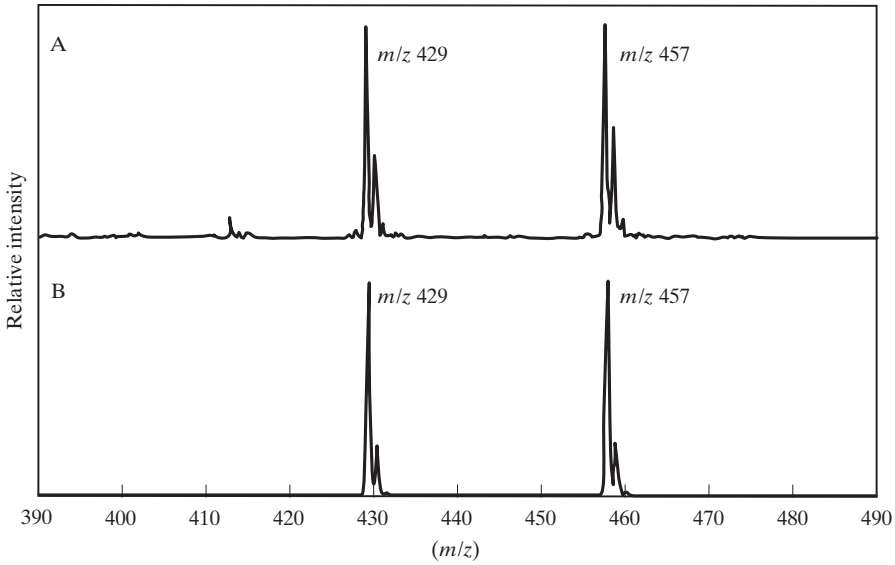


FIGURE 11 LC-MS spectrum of security ink currency (A) and on cotton (B), as might be encountered in casework. Reproduced with permission from Balko, L., and J. Allison, "The Direct Detection and Identification of Staining Dyes from Security Inks in the Presence of Other Colorants, on Currency and Fabrics, by Laser Desorption Mass Spectrometry." *Journal of Forensic Sciences* 48 (2003). Copyright 2003 ASTM International.

Because many dyes and pigments are not amenable to GC, HPLC methods have been explored as an alternative.[50,51] This makes sense in light of the relationship of HPLC to TLC, still a staple of colorant analysis. However, the lack of specificity in a detector system has been a limitation; consequently, until the advent of LC-MS instrumentation, HPLC has been used in the same way as pyrolysis GC and peak or pattern matching.

1.6 Elemental Analysis

Pigments are well suited to examination by elemental analysis techniques such as XRF (for elemental identification), XRD (for identifying structure), and SEM.[52,53] XRF provides elemental analysis in conjunction with SEM imaging and can identify the metallic constituents of pigments. XRD can reveal information about crystal structure and is useful for detecting very small amounts of inorganic colorants. XRD is capable of differentiating crystal forms of pigments, such as the two common forms of titanium dioxide (**rutile** and **anatase**).[52] SEM and XRF offer the ability to focus easily on individual layers and particles and obtain elemental ratios. Other elemental analysis procedures, such as ICP-MS have not been widely used for colorant analysis to date, although more applications are being reported using LA-ICP-MS.

2 AGING AND DATING

Ink in a pen and paint in a can (closed system) are chemically distinct from ink applied to paper and paint applied to a car (open system). In forensic casework, the latter is typically of more interest, but comparisons between ink or paint in containers with the same materials on a substrate can be used to estimate when the ink was put to paper or the paint applied to a surface (i.e., when the material leaves a closed system and enters and open system). Paper and inks age in chemically predictable ways, although

the time frame of the decay is dependent on many factors. The age of ink or paint in terms of when it was applied to the substrate can provide invaluable investigatory information, as well as inclusive or exclusive evidentiary information.

The aging process is illustrated in Figure 12 and is similar for paints and inks. When the solution or emulsion is applied to the substrate, solvent evaporation commences, in concert with film formation, which occurs by oxidation, polymerization, or cross-linkage. Materials diffuse across and into the substrate during film formation. Once formed, the protective film is subject to degradation by exposure to UV light, moisture, temperature extremes, and other environmental factors. The same is true of dyes and pigments. Colorants may fade, and primer layers can crack and peel away from a surface.

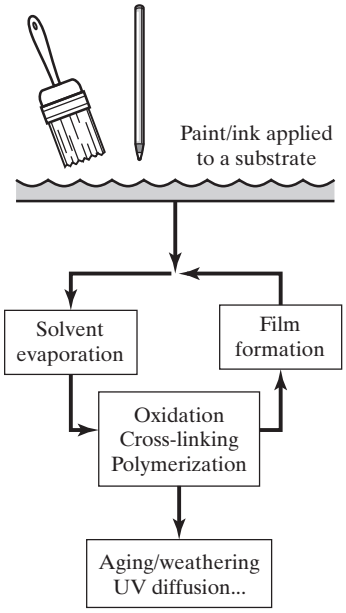


FIGURE 12 Processes involved in aging of colorant solutions applied to a substrate.

For most cases in which aging is an issue, there are two types of information that may be sought. First is the **absolute age** of the writing, meaning determination of a date when the ink was placed on the substrate. In such cases the comparisons of interest are between fresh inks, such as from a reference collection, and the ink in question. Second is a question of **relative age**, in which two separate samples are being compared to determine if they were applied at approximately the same time or at different times. One approach to this is the use of an aging curve, as illustrated in Figure 13. Typically, the evaporation rate is steady early in the process, which is measured in hours. As solvent evaporates, film-formation or hardening also occurs, and eventually, evaporative loss becomes negligible. Accordingly, aging of inks based on solvent composition is limited to a relatively narrow time window.

The historical time frame over which aging is thought to have occurred is an important consideration in the analysis. Synthetic colorants were not well developed or widespread until after 1900, so paints and inks produced before that time cannot contain synthetic colorants or vehicles. Similarly, ancient colorants were relatively crude, often containing particulates that were not as uniformly ground as their modern counterparts. Certain crystal forms were not available as well, making optical microscopy one of the most valuable tools for detecting counterfeit art and historical forgeries.

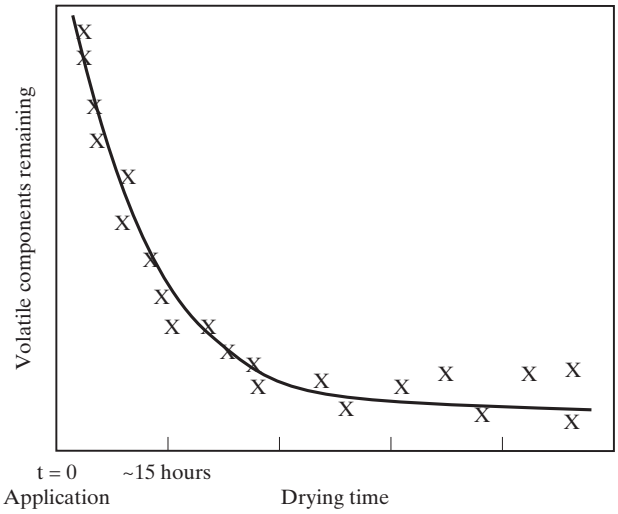


FIGURE 13 A generic aging curve depicting the loss of volatile materials from ink as a function of drying time.

For time frames of days to years, chemical analysis has proven valuable in estimating relative ages with techniques such as HPLC, MS, UV/VIS, and IR spectroscopy, targeting components and processes across the drying process. Solvent evaporation is generally the least useful analytical angle, whereas film formation and aging owing to UV light is the most useful for dating and aging studies. In the late 1980s, investigators proposed a solvent extraction technique for aging based on the idea that the older an ink is, the drier it is and thus the harder it is to extract.[54–58] In this scheme, samples are extracted from substrate, and the relative concentration of extracted dyes is used to evaluate the relative age of the writing. Investigators have also identified 2-phenoxyethanol as a volatile compound that could be useful for dating inks, given that the evaporation rate should allow for detection over roughly two years and that a high percentage of commercial inks appear to contain that compound.[4,54] GC-MS was used in this instance to isolate and identify the compound. An excellent summary of ink.

One of the challenges of dating inks is generating reliable standards. Because it is unusual to have an old (months to decades) ink sample that can be definitively linked to an unassailable source, artificial aging is required. By definition, this type of aging does not necessarily match gradual aging. Regardless, such studies are useful, and the casework is relevant. One study illustrates interactions in aging between the colorant (in this case, a triarylmethane dye) and the binder medium.[37] Figure 14 illustrates heat-accelerated aging and shows spectral peaks associated with the phenyl structure of the dye decreasing over time. A peak associated with the epoxy resin decreased as well, but at a slower rate than the dye features. The authors concluded that the

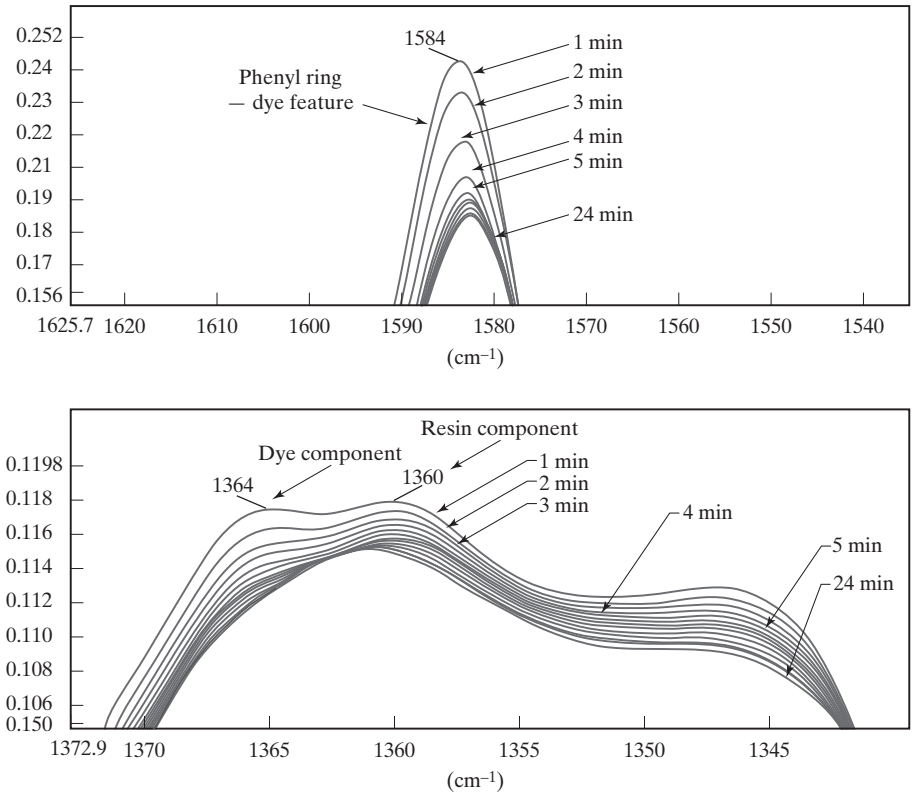
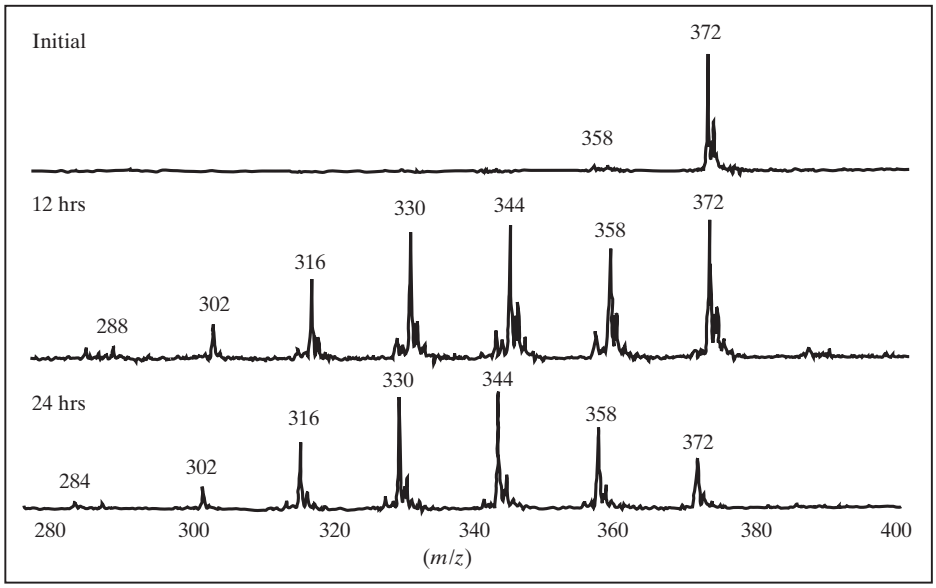
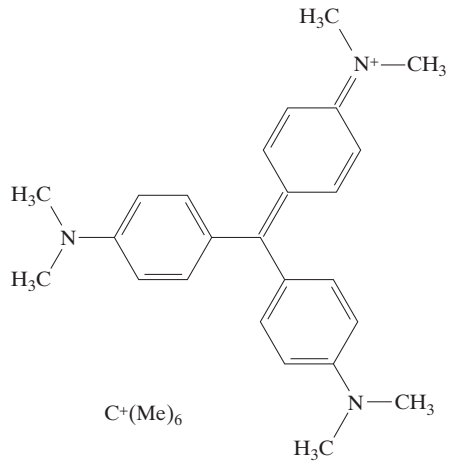


FIGURE 14 Decrease in peak intensity as a function of heating time meant to mimic aging. Reproduced with permission from Wang, F., et al., "Systematic Analysis of Bulk Blue Ballpoint Pen Ink by FTIR Spectrometry." *Journal of Forensic Sciences* 46 (2001): 1093–97. Copyright 2001 ASTM International.

dye initially broke down quickly but slowed as a protective film of cross-linked epoxy formed over it.

Degradation of components found in inks and similar products is characterized by the breakdown of larger molecules to small molecules, a phenomenon that is useful in chromatographic and mass spectral analyses. UV aging has been used to stimulate the aging and degradation of dyes in inks analyzed with laser desorption mass spectrometry (LDMS).[49] Degradation toward smaller compounds was clear and dramatic, as shown in Figure 15. Similar results are seen in an HPLC analysis in which the pigment crystal violet showed characteristic degradation products after a few months of natural aging. In this case (shown in Figure 16), the change in peak height was used to create a degradation curve for approximate dating.





m/z	Structure
372	$C^+(Me)_6$
358	$C^+(Me)_5$
344	$C^+(Me)_4$
330	$C^+(Me)_3$
316	$C^+(Me)_2$
302	$C^+(Me)_1$
288	$C^+(Me)_0$

FIGURE 15 LCMS spectra of dye exposed to UV radiation. Reproduced with permission from Grim, D. M., et al., "Evaluation of Desorption/Ionization Mass Spectrometric Methods in the Forensic Applications of the Analysis of Inks on Paper." *Journal of Forensic Sciences* 46 (2001): 1411–20. Copyright 2001 ASTM International.

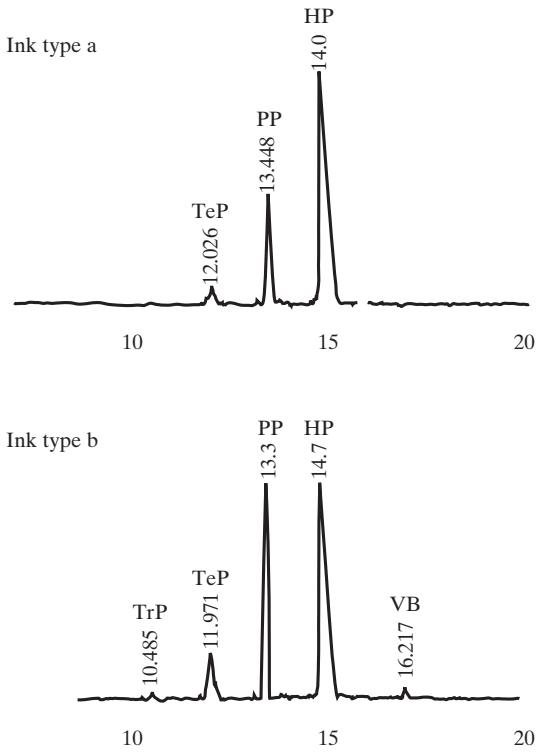


FIGURE 16 HPLC chromatogram of ballpoint pen ink types a and b from January to August and September to December, respectively. The ink types are distinguishable by the different concentration ratios of crystal violet (HP, at 14.8 min) and its degradation products (methyl violet: PP, at 13.4; TeP, at 12.0; and TrP, at 10.5 min) as well as by the ingredient Victoria blue (VB, at 16.9 min) that is only present in ink type b. Reprinted, with permission, from Journal of Forensic Sciences, copyright ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Summary

This concludes our discussion of materials that also contain colorants and some of the primary types of physical evidence that incorporate them. This chapter does not afford the opportunity for many quantitative questions, but you are encouraged to explore some of the following references to obtain a better understanding of how paints and colorants are analyzed and how the concept of pattern recognition, "chemical fingerprinting," and forensic comparison are applied in these types of analyses.

Key Terms and Concepts

Absolute age
Alternative illumination
Anatase
Chemical fingerprinting
Closed system
MALDI
Open system
Paint stratigraphy
PDQ database
Relative age
Rutile

Problems

FROM THE CHAPTER

1. Why are pigments typically more difficult than dyes to analyze with chromatographic techniques such as TLC?
2. How does the complementary relationship between FTIR and Raman spectroscopy enhance the analysis of inks and paints?
3. List and discuss some of the caveats and limitations of artificial-aging studies such as those discussed and shown in Figure 12.
4. Why is LA-ICP-MS used for paint and inks rather than typical acid digestion methods?
5. One method of measuring the relative rate of evaporation of volatiles from ink in aging studies is by monitoring

weight loss over time. As part of such an experiment, you preweigh a small clean filter paper and then quickly cover as much of the surface as possible with writing from a new ballpoint pen. You immediately weight it again and note a gain of 20,000 μg in mass, which you assume is all from the ink. You then reweigh the paper at regular intervals and obtain the following data:

Hours (*t*)	Mass (μg)
0.0	20000
0.1	19000
0.2	18000
1.0	16000
2.5	11000
5.0	6500
7.5	5005
10.0	3674
12.5	3203

Hours (*t*)	Mass (μg)
15.0	2894
20.0	2471
25.0	2599
30.0	2500
40.0	2499
50.0	2133
60.0	2301
70.0	1923

Use this information to perform the following tasks and answer the following questions:

Hint: Review first-order kinetics before proceeding. Assume it is ok to use mass as a measurement analogous to concentration.

a. Using an *XY* scatterplot, graph these points. When does the drying appear to cease? Support your statement.
b. What portion of the curve appears to follow first-order kinetics? Justify your answer.
c. What is the half-life of the evaporation process?
d. Using the 10 half lives rule of thumb, for how many days will evaporative processes be of any potential use?
e. What percentage of the solvent remains 7-1/2 hours after the writing was first made?

INTEGRATIVE

1. Which dye molecules would be amenable to typical GC-MS methods, and which would not? Why?
2. In fire debris analysis, weathering is manifest by an increase in chromatographic peaks with relatively higher molecular weights, whereas in ink evidence, weathering is manifest by a shift to lower molecular weights. Why?
3. Based on your knowledge of how IR and Raman spectroscopy work, explain why Raman differentiates the two forms of TiO2 discussed in the chapter while IR cannot.

FOOD FOR THOUGHT

1. Why are the solvents used in inks and paints usually of less forensic interest than the binders and colorants?

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Alphabetical Glossary of Terms

The following is a list of many of the important terms used in the text. Note that specific drugs are not discussed; refer to the index for that information.

5 Ps A moniker for the 5 most common forms of drugs and physical evidence: pills, powders, plant matter, precursors, and paraphernalia.

A

Absolute age In dating as with radioisotopes, the number of years that has elapsed since a material was formed.

Absolute error In any measurement, the difference between the expected (true) value and the experimental value.

Absolute uncertainty The uncertainty expressed in the same units as the measurand. For example, 45.67 g $\pm$ 0.46 g is an absolute uncertainty whereas 45.6 $\pm$ 1% is expressed as a relative uncertainty.

Accelerant A solid, liquid, or gas used to start and sustain an intentionally set fire.

Accreditation The process of reviewing a forensic laboratory against a set of accepted standards such as promulgated by ASCLD/LAB or ISO to insure that its policies, procedures, and practices meet these standards. If so, the laboratory is said to be accredited.

Accuracy How close the calculated value is to the true or accepted value; includes components of trueness and bias.

Achromatic Without color; white, gray, or black.

Active headspace method A sample preparation and preconcentration procedure in which vapors from a headspace are continually withdrawn and (often) concentrated outside of the original vessel for later analysis. The headspace is continually swept or purged.

Active metabolite A metabolite that is pharmacologically active and possibly toxic.

Addition polymerization Also called *condensation polymerization*; formed by a stepwise addition of a monomer to an active site, often (but not always) involving the loss of a small molecule such as water.

Additive color system A color system based on transmission where RGB colors are combined to yield a color, such as on a computer monitor.

Adiabatic combustion A combustion in which Q (heat evolved) is used only to heat the reaction products. It is the basis of some simple combustion models.

Admissibility hearing A hearing to determine if evidence will be admissible; a Daubert hearing is an example of an admissibility hearing.

Adulterant A material added to dilute a drug that is pharmacologically active. Caffeine and lidocaine are adulterants. The term diluent is often used interchangeably with adulterant and cutting agent, but they are not equivalent.

Adversarial system A system in which opposing arguments are presented to the party that makes the decision (trier of fact).

Agonist A substance such as a drug that binds to a receptor and causes the same effect as another substance. The first is an agonist of the second.

Alkaloid A basic molecule obtained (or at one time obtained) from a plant. In older literature, a vegetable alkali. Alkaloids are basic due to the presence of an amine group.

Alkyd A group of binders in paints and inks derived from acids and alcohols.

Alternative lighting The use of non-standard illumination (i.e., sunlight, indoor lighting, etc.) to assist in visualizing evidence such as fingerprints or body fluids.

Amine An organic compound or functional group that has a nitrogen but not an oxygen, of the form RNH_2 (primary amine), R_2NH (secondary), R_3N (tertiary).

Amorphous "Without form;" non-crystalline, no repeated structural units. Glass is an amorphous solid.

Amylopectin A highly branched and insoluble glucose polymer that is part of starch.

Amylose A linear glucose polymer that is a component of starch.

Anabolic steroids Steroids, natural or synthetic, that encourage muscle growth and purportedly improve athletic performance.

Analgesics Drugs that alleviate pain such as aspirin, acetaminophen, or morphine.

Anatase A crystal form of TiO_2, a white pigment.

Androgens/androgenic sex hormones Male sex hormones.

Angle aperture The angle of the cone of light exiting a sample observed by microscopy.

Anhydroglucose fibers Vegetable fibers; fibers based on glucose polymers.

Anisotropic Materials that have more than one refractive index.

Anomeric carbon A chain terminating carbon in a sugar, bound to a C = O that is converted to a chiral center as a result of ring closure.

Antagonist A substance such as a drug that binds with a receptor but that does not initiate the physiological response as another compound. The first is said to be an antagonist of the second because it blocks the response.

Antibody A substance produced in an organism in response to the introduction of an antigen.

Anti-Drug Abuse Act (ADA) A federal law passed in 1986 that regulated designer drugs.

Antigen A substance that when introduced into an organism stimulates an immunological response and production of an antibody.

Antiserum A solution of antibodies with a strength reported as the titer.

Anti-Stokes scattering Scattering interactions that start from an excited state; a type of inelastic scattering.

Aperture An opening, often adjustable, that determines how much light or electromagnetic energy passes through; usually designed to limit stray light.

Apparent volume of distribution A calculated quantity in toxicology that is used to express where and how a substance is distributed in the plasma and tissues. V_d depends primarily on the lipophilicity of the drug and the degree of protein binding.

Aryl Another term for a phenyl ring constituent in an organic molecule.

Associative evidence Evidence that tends to include or associate a person with a place or an item of evidence with a person or place. For example, finding soil embedded in a shoe that is consistent with soil found at a gravesite is associative evidence—the shoe was likely at the gravesite.

Atmospheric pressure chemical ionization Ionization using an electrical discharge used for LCMS instrumentation and some IMS instrumentation.

Atomic emission spectroscopy Spectroscopy in which thermal excitation is used to stimulate electronic transitions and photon emission.

Autolysis Self-lysis; bursting of cells after death.

Auxochrome A group or substituent on a chromophore that alters the absorption characteristics.

Aza linkage A linkage, such as in a dye molecule of the form —N═.

Aziridine A three-membered ring compound that can be associated with the Emde method of methamphetamine synthesis.

Azo/azo linkage A linkage of the type —N═N —.

B

Bandwidth Generically, the width of a band of electromagnetic energy measured in terms of wavelength. For example, the bandwidth of a monochromatic source such as an HCL is typically 1 nm or less.

Barbiturates A family of drugs based on the barbituric acid skeleton. Once widely abused, the introduction of benzodiazepines has reduced illicit use.

Baseline resolution In a spectrum, chromatogram, or other peak-based output, the situation that occurs when there is some portion of flat baseline in the space separating the peaks.

Batch A group of samples and related QC samples.

Bathochromic shift A shift of absorbance to a longer wavelength.

Bayesian statistics Statistical method that uses likelihood ratios as a part of estimating the probability of certain outcomes.

Bear claw An informal description of the morphology of the cystolythic hairs on the surface of the leaves of the marijuana plant.

Beating A process in paper making in which water is incorporated into the fiber matrix.

Becke line method A method of determining relative refractive index using mounting media of known refractive index and adjustment of focus.

Beer's law $A=\epsilon bc$; the law describing absorption quantitatively and the basis of linear calibration curves in spectroscopy.

Bell curve An informal term for the Gaussian curve derived from the characteristic shape.

Benzodiazepines A group of synthetic alkaloids used to treat anxiety, depression, and related ailments.

Bertrand lens A lens that, when inserted into the optical train of a microscope, allows the viewer to see an image of the filament; used to establish Koehler illumination. A lens that focuses on the rear apeture of the objective lens assembly.

Bias The difference between an experimentally determined value and an accepted true value; quantitative expression of trueness.

Bicomponent fibers Fibers that consist of two distinct polymers, such as in a core and sheath design.

Bile A bitter-tasting yellowish green material formed by the liver.

Binder Substance used in inks and dyes that bind pigments to the surface; materials that polymerize as they cure.

Binding strength The affinity of a substrate for a ligand.

Binomial distribution A distribution of possible outcomes of an event or events when the outcome of each is binary, as in flipping a coin (heads or tails).

Bioavailability (F) The amount of an orally ingested substance the remains unaltered after first pass metabolism.

Biopolymer Polymer derived from a natural source such as cellulose or proteins.

Biosensor A sensor that responds to a target analyte or analytes based on a biochemical mechanism.

Biotransformation The conversion of an ingested xenobiotic substance by biological processes; metabolism is a type of biotransformation.

Birch method A method of making methamphetamine.

Birch reduction A reduction that reduces a benzene ring by removing one double bond.

Birefringence The difference in the refractive indexes of an anisotropic material; can be calculated by subtraction or with a Michel-Levy chart.

Blackbody radiator/emitter A theoretical material that emits wavelengths of light that correlate to the temperature of the body.

Blank A sample that contains no analyte of interest.

Bleaching agent A treatment applied to a drug sample to remove tan/brown coloration.

Blind samples QC samples provided to the analysis without a known value.

Boltzmann distribution A function that describes the fraction of atoms in a given excited state as a function of temperature, energy gap, and degeneracy.

Bottom-up model An approach to the estimation of uncertainty based on a dissection of a method or process from the ground up. A fishbone (cause and effect) diagram is often used in this process.

Brisance The shattering power of an explosive or explosive device.

Buoyant flame A flame in which the hot gas products and heated air drift up and away from the reaction zone due to density differences generated by heating.

Bulk method An analytical method yielding results from the totality of a sample.

C

Cahn-Ingold-Prelog convention The most common method used to describe bonding around a chiral atom; uses the R/S notation and assigned priorities.

Caliber The nominal diameter of the barrel of a firearm.

Calibration The process of establishing a link between the output of an instrument or equipment and sample concentration.

Calibration check sample A sample prepared independently of the calibration curve and used to detect problems with the curve.

Calibration curve A plot and mathematical relationship (usually linear) between the concentration of an analyte and an instrument response.

Cannabimimetics Drugs that bind with the cannabinoid receptors and produce similar effects to a greater or lesser extent depending on structure.

Capillary electrophoresis/capillary zone electrophoresis Electrophoresis in a capillary tube that exploits electroosmotic flow.

Carbitol Similar to *cellusolve*; a solvent created from glycols and epoxides.

Carbocation A species that contains a carbon atom that carries a positive charge.

Cartridge A complete bullet, propellant, and primer assembly.

Cassegrain lens/system A focusing device that utilizes highly polished mirrors in lieu of glass lenses.

Cause of death The event or injury that starts the chain of events that leads to death. Blunt force trauma is an example of a cause of death.

Cause-and-effect diagram (fishbone diagram) A diagram used in uncertainty estimation procedures that involves diagramming and associating individual contributors to uncertainty of a measurement process and how they are related to each other; also called a fishbone diagram.

Cellophane Regenerated cellulose cast in a film.

Cellulose A biopolymer consisting of glucose monomers; the fiber of cotton and used in paper.

Cellulose acetate Regenerated cellulose fibers made by treating cellulose with acetic anhydride.

Cellusolve A type of solvent made from glycols and epoxide.

Center fire A type of ammunition cartridge in which the primer is in the center of the base of the cartridge case.

Central nervous system The physiological system consisting of the brain and the spinal cord.

Certification The process of insuring that a forensic practitioner is competent to conduct analyses based on written and laboratory testing.

Chain growth polymerization Polymer that grows via a free radical, anion, or cation chain reaction process. Involves the typical generic steps of any free radical reaction: initiation, propagation, and termination.

Chain of custody A cradle-to-grave document that tracks evidence.

Chemical Diversion and Trafficking Act (CDTA) A federal law passed in 1986 designed to limit access to precursor chemicals and pharmaceutical drugs used in clandestine synthesis.

Chemical fingerprinting A generic term to describe the use of chemical data, typically pattern-based such as spectra or chromatograms, to classify materials.

Chemical imaging The use of chemical reagents and developers to assist in the visualization of evidence; also refers to indirect imaging of samples by conversion of signals such as IR or MS data to viewable data.

Chitin A biopolymer that forms the exoskeleton of insects.

Chroma The degree of saturation of a color.

Chromatic Capable of displaying or generating color.

Chromaticity diagram A 2D plot of the chromaticity values x and y derived from the tristimulus values XYZ. A plot of CIE color space.

Chromophore That portion of a molecule that is capable of absorbing light in the UV or visible range. In organic compounds, this is a conjugated system.

CIELAB A mathematical transform applied to chromaticity coordinates to address the asymmetry in a chromaticity diagram.

Circumstantial evidence Evidence that alone proves nothing directly; requires additional inference to prove a fact in dispute.

Civil law Law that deals with disputes between parties.

Classification To assign an exhibit of evidence or other object to a group of like objects based on descriptors such as chemical and physical properties.

Clearance rate The rate at which a drug or other substance is removed or eliminated from the body.

Closed system In combustion, a system in which the reaction occurs in an isolated environment. Firing a gun or igniting gasoline in a cylinder are examples of closed systems.

Club drugs Drugs such as MDMA, GHB, LSD, and methamphetamine used by young people and young adults at clubs and parties.

Coagulation One process by which film-forming agents in paints and inks create a protective polymer coating; latex paints work this way.

Cobalt thiocyanate A reagent used for a color test for cocaine and related tropane alkaloids.

Coca paste The pasty material that results from crushing and mashing cocoa leaves for purposes of extracting cocaine.

Collision cell A component of mass spectrometers in which collision gases are introduced into a high vacuum region to facilitate collisional dissociation of complexes and compounds.

Color matching functions Functions used in the CIE system to express a color in terms of RGB equivalents.

Color test A test that involves the addition of a reagent or reagents to a sample and if positive, results in production of a color or a color change.

Colorant A substance that can impart or is colored such as dyes or pigments; a substance that absorbs or emits energy in the visible range.

Colorimeter A spectrometer that operates in the VIS range only.

Combined standard uncertainty The sum of the squares of the uncertainty contributors, typically in an uncertainty budget form.

Common ion effect In solution, the effect of ions present but not part of the reaction of interest. For example, if a sodium salt is dissolved in a solution with an existing high concentration of sodium that ion is the common ion and it will affect the reaction of interest according to Le Châtelier's principle.

Common source Associating two or more items or exhibit of evidence to one and only one possible source.

Compensator (wedge) A crystal with a known retardation factor; used to distinguish small retardation values.

Competitive assay A category of immunoassay that involves competition by antigens for a limited number of antibody binding sites.

Concentration gradient A situation in which differences exist in concentration between two zones or compartments. For example, when a drug is ingested orally, a concentration gradient exists between the gastric contents and the bloodstream.

Condensation polymerization See *addition polymerization*.

Condensation reaction An organic reaction in which two separate molecules combine and a small molecule is lost from the combination as a result.

Condenser/condenser lens The assembly in a microscope below the sample stage; focuses the beam into a tight cone of light.

Confidence interval An expression of the standard deviation of a relatively small data set adjusted by the use of the student t value.

Conjugation (conjugated system) A series of consecutive alternating single and double bonds.

Control chart A running record of the performance of a device or solution that identifies when performance is no longer within accepted uncertainty ranges.

Controlled Substances Act (CSA) A federal law first passed in 1970 that placed abused drugs on five schedules based on acceptable medical uses and potential for abuse.

Cook A slang term for those that manufacture illicit methamphetamine.

Coordination complex A stable complex formed between a transition metal cation such as copper or cobalt and ligands.

Copolymer A polymer made of different monomers; nylon 66 is a copolymer.

Correlation coefficient A value calculated to gauge the goodness of fit of points to a line generated by a linear regression algorithm.

Coverage factor (k) The multiplier used to calculate an expanded uncertainty; typically values of 2 or 3 are used, roughly corresponding to the 95% and 99% confidence intervals.

Cracking Another term for *thermal distillation*; applied to refining crude oil. Cracking also incorporates degradation, or catalytic splitting of large molecules into smaller ones.

Crash and shoot A sample preparation method used for LC-MS^n analysis that involves the addition of a small volume (μL) of an organic solvent to a small volume of sample (μL) to precipitate proteins.

Criminal law Law that deals with crimes committed against society as defined by law and administered by government.

Crossed polars In PLM, the situation when the analyzer and polarizer are in the light path oriented at 90 degrees to each other.

Cross-reactivity In immunoassays, the tendency of an antibody to react with antigens other than the target antigen.

Crystal Field Theory (CFT) A theory that describes bonding in transition metal complexes and explains color and magnetic properties. It is based on electrostatic forces.

Crystal test A presumptive test performed on a microscope slide in which the color, crystal morphology, and behavior under PLM are used to judge the outcome.

Crystal test See *microcrystal tests*.

Cutting agent Substances used to dilute a drug; can be pharmacologically active (adulterant) or inactive (diluent). Caffeine is an adulterant while cornstarch is a diluent.

Cystolith A nodule of calcium carbonate $CaCO_3$ found in cystolythic hairs.

Cystolithic hair A fine hair-like structure on the leaves of marijuana informally referred to as "bear claws".

D

Daubert/Daubert decision A court ruling on admissibility that among other things tasked judges with the role of gatekeeper for admissibility of scientific evidence and expert testimony.

Daubert hearing An admissibility hearing based on the Daubert standards held to determine if scientific evidence and testimony is going to be admitted into the court proceeding.

Daubert trilogy Three cases – *Daubert*, *GE.* v. *Joiner*, and *Kuhmo Tire* related to the admissibility of scientific evidence; all three are federal cases.

Deflagration Burning or combustion that propagates at less than the speed of sound.

Degressive burning powders/propellants A propellant that burns from the outside of the granule inward.

Delusterants Particulates applied to a fiber to decrease the shine or brightness; works by scattering light.

Dependent variable A variable that has a value derived from or dependent on the value of another.

Depressants A class of drugs that causes depression of the CNS, resulting in slowed breathing and heart rate and sleepiness, among other symptoms. Alcohol is a depressant.

Depth of field How far into a sample the field can be viewed and remain in focus.

Deterrent A material used to treat propellants in order to slow the rate of burn.

Detonation Explosive combustion driven by pressure and a compressive shockwave.

Detonation wave/detonation shock wave A compressive pressure wave (shockwave) that propagates through an explosive and causes combustion.

Diazo coupling The creation of an azo compound. This is a common way to make dyes and indicators.

Diazoniation Another term for diazo coupling.

Diazonium salt The combination of an anion and the cation $R—N^+ \equiv N$.

Dichroic A material that has two colors due to different absorbances depending on orientation.

Dichroic ratio The ratio of absorbances at a given wavelength in the parallel and perpendicular directions.

Diffuse reflection An older form of micro-IR in which the diffuse reflections off a sample act as the attenuated IR signal.

Diffusion Natural spreading of a concentrated band of analyte.

Digestion A sample preparation technique that attacks and destroys most of the matrix, leaving analytes behind; primarily for inorganics and metals.

Digressive burning powders A propellant that burns from the inside out.

Dilli-Koppanyi A presumptive test used mostly for barbiturates and based on formation of a colored complex with cobalt.

Diluent A material added to dilute a drug that is pharmacologically inactive. Cornstarch and sugars are diluents. The term diluent is often used interchangeably with adulterant and cutting agent, but they are not equivalent.

Dipole-dipole interactions Electrostatic attractions or repulsions between partially (+) or (−) regions of two polar molecules.

Direct evidence Evidence known to a person directly by personal knowledge.

Dispersing agent Substance added to paints or similar formulations to prevent settling.

Dispersion Physical separation of constituent wavelengths by a device such as a prism or grating.

Dissociative anesthetics Anesthetics that produce what is often described as an "out-of-body" sensation. Includes ketamine and PCP.

Distance determination Experiments conducted to estimate the distance from a firearm barrel to a target.

Distant precursor A chemical that can be converted to a controlled substance only after several steps.

Dixon's test See *Q test*.

Double base (smokeless) powder Propellant in which the main ingredients are guncotton and nitroglycerin.

Dragendorff test A color test reagent and TLC developer based on ion pairing with bismuth-iodide complexes.

Dronabinol Synthetic THC.

Drug A substance that when ingested is capable of inducing a physiological change.

Drug facilitated sexual assault A sexual assault that involves the use of a predator drug such as rohypnol or ketamine.

Dry extraction In drug analysis, a simple one-step extraction method in which a solvent is added to a solid sample. Typically, the next step is GCMS analysis of the extract.

Drying oils Oils that speed the drying and curing of binders in paint and inks; linseed oil is among the best known.

Duplicates Separate samples taken from the same source; not the result of subdividing one sample.

Duquenois-Levine A presumptive test for THC, the active ingredient in marijuana.

Dye A colorant that is soluble in the solvent or vehicle being used.

E

Effluent See *eluant*.

Electron impact ionization Ionization and fragmentation of molecules entering a mass spectrometer achieved by collision with a stream of electrons produced by a heated filament.

Electronic transition Transition of an atom or ion from the ground to an excited state via electron promotion among atomic orbitals.

Electroosmotic flow Flow of ions that occurs in a silica capillary tube exploited in capillary electrophoresis.

Electropherogram Output of CE or MEK analysis.

Electrophoresis Separation of charged and neutral species based on size-to-charge ratio.

Electrospray ionization (ESI) An ionization source for LC/MS that applies charge to solvent droplets that evaporate and eventually disperse via Coulombic explosion.

Eluant The material that exists in a SPE or chromatographic column.

Eluent The solvent or mobile phase used in SPE or chromatography.

Emde method A type of methamphetamine synthesis.

Enantiomers Isomers of a compound with a chiral center that are mirror images of each other.

Enantioselectivity The ability of a sorbent (typically a cyclodextrin) to separate chiral compounds.

Endogenous substance A substance, compound, element, or material that is naturally present in the body. Arsenic is such a substance, but it is found in very small amounts.

Endorphins A term derived from endogenous morphine, compounds that mimic the psychological effects of morphine and that can produce a feeling of well-being and euphoria.

Entomotoxicology The analysis of insects and associated materials found at a death scene for the presence of drugs or poison. The results are used to help reconstruct the cause or contributing factors in a death.

Enzyme linked immunoassay A heterogeneous immunoassay that is based on an enzyme catalyzed colorimetric reaction.

Ephedra A grass, herbal supplement, and natural source of ephedrine and pseudoephedrine.

Equilibrium constant The ratio of products to reactants raised to the power of the coefficients.

Ergot alkaloid Alkaloids with an indole structure derived from fungus. LSD is an ergot alkaloid.

Ergotism Ergot alkaloid poisoning with symptoms such as hallucinations.

Error The difference between an accepted true value and an experimentally determined value; bias. Error is not the same thing as uncertainty.

Erythrocytes Red blood cells.

Euclidean distance The distance between two points in data space, be it 2, 3, or more dimensional.

Evanescent wave In an ATR, a series of reflective absorptive interactions; multiple internal reflections.

Excipients An inactive or inert ingredient found in commercial drugs and medicines.

Exclusive evidence Evidence that by itself excludes a person or a possibility.

Exhibit A piece or individual item of physical evidence.

Expanded uncertainty (U) The product of the combined uncertainty (u) multiplied by the coverage factor k.

Explosion Combustion that propagates faster than the speed of sound.

Explosive train The series of explosive materials used to detonate a high explosive.

Explosive power index/explosive index The power of explosives scaled to that of picric acid and expressed as a percentage.

Extenders Compounds, often pigments such as calcium carbonate, added to inks or paints.

External standard curve A calibration curve in which the standards are made in simple solvents that may not match the matrix.

Extinction/extinction angle In PLM, the point at which a birefringent material is not visible.

Extraction A sample preparation technique that removes the analyte from the matrix.

Eyepiece See *ocular lens*.

F

Femoral blood Blood drawn at autopsy and found in the femoral arteries.

Field of view How large a portion of the sample can be seen in focus at one time.

Figures of merit Terms used to describe the performance of a validated analytical method.

Film-forming agent A binder or resin that polymerizes and protects a colorant in a paint or ink.

First order process A chemical reaction or other process, the speed of which depends only on the concentration of one reactant.

First pass metabolism Metabolic changes that occur to a drug after absorption in the GI tract but before any pharmacological effect can occur.

Fishbone diagram See *cause and effect diagram*.

Fitness for purpose/fitness of purpose A criteria used to gauge the utility of a given method to answer the forensic question and to provide the pertinent data in the most expedient and reliable way.

Flame ionization detector A GC detector selective to organic carbon and C—H bonds; based on creation of charged species in the flame.

Flash point The lowest temperature at which an ignitable mixture is capable of combustion.

Flow cell A cell used to isolate separated analytes in a flowing system long enough to obtain a spectrochemical measurement with an adequate pathlength.

Fluorescence Emission of a photon from an excited state; immediate.

Fluorescent polarization immunoassay A homogenous immunoassay in which the label is a fluorophore.

Focal length The distance along the optic axis of a lens that covers the distance from the lens to the principal focus.

Focal plane The plane at which a real image will be focused; the plane is centered about the focal point.

Forensic question The question that directs a forensic analysis; the question that dictates how a piece of evidence is analyzed and what data is provided. The forensic question may or may not be the same as the scientific and legal questions.

Fourier transform A mathematical procedure that converts a function from the time domain to the frequency domain; a function that translates an interferogram into a spectrum.

Free radical/free radical mechanism A species that has an atom with an unpaired electron; a reaction that involves free radicals.

Frye rule A rule of admissibility of scientific evidence and expert testimony that relies on general acceptance by the scientific community.

Fuel/air ratio The ratio of a fuel to air relative to a combustion reaction; typically expressed as a weight or volume percent comparison.

Functional groups A group of atoms within an organic molecule that are reactive. The functional group in alcohols is —OH for example. Many drug molecules have multiple functional groups.

G

GABA A key neurotransmitter.

Galvanization A treatment applied to steel that prevents rust; zinc is used.

Gastric contents Stomach contents.

Gatekeeper The role of the judge in regards to the admissibility of scientific evidence under the Daubert ruling.

Gauge In a shotgun, the size of the barrel.

Gaussian curve The graphical depiction of the Gaussian or normal distribution.

Gaussian distribution A type of distribution that can be assumed by a set of replicate measurements of the same criteria. The data is centered about a mean value and the spread is defined by the standard deviation.

General acceptance Under the Frye decision, the criteria for admitting scientifice evidence.

General Electric v. Joiner (GE v. Joiner) The second case in the Daubert trilogy; the ruling in the case stressed the need to weigh the relevancy of the data to the question at hand.

General unknown An exhibit for which there is no prior knowledge or outward signs that point to its composition. Forensic samples are often treated as general unknowns even if there is some prior knowledge.

Glass transition temperature (T_g) Temperature at which a polymer is transformed to a more rubbery material, but distinct from a melting point.

Glucuronide A conjugate form of drugs made by conjugation with glucuronic acid.

Glycogen A highly branched glucose polymer; primary energy storage molecule (glucose based) in animals.

Griess reagent/test A presumptive test used in the analysis of GSR.

Grubbs test A hypothesis test to identify outliers.

Guide to the Expression of Uncertainty in Measurement (GUM) An internationally accepted guide that describes in detail how to estimate the uncertainty of a measurement.

Guncotton A propellant made by treating cotton with nitric and sulfuric acid.

H

Half-life ($t_{1/2}$) The amount of time for half of the original amount of a substance ingested or existing in the body to be eliminated or converted to another substance.

Hallucinogens Drugs that can cause hallucinations of all types (visual, audio, etc.); causes changes in thoughts and perceptions.

Hapten The large protein portion of a drug-protein complex used to stimulate the production of antibodies.

Headspace The gas above a solvent or sample into which analytes can volatilize.

Hemicellulose A saccharide-based biopolymer composed of subunits of glucose, galactose, mannose, and xylose.

Hemp Marijuana; a term used to describe the plant when it is used to obtain fibers and other products unrelated to illicit drug use.

Henderson-Hasselbalch equation An equation used to describe buffers and other weak acids; relates pH to pK_a and concentrations of acid and conjugate base.

Henry's Law The law that relates partial pressure of a substance above a solution to the concentration in the solution; $K_H{*}P_a=[A]$.

Heterogeneous assay A category of immunoassay in which the bound and unbound phases must be separated prior to measurement.

High explosive An explosive that is relatively stable and insensitive to heat and shock.

High spin complex A complex in which electrons are distributed among the d-orbitals with the maximum number of unpaired electrons.

Hollow viscose A rayon produced such that there is a hollow space inside.

Homemade explosives Explosives that are created by combining easily obtainable ingredients such as sugar and fertilizer.

Homogeneous A substance or sample that is the same no matter what portion is examined.

Homogeneous assay A category of immunoassay in which the bound and unbound phases do not have to be separated prior to measurement.

Homolytic cleavage During formation of free radicals, an even splitting of an electron pair, one electron per involved atom.

Homopolymer A polymer consisting of all the same monomers.

Hot stage/hot stage microscopy An accessory for a microscope that allows for gradual and accurate heating. Used to determine melting points of fibers as one example.

Hue The descriptive color such as red, blue, green, etc.

Human performance toxicology Area of toxicology that concentrates on substances that alter performance, such as alcohol and steroids.

Hybridoma cells Cells created by a fusion of antibody-producing cells and cancer cells; used to produce monoclonal antibodies.

Hydrophilic "Water loving"; water soluble. Hydrophilic compounds are usually lipophobic.

Hydrophobic "Water hating"; water insoluble; hydrophobic compounds are usually lipophilic.

Hyperchromic shift An increase in absorptivity at of a chromophore resulting in a more intense color.

Hypergeometric distribution A distribution of possible outcomes of an event or events when samples are withdrawn or replaced.

Hyphenated instrument A combination of a separation module with a detector module such as GC-MS (GCMS); hyphen is often omitted.

Hypochromic shift A decrease in the absorptivity at of a chromophore resulting in a less intense color.

Hypothesis and hypothesis tests Statistical tests that compare two quantities, one calculated and one tabulated, to determine the acceptance or rejection of a hypothesis.

Hypsochromic shift A shift of λ_{max} to a shorter wavelength.

I

Identification A term with various meanings in forensic science including linking a piece of evidence to a single source (such as a fingerprint to a person) or identification of a chemical compound using libraries and reference materials

Ignition energy The amount of energy that must be transferred to a fuel/air mixture within flammability limits to cause ignition.

Illuminant In the CIE lab system, the lighting system or source selected.

Immediate precursor A chemical precursor that can be converted to the controlled substance by 1 or 2 simple steps and easily obtainable materials.

Immunoassay Analytical technique based on antigen-antibody reactions.

Immunogen The drug-hapten complex that stimulates the production of antibodies.

Immunological reaction The binding of an antigen to an antibody.

Improvised explosive An explosive or explosive device made from existing or homemade components.

Impurity A substance other than the drug or analyte of interest; can be present as a result of processing for example.

Incendiary device In arson fires, the device used to supply ignition energy.

Inclusive/inclusionary evidence Evidence that by itself includes a person or a possibility.

Independent variable A variable with a value that does not depend on or derive from any other variables.

Individualization Linking a piece of evidence to a single source.

Indole A molecule that contains a heterocyclic structure of a benzene ring connected to a 5-membered ring containing a nitrogen.

Inductively coupled plasma (ICP)/ICP torch An extremely hot excitation source for elemental analysis via emission or mass spectrometry. A plasma is not a flame and reaches much higher temperatures, so it can excite a significant portion of the ground state atoms. The plasma is created in the torch portion of the inlet.

Inhalants Volatile substances that are abused by inhalation and that produce effects similar to anesthetics.

Intaglio printing A process used in making currency that creates ridges and grooves in the printed substrate; an anti-counterfeiting measure.

Interference colors In PLM, colors observed for a birefringent material under crossed polars away from extinction.

Interferogram A plot of the distance traveled in an interferometer by the mirror versus intensity; can also be plotted versus time.

Interferometry Techniques that exploit interference (constructive and destructive) to limit or control the wavelengths of light transmitted from a source to a detector.

Internal standard curve A calibration method that involves addition of internal standards to all samples and standards and to which concentration and responses are ratioed.

Intramuscular "Into the muscle"; a method of injection for drug delivery.

Intravenous "Into a vein"; a method of injection or drug delivery.

Intrinsic solubility (S_0) The water solubility of a drug alone, not in the ionized form.

Ion mobility spectrometry Gas phase separation of ion/molecule clusters at atmospheric pressure; generically gas phase electrophoresis.

Ion pair/ion pair compound A tightly bound cation and anion that behave as a molecular compound or particle in solution.

Ion-dipole interactions Electrostatic attractions or repulsions between an ion and a partially (+) or (−) region of a polar molecule.

Ion-ion interactions Electrostatic attractions and interactions between two ions.

Ionization center In a large molecule, a site where ionization can occur typically in an acid/base manner.

Isobaric interference In mass spectrometry, a substance that has the same molecular weight or atomic weight of the substance of interest.

Isoelectric point (isoelectric pH) The pH at which a molecule with multiple ionization centers is neutral.

Isotope ratio The ratio of a heavier stable natural isotope to a lighter more abundant isotope. Ratios are used in drug profiling.

Isotropic Materials that have the same refractive index regardless of orientation.

K

K Generic symbol for the equilibrium constant; the subscript indicates what type of reaction. For example, K_a is the acid dissociation constant, K_b the base dissociation constant, and K_{sp} is the solubility constant.

Kinetic energy discrimination A method of filtering ions in a collision cell such as in an ICP-MS or LC-MSn instruments.

Knowns Samples with known accepted values.

Koehler illumination In a microscope, the alignment of lamp and lenses that produces optimal and even illumination.

Kraft pulping A pulping process used in paper product conducted at alkaline pH; principle lignin dissolution stage.

Kuhmo/Kumho Tire v. Carmichael The third decision in the Daubert trilogy; the ruling extended scope of *Daubert* and the judge's gatekeeper role to *all* expert testimony, not just scientific.

L

Lactone A cyclic ester that can form from an internal condensation between an alcohol functional group and a carboxylic acid functional group.

Laminar flame A flame with defined regions delineated by temperatures and flame color.

Lands and grooves A series of grooves spiral cut into the barrel of a firearm. The elevated portions are referred to as the lands; also called rifling. Rifling of a barrel imparts spin to projectiles as they exit the weapon.

Latex The thick, milky liquid that can be extracted from an unripe seed pod of the opium poppy. The consistency is like that of latex paint.

Latex particle A micelle particle in latex paint

Le Châtelier's Principle When a chemical equilibrium exists, any disturbance of the system will result in the system compensating.

Lean mixture A combustive mixture in which the concentration of oxidant is greater than the stoichiometric ratio.

Least-squared regression/least-squares fit A fit of a line or other curve to a set of points that is optimized by minimizing the total distance of all points to the curve. Distances are squared to eliminate potential canceling with some (+) and others (−) relative to the curve.

Legal question The question that is at the center of a case or court proceeding.

Leuckart method A synthetic method to make methamphetamine from P2P.

Leucocytes White blood cells.

Leuco form/leuco dye A soluble form of a vat dye.

Lewis acid A species that can accept electrons, such as a transition metal cation in a coordination complex.

Lewis base A species that can donate electrons such as nitrogen or oxygen.

Lieberman reagent/test A presumptive test based on nitrous acid. Also spelled as Liebermann.

Ligand An ion or molecule that coordinates with the central metal cation in a coordination complex.

Ligand field theory A theory that is used to describe bonding and interactions in transition metal complexes.

Lightness A characteristic of color; depth on a white to black scale.

Lignin A family of compounds of related biopolymers based on phenylpropyl units that are linked by strong C — C bonds resistant to many degradation pathways.

Lignocellulose The complex of cellulose-based materials that impart rigidity and structure to plants.

Like dissolves like (LdL) A rule of thumb concerning polarity and solubility. A polar solute will dissolve in a polar solvent but not in a non-polar one.

Lime method A procedure used to extract morphine from opium using lime (calcium hydroxide) to make extract basic.

Linear correlation A relationship between two variables that can be described by a linear equation of the form $y = mx + b$.

Linear dynamic range The concentration range over which a calibration curve demonstrates linear response with concentration. LDR is usually reported in terms of orders of magnitude.

Linearity of a balance The deviation of a balance from perfect correlation between certified reference weights and the weights obtained on the balance.

Linear regression The process of creating a straight line and linear equation to describe the relationship between a dependent and independent variable.

Lipophilic/lipophilicity Literally, "fat loving"; molecules that are more soluble in fats and oils than in water. The degree of lipophilicity is gauged by the logP value (octanol/water partition coefficient).

Lipophobic "Fat hating"; insoluble in nonpolar solvents. Compounds that are lipophobic are usually hydrophilic.

Liquid/liquid extraction (LLE) Separation and isolation of analytes based on preferential affinity for one solvent over the other; solvents must not be miscible.

Living polymerization A self-perpetuating anionic or cationic polymerization that is stopped by quenching ("killing").

Log P/K_{ow} The relative solubility of an analyte on octanol; a measure of lipophilicity/hydrophobicity.

Low explosive An explosive that detonates easily and is sensitive to heat or shock.

Low spin complex A complex in which electrons are distributed among the d-orbitals with the least number of unpaired electrons.

Lower flammability limit (LFL) The lowest mixture ratio of fuel and oxidant that will ignite and sustain combustion.

M

Manner of death The description of the way in which a death occurred such as accidental, suicide, homicide, natural, or indeterminate. The acronym NASH is sometimes used.

Marquis reagent/test A color test reagent that reacts with a variety of controlled substances, principally alkaloids.

Mass spectrometer A detector that ionizes and fragments molecules and creates a reproducible and usually unique fragmentation pattern that can be used for identification.

Mass transfer The process of a solute moving in and out of a stationary phase.

Matrix controls Samples of pristine background material that must be analyzed to facilitate interpretation of analysis.

Matrix mismatch The situation that arises when the solvent system used to generate a calibration curve does not match the matrix of the sample.

Mayer test An older and rarely used presumptive test based on mercuric compounds.

Mean (average) The sum of all values in a population or sample divided by the number of samples or measurements.

Measurable quantity Legally, similar to a "useable quantity," but in chemical terms, more akin to the limit of detection (LOD).

Measurand That which is being measured; for example, if determining the weight of a white powder, the measurand is the weight.

Meconium The first fecal products produced by a fetus.

Medicine A mixture of drugs or other physiologically active materials. Aspirin is a drug while a cold preparation is a medicine.

Melt extrusion A method of casting or making fibers in which raw polymer is heated until liquid and then extruded into a film or fiber.

Metabolites Products of metabolic reactions and conversions.

Metamerism Occurs when a colorant takes on a different perceived color based on a change in illumination.

Methamphetamine Anti-Proliferation Act (MAPA) A federal law passed in 2000 to address the availability of methamphetamine precursors.

Metrology The science of measurement as a general topic.

Micelles Structures formed by surfactants above a critical concentration in water.

Micellular electrokinetic chromatography (MEKC) Capillary electrophoresis using micelles and selective partitioning to separate neutral species.

Michel-Levy chart A chart showing interference colors and relating them to birefringence and thickness; shown in the color insert.

Microcrystal tests Another term for crystal tests; refers to the use of the microscope to study crystal form.

Microspectrophotometry Use of a microscope in conjunction with a spectrometer.

Minimum ignition temperature The temperature at which molecules in the environment can transfer sufficient energy to the fuel to overcome E_a and begin the reaction.

Mobile phase In solid phase extraction or chromatography, the phase that moves over the solid or stationary phase; may be polar, nonpolar, or inert.

Mobility spectrum Output of an ion mobility spectrometry.

Mode of ingestion The route or pathway by which a drug or poison enters the body.

Molecular explosive An explosive that detonates relatively easily when in the pure form.

Molecular orbitals (MO) An orbital that forms from the overlap and combination of atomic orbitals and that belongs to a molecule; a covalently bonded compound.

Molecular transition Transition of a molecule (covalently bonded) from the ground to an excited state via electron promotion among molecular orbitals.

Monochromator A device such as a prism, filter, or grating that selectively removes all but a narrow range of electromagnetic energy from an impinging source.

Monoclonal antibody A nearly pure antibody produced by a several step procedure that includes the use of hybridoma cells.

Monomer The base unit of a polymer such as vinyl chloride in PVC.

Mordant A substance that is used to fix a dye to a fiber or fabric.

Multiplex assay An assay such as an immunoassay that detects more than one compound or class at a time.

Munsell Color System A color space based on a catalog of standard colors and a uniform 3D color space.

Muzzle velocity Speed at which a projectile leaves the barrel of a firearm.

N

NA The numerical aperture of a lens; an ability of the lens to collect light. As magnification increases, NA decreases.

Narcotic Analgesic that also acts as a CNS depressant.

Narcotics A class of drugs that relieve pain and encourage sleep. Morphine and heroin are narcotics.

Natural drug/natural product A drug that is derived directly from a plant. THC and morphine are natural products.

Nazi method Another term for the Birch method, a clandestine methamphetamine synthesis.

Nebulizer A sample introduction device used with ICP-MS and other techniques. A nebulizer creates a fine mist for introduction into the instrument.

Negative control A sample that should produce a negative or no reaction or response in an analytical procedure; part of QA and QC.

Neurotransmitter A compound vital in the process of transmitting a nerve impulse.

Neutral burning powder/propellant A propellant that burns evenly throughout the granule due to pores and holes that expose more surface area.

Nitrocellulose Informally, *guncotton*; made by treating cotton with nitric and sulfuric acids; attaches nitro groups to cellulose.

Nitrogen phosphorus detector A GC detector selective to N- and P-containing compounds; similar in design to an FID and includes an alkali salt.

Nitroprusside A complex species with the formula $[Fe(CN_5)NO]_2^-$.

Non-competitive assay A category of immunoassay that does not involve competition by antigens for a limited number of antibody binding sites.

Non-invasive sampling Sampling that does not require a puncturing of the body; collection of saliva is an example.

Normal phase Separation or chromatography using a polar stationary phase and a nonpolar mobile phase.

Normalization The process of scaling data across a data set such that large variables do not overwhelm small ones.

Noscapine An opiate alkaloid sometimes encountered as an adulterant of heroin or related drug samples.

NSAID Non-steroidal anti-inflammatory drugs such as aspirin that relieve pain by reducing inflammation at the sight of an injury.

Neutral burning powder A propellant that burns at an even rate.

Nylon A class of completely synthetic fibers and the first completely synthetic fiber.

O

Objective lens The lens or lens assembly closest to the sample in optical microscopes.

Ocular lens The lens or lens assembly closest to the viewer's eyes in optical microscopes.

Off-axis Light path that does not follow the principle optical axis; set off at an angle.

Open system In combustion, a reaction that takes place in a system that is open to the atmosphere.

Opiate alkaloids Alkaloids derived from the opium poppy.

Optic axis The imaginary line that runs through the point of a curved lens where the curvature is at a maximum; may or may not be the geometric centerline.

Optical isomers Enantiomers; compounds with chiral atoms that rotate plane polarized light in opposite directions (or d/l).

Oral fluid Saliva.

Oriented polymer A strong polymer in which all the chains are aligned parallel.

Outlier A sample result that appears to be unusually far from the mean in a normal distribution; can be evaluated using significance tests.

Oxidation A gain of oxygen; a loss of hydrogen; or a loss of electrons.

Oxygen balance A measure of the amount of oxygen in a molecule that undergoes combustion. A positive oxygen balance means that all the oxygen needed for complete combustion is available intramolecularly.

P

Paint stratigraphy The use of paint layers as a aid to forensic analysis.

Paracetamol A term used for acetaminophen outside of the United States.

Paraphernalia Equipment and supplies used in the process of ingesting a drug. Pipes, mirrors, and syringes are examples.

Partitioning A preference or affinity for one physical phase or state over another; the basis of solvent extraction and chromatography.

Passive diffusion Diffusion of a substance from a zone of relatively higher concentration to a zone of relatively lower concentration.

Passive headspace method A sample preparation procedure in which vapors from a headspace are withdrawn for analysis.

Pattern matching The process of comparing patterns of chemical data such as spectra or chromatograms to group or classify them.

p-DMAB p-Dimethylaminobenzaldehyde, a color test reagent also called Erlich's reagent. It is particularly valuable for detecting ergot alkaloids such as LSD.

Peak capacity The number of distinct peaks that can be detected across a chromatographic run; a term used in conjunction with UPLC assays.

Pedigree In the NUSAP model, the data and information that supports a measurement and spread; examples include peer review, laboratory accreditation, and analyst certification.

Percussive explosive An explosive that is shock-sensitive, such as a primer used in ammunition.

per-Fluorotributylamine (PFTBA) A compound used to standardize the performance of mass spectrometers to insure intercomparability of spectra.

Peripheral blood Blood collected away from the core such as arms or legs at autopsy.

Persistence The tendency of trace or transfer evidence such as GSR to stay where it is originally deposited during the primary transfer.

Petroleum distillate A product that is or was at one time derived from crude oil by distillation techniques.

Pharmacodynamics The study of effects of drugs over time and is concerned with the interaction of the drug with its target.

Pharmacogentics The influence of genetic factors such as enzyme polymorphism on drug metabolism.

Pharmacokinetics The study of the movement of the drug and metabolic products through the body; studies the traversal of a drug or foreign substance (xenobiotic) by dividing it into stages of absorption, distribution, metabolism, and elimination.

Pharmacology The study of how drugs behave once ingested; can be broadly divided into pharmacodynamics and pharmacokinetics; includes metabolisms, rates of elimination, etc.

Phenethylamines The family of stimulants based on a phenylethylamine structure. The family includes amphetamine and methamphetamine.

Phenobarbitone A barbiturate; occasionally encountered as an adulterant of heroin or related drug samples.

Phosphorescence Emission of a photon from an excited state; emission is not immediate but rather delayed.

Photodegradation Degradation initiated by exposure to ambient indoor or outdoor light.

Photodiode array (PDA) A detector that works by detecting photons dispersed in space; one photodiode per geometrical location.

pH_{max} The pH at which a drug with an ionizable center is most soluble.

pH-partition hypothesis The theory that states charged species (here, A^- or BH^+) are not appreciably soluble in lipids and as such will not partition to any significant degree across a lipid membrane.

Physical match A match based on piecing or comparing edges; fitting together as one would fit puzzle pieces.

Physiological pH 7.4; assumed to be the typical normal pH of the plasma.

Pigment A colorant that is insoluble in the solvent; exists as a suspension and not a solution.

Pills A common form of drug evidence.

pK value The –log of the quantity of interest such as pH or pK_a.

Planar chromatography See *Thin layer chromatography*.

Plant matter A common form of drug evidence.

Plasticizer An additive to a polymer, typically a phthalate, that imparts softness and pliability.

Pleochroic pleochroism A material that has two or more colors due to different absorbances depending on orientation.

Point of origin The location(s) where a fire began.

Polarity Arises from asymmetric electron distribution around a molecule; leads to partially positive and partially negative areas.

Polarizable bonds Chemical bonds that are altered by passage of light; bonds in which the electron clouds can be distorted by scattering interactions.

Polarized light Light that vibrates in a single plane; also called *plane polarized light*.

Polarized light microscopy (PLM) A technique that uses polarizing filters in the optical train of a microscope.

Polyclonal antibody A mix of related antibodies produced when an antigen is introduced into an organism.

Polysaccharide A polymer consisting of sugars such as cellulose.

Population The larger group of possible measurements from which a subset is drawn; has N member.

Positive control A sample that should produce a positive reaction or response in an analytical procedure; part of QA and QC.

Postmortem redistribution Redistribution of drugs and metabolites after death and not associated with normal processes that occur in the living.

Post-mortem (toxicology) Literally "after death;" toxicology that analyzes biological materials collected at autopsy.

Power (of a color) A value derived from the location of a color as plotted on a chromaticity diagram.

Precedent That which has gone before; previous rulings and approaches made by courts and supported over time.

Precision Reproducibility of replicate measurements.

Precursors Chemical compounds, including pharmaceuticals that are used as the starting point for clandestine synthesis of controlled substances. A precursor can be immediate (one step from product) or distant (several steps).

Predator drugs A class of drugs used in drug facilitated sexual assault.

Presumptive test A test used to narrow down the possible identity of a sample or to classify it. Results are not conclusive and a positive result is best phrased as "more likely than not."

Primary explosive A sensitive high explosive used to initiate detonation of a relatively insensitive high explosive.

Primary transfer The transfer of trace evidence from the original source to another location. The deposition of GSR on the hands of a shooter is an example of primary transfer.

Primers Devices used in ammunition to ignite the propellant; consist of low explosives that are shock sensitive (percussive explosives).

Principal component analysis A exploratory technique in multivariate statistics.

Principal focus The point in space where light rays that have passed through a lens will converge.

Probability sampling The selection of an unbiased subset for sampling a large population such that the subset adequately and appropriately represents the characteristics of the population.

Prodrug A substance that metabolizes to an active drug; codeine is a prodrug of morphine.

Profiling A thorough organic and inorganic analysis of a drug, diluents, adulterants, contaminants, and in some cases, isotope ratios and DNA profile. The goal is to link a sample to a batch and/or place of origin.

Progressive burning powders/propellants A propellant that burns slowly at first due to a deterrent. Once the deterrent is burned away, the burn speed increases.

Propagation of uncertainty A technique that combines uncertainties from individual steps to obtain an estimate of the uncertainty of a process.

Propellant A powdered combustive fuel used in firearms; typically contains nitroglycerin and guncotton.

Prostaglandin (PG) Fatty acid derivatives found associated with cell membranes that affect many processes, including inflammation.

Provenance Location or place of origin. In drug profiling of natural products, the geographic area where the plant grew.

Pseudocrystalline A material such as a synthetic fiber that is ordered but not crystalline in the sense that a solid inorganic material is.

Purge-and-trap A type of active headspace method in which analytes are trapped and pre-concentrated before being desorbed and analyzed.

Pushing power Brisance.

Pyranose A 5-membered ring form of a sugar.

Pyrogens Literally, "fire starters"; compounds released by white blood cells in response to injury or infection. Pyrogens act on the hypothalamus and stimulate the release of prostaglandins.

Pyrolysis (combustion reactions) A high temperature decomposition that occurs in a reducing environment; an inlet for instrumentation and also an element of combustion reactions. Pyrolysis is often combine with gas chromatography in forensic applications.

Q

Q test A hypothesis test to identify outliers.

Quadrupole A type of mass filter for a mass spectrometer that uses DC/Rf voltages to control ion trajectories.

Quality assurance (QA) The philosophy and practices used to insure the goodness and reliability of data.

Quality control (QC) Procedures used as part of quality assurance.

Quiescent Quiet, an unstirred solution.

R

Radiationless transition A transition of an excited state to a lower state that occurs without emission of a photon; a transition in which energy is dissipated typically as heat.

Radioimmunoassay A heterogeneous immunoassay in which the label is a gamma or beta emitter.

Raman spectroscopy A vibration technique based on scattering and polarizable bonds.

Random errors Errors that are not the same, not reproducible, equally plus and minus, and generally small.

Random sample A sample or set of samples collected in such a way that there is an equal probability of any one sample being selected.

Rayleigh scattering Scattering that results in no change in wavelength; elastic scattering.

Rayon A semi-synthetic fiber made from regenerated cellulose.

Reaction cell See *collision cell*.

Readability of a balance How many decimal places can be read off the display with the understanding that there is uncertainty associated with this last place.

Real image An image created by a lens that can be projected onto a screen; an image that exists at a plane in space and does not require one to look through a lens to see it.

Rectangular distribution A form of distribution in which there is equal probability across a range; the readability of a balance is an example.

Red cook A clandestine synthesis of methamphetamine that utilizes phosphorus and iodine.

Reduction A loss of oxygen; a gain of hydrogen; or a gain of electrons.

Reductive amination The chemical conversion required to go from P2P to methamphetamine.

Refluxing Prolonged heating in an enclosed container in which evaporated solvent condenses and is recovered.

Regression line A line describing points that have a linear relationship or correlation.

Relative affinity The comparative affinity of a compound to different phases such as two different solvents; the basis of partitioning, separations, and chromatography.

Relative age In dating studies, the age of one material relative to another.

Relative error In any measurement, the difference between the expected value and the experimental value expressed as a percentage or other unitless way.

Relative explosive power (REP) The power of an explosive as compared to some standard such as picric acid or TNT.

Relative uncertainty Uncertainty contribution expressed as a fraction or percentage. For example, a weight reported as 12.34 g $\pm$ 1.0% is based on relative uncertainty.

Relevancy In the forensic context, the applicability of the data or information to the question at hand.

Reliability The degree of trustworthiness of data.

Repeatability Closeness of the agreement between the results of successive measurements of the same measurand under similar and controlled conditions.

Replicates Repeat measurements of the same criteria under similar conditions; multiple samples derived from one larger sample.

Representative sample A sub sample of a larger sample or group that accurately reflects the composition of the whole.

Reproducibility Closeness of the agreement between the results of measurements of the same measurand under controlled conditions; typically broader that repeatability.

Resins Natural binders used in paints and inks.

Resolving power The ability of a lens or optical train to distinguish two objects; = 0.6/NA.

Retardation An expression of the slowing of light as it propagates along and perpendicular to the optic axis of the material. The greater the difference in speed, the greater the retardation.

Retardation distance In polarizing light microscopy, the distance (in nm) that separates the emerging perpendicular and parallel rays emerging from a birefringent sample.

Retention factor In TLC, the distance that a compound migrates relative to the starting point.

Reversed phase Separation or chromatography using a nonpolar stationary phase and a polar mobile phase.

Rich mixture A combustive mixture in which the concentration of fuel is greater than the stoichiometric ratio.

Rifling rifled Scoring of lands and grooves in a spiral pattern in the barrel of a firearm.

Rimfire A type of ammunition, typically small caliber (0.22) in which the primer is wrapped around the periphery of the case.

Ring opening polymerization Polymerization that involves a ring opening such as the opening of an epoxide ring.

'Roid rage An informal term referring to excessively aggressive behavior that can be associated with steroid abuse.

ROYGBIV An acronym for the simple colors of visible light: red (700 nm wavelength), orange, yellow, green, blue, indigo, and violet (400 nm).

Rutile A crystal form of TiO_2, a common white pigment.

S

Salicylates Drugs such as aspirin based on the salicylic acid skeleton.

Saltpeter An older name for KNO_3, potassium nitrate.

Sample A subset of a larger population selected for analysis.

Sampling statistics The application of statistical techniques to sampling to insure that the samples are sufficient to adequately represent the larger population or sample.

Saturation Overloading; in enzymes, a situation where all available binding sites are occupied.

Scanning electron microscopy (SEM) An imaging technique that uses interaction of a sample with electrons to create an image.

Schiff base A compound of the form $R_2C = NR$.

Secondary explosive An relatively insensitive explosive that is detonated by a primary high explosive.

Secondary transfer The transfer of trace evidence from the place it was originally deposited to a secondary location.

Seed fiber A natural fiber such as cotton that is derived from the seed pod of a plant.

Semi-synthetic A drug that is derived indirectly from plant matter. Heroin is a semi-synthetic that is made by acetylation of morphine, which is derived from opium.

Sensitizers Compounds added to propellants and explosives to increase sensitivity to detonation.

Sequential In a detector, the process of scanning one-at-a-time through masses or wavelengths.

Shade Describes the variation in the hue of colors.

Shock wave See *detonation wave*.

Sign of elongation The sign of the birefringence; positive SE is where the parallel RI exceed the perpendicular under crossed polars.

Significance test See *hypothesis test*.

Significant figures These arise from instrumentation and consist of every digit that is certain plus the first uncertain one.

Simon test See *sodium nitroprusside test*.

Simultaneous In a detector, the ability to detect a range of masses or wavelengths simultaneously rather than by traditional scanning.

Single base (smokeless) powder Propellant in which the main ingredient is guncotton.

Snell's Law An expression that described the angles of refraction of light when it passes through an interface;

$$\frac{\sin i}{\sin r} = \frac{n_2}{n_1}$$

where i = angle of incidence and r = angle of refraction.

Sodium nitroprusside test A presumptive test using the nitroprusside complex and the NO associated with the central iron cation.

Solid phase extraction An extraction in which one of the phases involved in the selective partitioning is bound to a solid support.

Solid phase microextraction Extraction into a solid phase coated on a microfiber. The pre-concentrated analytes are typically introduced directly in the injector port of a GC.

Solubility S, calculated using K_{sp}.

Solution casting A method of making thin polymer films by pouring a solution in a thin film and allowing it to dry or cure.

Solvent extraction Use of a solvent to extract an analyte or analytes from a matrix.

Solvent strength A measure of the ability of a solvent to elute a material in solid phase extractions or chromatography, based on relative polarity.

Specular reflection "Perfect reflection"; angle of reflection = angle of incidence; occurs at a surface where there is a change in the refractive index.

Spikes Analytes purposely added to a sample to gauge recovery and flag potential matrix problems and affects.

Spinning The physical process of converting polymer into a synthetic fiber product.

Spontaneous ignition temperature See *minimum ignition temperature*.

Spot plate A plate, typically in the range of 3×4" made of white or black ceramic or glass. Spot plates have depressions in which the sample is placed and color test reagents are added. The design and color helps with interpretation of colors produced.

Spot test Used as a synonym for color and presumptive testing. The name arose from an early method of qualitative analysis in which small spots were applied to paper and the paper rotated to disperse reagent in a radial manner.

Springall Roberts rules One of several sets of rules used to approximate the mixture of products produced in an explosion.

Stabilizer An organic constituent of propellants.

Stable isotope ratio (SIR) The ratio of stable isotopes ratios such as $^{13}C/^{12}C$ that are determined by mass spectrometry and that can be incorporated as part of drug profiling of plant-derived substances such as heroin.

Standard addition A calibration method which uses the sample as the matrix and to which increasing aliquots of the target analyte are added.

Standard deviation The average deviation of all points in a data set from the mean of that data set.

Standard observer curves In the CIE colorant space system, three spectral curves that describe the contribution of red, green, and blue light to a perceived color.

Stand-off detection Indirect detection at a distance such as sensing explosives using electromagnetic energy

Standard operating procedure A laboratory procedure or analytical method that has been validated and accepted for routine use in a forensic or other laboratory; SOP.

Standards Analytical solutions used for calibration; ASTM documents describing a standard procedure or method.

Standard uncertainty (u) The uncertainty contribution from a single factor, expressed as the equivalent of a standard deviation.

Stand-off detection Detection at a distance that does not require physical contact between the sample and the detector.

Starches Glucose polymers that store energy; include glycogen, amylopectin, and amylose.

Stationary phase A solid immobile material to which an active material is bound; the stationary phase in solid phase extraction or chromatography; may be polar or nonpolar.

Steroid profile An initial step in doping assays to determine if the compounds present or their concentrations indicate the possibility of ingestion of banned substances.

Stimulants A class of drugs that stimulates the CNS resulting in elevated heart rate and less of a need for sleep. Methamphetamine is a stimulant.

Stoichiometric equivalence (STE) The point at which fuel and oxidant create a zero oxygen balance.

Stokes scattering Scattering interactions that start from the ground state; a type of inelastic scattering.

Subclavian blood Blood collected at autopsy from below the clavical.

Subcutaneous Below the skin surface; a method of injection for drug delivery.

Subtractive color system A color system based on reflection where colorants absorb or subtract colors; used in color laser printing such as CMY.

Surface absorption-reflection A process where energy is absorbed to some degree by a substrate before being reflected.

Surface method An analytical method yielding results from the surface of a sample, typically within a few microns.

Synthetic A drug that is synthesized in the traditional organic chemistry sense as opposed to extracted from a plant or obtained by simple chemical processing of a plant extract.

Systematic errors Errors that are the same size and magnitude each time; reproducible errors.

T

Taggant A substance or compound added to a material to allow it to be traced to a manufacturer or date of manufacture.

Tandem mass spectrometry A series of mass filters placed in tandem (in space or time) such as a triple quadrupole mass spectrometer.

Temperature (color) The temperature of a color refers to how it correlates to light emitted by a blackbody emitter.

Tertiary explosive An extremely insensitive explosive.

Testosterone A male sex hormone that is produced in the testes. It plays a central role in the development of secondary sex characteristics. It is a steroid hormone derived from cholesterol.

Theoretical plates A measure of the efficiency and resolving power of a chromatographic column; based on a distillation model.

Thermal transfer printing Mechanical printing in which colorant is delivered to a surface by heating of a waxy substrate.

Thermoplastic behavior Physical changes observed in a polymer as it is heated.

Thermoplastic polymers Polymers with dispersed ordered and amorphous regions that can be molded by heating.

Thermosetting polymers Polymers that set with heat but that cannot be reheated and remolded, in contrast to thermoplastic polymers.

Thin layer chromatography (TLC) Chromatography in which the solid phase is coated on a support such as glass and solvent is drawn up by capillary action.

Thinner Diluent in a street drug sample.

Three-dimensional data Data from a hyphenated instrument that produces three dimensions of data such as GC-MS or LC-PDA.

Threshold weight A weight of a controlled substance that defines the severity of a crime or penalty.

Thrombocytes Cells that are involved in clotting; platelets.

Titer A measure of the relative strength of an antiserum.

TMS Tetramethylsilane.

Toner A waxy particulate mixture used in laser printers and copiers to deliver colorant to a substrate that is then heat-affixed.

Top-down model An approach to the estimation of uncertainty in which individual contributors are captured using techniques such as control charting.

Torch In an ICP-MS, the plasma source.

Total quality management (TQM) A cradle-to-grave approach to quality assurance that integrates all aspects inside and outside of a particular organization or lab.

Toxicodynamics Same as pharmacodynamics except describing materials are ingested at toxic levels.

Toxicokinetics Same as pharmacokinetics except describing materials are ingested at toxic levels.

Traceability The ability to relate a measurement or piece of equipment to an unassailable standard.

Transducer A device that converts whatever arrives at a detector (photons, ions, etc.) into an electrical signal.

Transition metals Metals that have electrons in their d-orbitals starting with scandium on the periodic table. The d-orbital structure can be exploited to produce colorful compounds and complexes.

Triangular distribution A form of distribution in which there is a higher probability of a value occurring in the middle of the range than at the extremes. The uncertainty associated with reading a meniscus in volumetric glassware could be reasonably modeled as a triangular distribution.

Trichorme A feature of marijuana.

Trier-of-fact In a court or legal proceeding, the person or persons that make the ultimate decisions; can be a judge or jury.

Triple base powder Propellant in which the main ingredients are guncotton, nitroglycerin, and nitroguanidine. Used in high caliber weapons.

Tristimulus values Three values (XYZ) calculated using a spectrum in the visible range, standard illuminate values, and weighting factors; associated with the CIE color space system.

Tropane alkaloid An alkaloid characterized by a bridged structure across a ring. Cocaine is a tropane alkaloid.

Trueness Closeness of agreement between the expectation of a test result or a measurement result and a true value. It is usually expressed in terms of a bias. Trueness is the systematic component of accuracy.

Tryptamines A class of amine drugs based on tryptamine, a double ringed structure similar to the neurotransmitter serotonin. Psilocin is a tryptamine.

Type A contribution A contribution to uncertainty that can be described by a normal distribution.

Type B distribution A contribution to uncertainty that has not been characterized as a normal distribution and derived from other sources such as manufacturer specifications or experience.

Type I error An error in which the null hypothesis is incorrectly rejected.

Type II error An error in which the null hypothesis is incorrectly accepted.

U

Uncertainty The range or expected spread around a measurand that arises from sample, analyst, procedure, or other factors. Uncertainty does not imply lack of trust or knowledge; it is a description of a range.

Uncertainty budget A tabular approach to estimation of uncertainty based on the contributions of individual factors.

Univariate Involving a single variable.

Upper flammability limit (UFL) The highest mixture ratio of fuel and oxidant that will ignite and sustain combustion.

Urochrome The compound responsible for the yellow color of urine.

Useable quantity An amount of a controlled substance that is deemed as usable; an amount that would evoke a response; an amount worth taking.

Utility A description of how useful information or data is to answering a specific question at hand.

V

Value (of a color) The depth of a color analogous to a scale of white (lower value) to black (higher value); also referred to as "lightness."

Van Deemter curve A plot of HETP versus flow rate; describes three contributing factors to band broadening and thus column efficiency.

Variance The square of the standard deviation.

Vegetable fibers Fibers based on cellulose; also called *vegetable fibers*.

Vehicle The solvent used in a paint or ink; the solvent used to suspend a pigment or dissolve a dye. A solvent system used to deliver colorants; typically includes materials that will polymerize as they cure.

Virtual image An image created by a lens that does not exist in a point in space and one that can't be captured on a screen. It exists only when viewed through the lens.

Vitreous fluid Fluid inside of the eyeball.

Volume of distribution V_d See *Apparent volume of distribution*.

W

Walker Test A presumptive test used in distance determinations and analysis of GSR.

Wave plate A transparent quartz plate used in PLM analyses to provide a known retardation that adds to any retardation provided by the sample. It is used to increase color and contrast and assists in application of the Michel-Levy chart.

Wear characteristics Characteristics of a material that are acquired over time and assumed to be unique. For example, two pairs of shoes may come off the assembly line in nearly identical condition, but the different ways in which the shoes are used and worn will lead to wear characteristics that could be used to distinguish them at a later time.

Weathering The loss of lighter components of hydrocarbon mixtures used as accelerants.

Wet chemical methods Analytical testing that utilizes relatively large quantities of liquid reagents.

Working distance The amount of space available between the stage and the lens of a microscope for sample insertion.

X

Xanthine alkaloids Alkaloids that include caffeine, theophylline and other compounds found in coffee, tea, and chocolate.

Xenobiotic A substance that is foreign to the body; one that is not normally ingested or that is present but in much smaller quantities than the dosage in question.

X-ray diffraction (XRD) A technique in which electrons analogous to light where the diffraction pattern relates to crystal structure.

Abbreviations

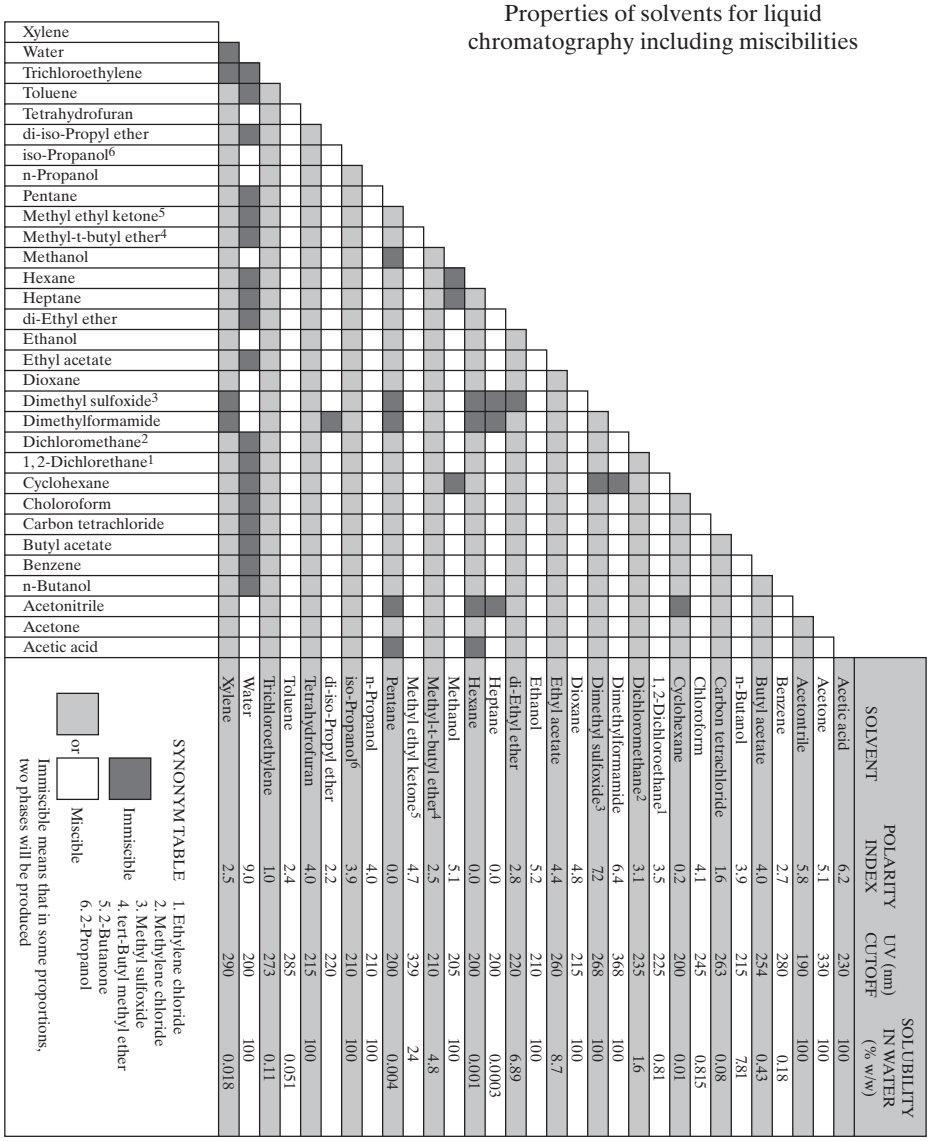
A	Absorbance
AA	Angle of acceptance; related to light exiting the condenser and entering the objective lens of a microscope
AA/AAS	Atomic absorption spectrometry
AAS	Anabolic agent steroids
AAFS	American Academy of Forensic Sciences
AATCC	Association of Textile Chemists and Colorists
Ab	Antibody
ABC	American Board of Criminalists
ABFT	American Board of Forensic Toxicologists
ADME	Absorption, distribution, metabolism, and elimination; stages of pharmacokinetics
AES	Atomic emission spectroscopy
Ag	Antigen, can also be the symbol for silver.
ANSI	American National Standards Institute
APCI	Atmospheric pressure chemical ionization
ASCLD	American Society of Crime Laboratory Directors
ASQ	American Society of Quality
ASTM	American Society for Testing and Materials
ATR	Attenuated total reflectance; a mode of infrared spectroscopy that requires surface contact and internal reflections.
AUC	Area under the curve
B	Birefringence; also sometimes Bi but not used here to avoid confusion with the element bismuth
BAC	Blood alcohol content
Bi	See *B*
BrAC	Breath alcohol
BSTFA	N,O-bis(trimethylsilyl) trifluoroacetamide, a derivatization reagent used prior to chromatographic analysis of liable or low volatility compounds
CBD	Cannabidiol
CBN	Cannabinol
CC	Calibration check
CDR	Cartridge discharge residue
CDTA	Chemical Diversion and Trafficking Act
CE	Capillary electrophoresis
CFT	Crystal field theory
CI	Color index
CIE	International Commission on Illumination (color spaces)
CMC	Color Measurement Committee of the Society of Dyes and Colorists
CMY	Cyan magenta yellow; a coloring system used in printing; a subtractive system
CMYK	Cyan/magenta/yellow/black
CNS	Central nervous system
CRM	Certified reference material from NIST
CSA	Controlled Substances Act
CV	Coefficient of variation; same as %RSD
CYP	Cytochrome P450
CZE	Capillary zone electrophoresis
DEA	Drug Enforcement Adminstration (US Department of Justice)
DFSA	Drug facilitated sexual assault
DHS	Dynamic headspace
DP	Degree of polymerization
DPA	Diphenylamine
DRE	Drug recognition expert
DTGS	A deuterium-triglyceride sulfate detector used in IR
E_a	Energy of activation
EC	Ethyl centralite, an ingredient in propellants
ECD	Electron capture detector
ED_{50}	Effective dose-50; The dose of a drug that generates the desired therapeutic effect in half of the test population
EDA	Exploratory data analysis
EDS	Energy dispersive x-ray fluorescence spectroscopy
ELISA	Enzyme-linked immunoassay
EMIT	Enzyme multiplied immunoassay
EOF	Electroosmotic flow
ESI	Electrospray ionization
F	Bioavailability
F/A	Fuel/air ratio
FDR	Firearms discharge residue
FID	Flame ionization detector
FIR	Far infrared region of the electromagnetic energy spectrum, wavelengths of 50–10,000 um

FPIA	Fluorescent polarization immunoassay
FTIR	Fourier transform infrared spectroscopy
GABA	Gamma aminobutyric acid
GBL	The lactone form of GHB
GC-MS	Gas chromatography-mass spectrometry
GHB	γ hydroxybutyric acid or γ hydroxybutyrate
GSR	Gunshot residue
GUM	Guide to the Expression of Uncertainty in Measurement
HETP	Height equivalent of a theoretical plate
HMX	A high explosive also known as octogen or cyclotetramethylenetetranitramine
HOMO	Highest occupied molecular orbital
HPTLC	High performance thin layer chromatography
HSDB	Hazardous Substance Database; a database available on-line through the National Library of Medicine Gateway that contains information on drugs and other compounds
HSV	Hue-saturation-value; a color description system
ICP	Inductively coupled plasma
IED	Improvised explosive device
IGSR	Inorganic gunshot residue
IMS	Ion mobility spectrometry
IR	Infrared region of the electromagnetic spectrum, wavelengths of 2.5 um–50 um
IRMS	Isotope ratio mass spectrometry
ISO	International Standards Organization
JCGM	Joint Committee for Guides in Metrology
JWH	John W. Huffman (cannabimimetics)
K	Known or known sample
K_a	Acid dissociation constant
K_b	Base dissociation constant
K_D	Distribution coefficient
K_H	Henry's Law constant
K_{sp}	Solubility product constant
K_w	Water dissociation constant, $= 1.0 \times 10^{-14}$
KED	Kinetic energy discrimination
LA	Laser ablation
LAMPA	Lysergic acid methylpropylamide
LD_{50}	Lethal dose-50; The dose of a drug that kills half of the test population
LD-MS	Laser desorption mass spectrometry
LDL	Like dissolves like
LDR	Linear dynamic range
LEL	Lower explosive limit; same as LFL, lower flammability range
LFL	Lower flammability range
LFT	Ligand field theory
LIBS	Laser induced breakdown spectroscopy
LLE	Liquid/liquid extraction such as performed in a separatory funnel or Soxhlet extraction unit
LOD	Limit of detection
LOQ	Limit of quantitation; lowest point on a calibration curve
LSD	Lysergic acid diethylamide
LUMO	Lowest unoccupied molecular orbital
MALDI	Matrix-assisted laser desorption/ionization
MAM	Monoacetylmorphine
MAOI	Monoxamine oxidase inhibitors
MAPA	Methamphetamine Anti-Proliferation Act
MCT	A mercury-cadmium-tellurium detector used for MSP; requires liquid nitrogen cooling
MDEA	3,4-methylenedioxyethylamphetamine
MDMA	3,4-methylenedioxymethamphetamine, Ecstasy
MEK/ MEKC	Micellular electrokinetic chromatography
MIR	Multiple internal reflections
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS^n	Tandem mass spectrometry
MSP	Microspectrophotometry
MVA	Multivariate analysis
N	Theoretical plates; Number of samples in a large population or parent set
NA	Numerical aperture, a measure of a lens to collect light
NASH	Natural/accidental/suicidal/homicidal
NC	Nitrocellulose
NG	Nitroglycerin
NIDA	National Institute on Drug Abuse
NIR	Near infrared region of the electromagnetic spectrum, wavelengths of 770–2500 nm
NIST	National Institute of Standards and Technology
NLM	National Library of Medicine
NPD	Nitrogen-phosphorus detector
NSAID	Non-steroidal anti-inflammatory drugs
NUSAP	Number/units/spread/assessment/ pedigree
OB	Oxygen balance
OGSR	Organic gunshot residue

OIML	International Organization of Legal Metrology
OTC	Over-the-counter; drugs and medicines that can be purchased without a prescription
P2P	Phenyl-2-propanone, a methamphetamine precursor
PCA	Principal component analysis
PCP	Phencyclidine. A potent synthetic hallucinogen. Likely derived from the description "PeaCe Pill"
PDA	Photodiode array
PDB	Peedee Belemnite, a calcium carbonate used as a reference material in SIR measurements
PDMS	Polydimethylsiloxane
PDQ	Paint data query
PDR	Physician's Desk Reference
PFTBA	per-Fluorotributylamine, a compound used to tune mass spectrometers
PG	Prostaglandins
PI	Power index of an explosive relative to picric acid
PLM	Polarized light microscopy
PMR	Post-mortem redistribution
PPA	Phenylpropanolamine
PT	Purge and trap
Py-GC	Pyrolysis gas chromatography
Q	Heat released by a combustion reaction, $-\Delta H$; also a questioned sample.
QA	Quality assurance
QC	Quality control
QQQ	Triple quadrupole
QToF	Quadrupole/time-of-flight
R	Retardation
RBC	Red blood cell
RDX	A high explosive also known as hexogen or cyclotrimethylenetrinitramine
REP	Relative explosive power
Rf	Radiofrequency
RGB	Red-green-blue; additive color system used in computer monitors and other projection systems
RIA	Radioimmunoassay
%RSD	Percent relative standard deviation; same as CV
S	Solubility
SANE	Sexual assault nurse examiner
SAR	Surface absorption-reflection
SAX	Strong anion exchange
SCX	Strong cation exchange
SDC	Society of Dyes and Colourists
SEM	Scanning electron microscope
SOFT	Society of Forensic Toxicology
SOP	Standard operating procedure
SORS	Spatially offset Raman spectroscopy
SPE	Solid phase extraction
SPME	Solid phase microextraction
SPSE	Solid phase stirbar extraction
SRM	Standard reference material from NIST
SSRI	Selective serotonin re-uptake inhibitors
STE	Stoichiometric equivalence
SWGDRUG	Scientific Working Group for the Analysis of Seized Drugs
T_g	Glass transition temperature
T_m	Temperature of melting (polymer)
TATB	A high explosive, 1,3,5-triamino-2,4,6-trinitrobenzene
TEA	Thermal energy analysis
THC	Tetrahydrocannabinol
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TNT	Trinitrotoluene
TQM	Total quality management
UEL	Lower explosive limit; same as UFL, upper flammability range
UFL	Upper flammability range
UPD	Uridine diphosphate
UPLC	Ultrahigh pressure liquid chromatography
UV	Ultraviolet region of the electromagnetic spectrum, wavelengths of 200–400 nm
V_d	Apparent volume of distribution
VIS/Vis	Visible region of the electromagnetic spectrum, wavelengths of 400–700 nm
VPDB	Vienna Pee Dee Belemnite
WADA	World Anti-Doping Agency
WBC	White blood cells
WDS	Wavelength dispersive x-ray fluorescence spectroscopy
XRD	X-ray diffraction
XRF	X-ray fluorescence

Solvent Properties for SPE and HPLC

Properties of solvents for liquid chromatography including miscibilities



SOLVENT	POLARITY INDEX	UV (nm) CUTOFF	SOLUBILITY IN WATER (% w/w)
Acetic acid	6.2	230	100
Acetone	5.1	330	100
Acetontrile	5.8	190	100
Benzene	2.7	280	0.18
Butyl acetate	4.0	254	0.43
n-Butanol	3.9	215	781
Carbon tetrachloride	1.6	263	0.08
Chloroform	4.1	245	0.815
Cyclohexane	0.2	200	0.01
1, 2-Dichloroethane[1]	3.5	225	0.81
Dichloromethane[2]	3.1	235	1.6
Dimethylformamide	6.4	368	100
Dimethyl sulfoxide[3]	72	268	100
Dioxane	4.8	215	100
Ethyl acetate	4.4	260	8.7
Ethanol	5.2	210	100
di-Ethyl ether	2.8	220	6.89
Heptane	0.0	200	0.0003
Hexane	0.0	200	0.001
Methanol	5.1	205	100
Methyl-t-butyl ether[4]	2.5	210	4.8
Methyl ethyl ketone[5]	4.7	329	24
Pentane	0.0	200	0.004
n-Propanol	4.0	210	100
iso-Propanol[6]	3.9	210	100
di-iso-Propyl ether	2.2	220	
Tetrahydrofuran	4.0	215	100
Toluene	2.4	285	0.051
Trichloroethylene	1.0	273	0.11
Water	9.0	200	100
Xylene	2.5	290	0.018

Key Organic Chemistry Terms and Reactions

TABLE 1 Common Functional Groups

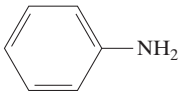
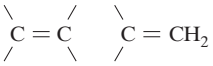
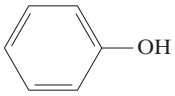
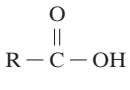
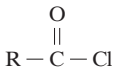
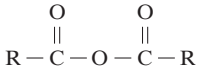
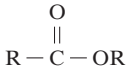
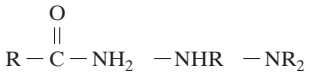
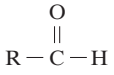
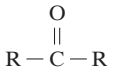
Alkane	RCH_3	Aniline	
Alkene	 Internal Terminal	Phenol	
Alkyne	$RC \equiv CR$ $RC \equiv CH$ Internal Terminal	Carboxylic acid	
Nitrile	$RC \equiv N$	Acyl chloride	
Ether	$R-O-R$	Acid anhydride	
Thiol	RCH_2-SH	Ester	
Sulfide	$R-S-R$	Amide	
Disulfide	$R-S-S-R$	Aldehyde	
Epoxide		Ketone	

TABLE 2 Substitution Notation

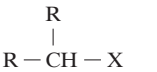
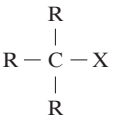
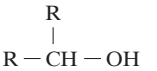
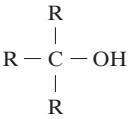
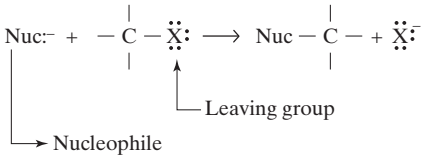
	Primary	Secondary	Tertiary
Alkyl halide	$R-CH_2-X$ X = F, Cl, Br, or I		
Alcohol	$R-CH_2-OH$		
Amine	$R-NH_2$		

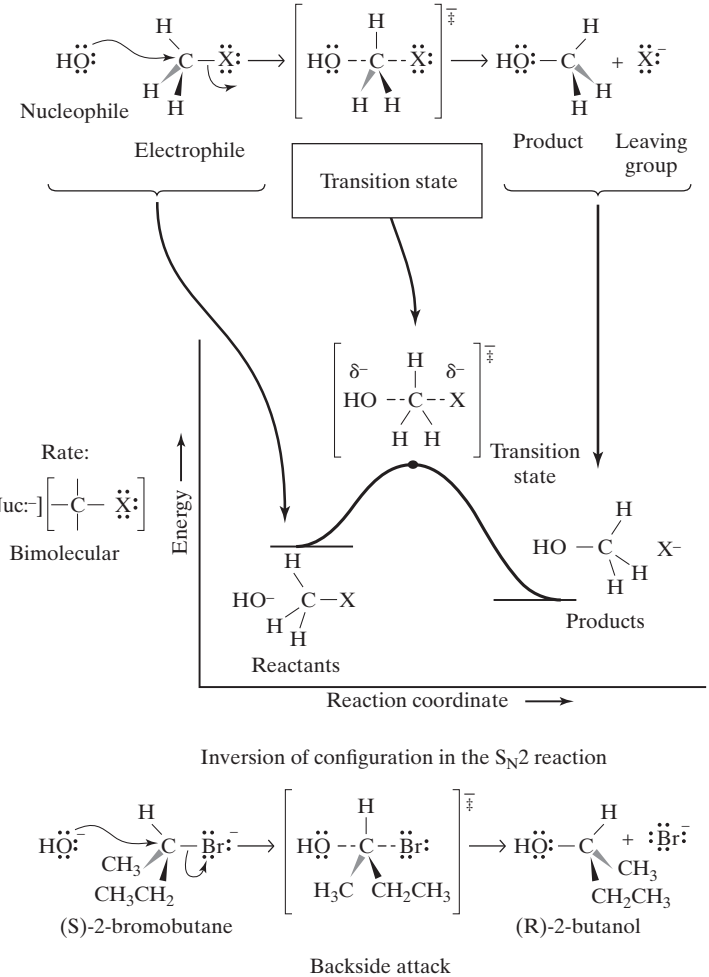
TABLE 3 Mechanisms: S_N2: Substitution, Nucleophilic, Bimolecular. The following summarizes the four generic organic reaction mechanisms using alkyl halids (RX) as examples.

Generic substitution reaction



S_N2

The S_N2 reaction takes place in a single (concerted) step:



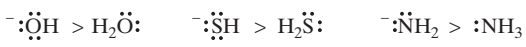
Inversion of configuration in the S_N2 reaction

Backside attack

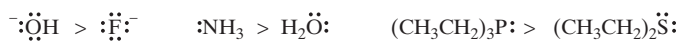
Source: The information is summarized from Wade, L.G., *Organic Chemistry* (4th ed.), Chapter 6, "Alkyl Halides: Nucleophilic Substitution and Elimination." 2003, Prentice Hall: Upper Saddle River, NJ, pp. 212–271.

TABLE 3 (Continued)

1. A species with a negative charge is a stronger nucleophile than a similar neutral species. In particular, a base is a a stronger nucleophile than its conjugate acid.



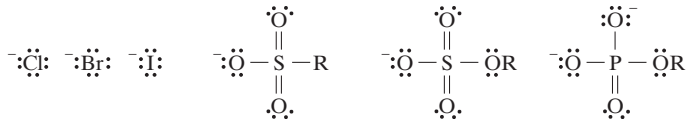
2. Nucleophilicity decreases from left to right in the periodic table, following the increase in electronegativity from left to right. The more electronegative elements have more tightly held nonbonding electrons that are less reactive toward forming new bonds.



3. Nucleophilicity increases down the periodic table, following the increase in size and polarizability.

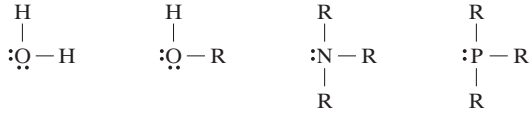
Weak bases that are common leaving groups

Ions:



Halides | Sulfonate | Sulfate | Phosphate

Neutral molecules:



Water | Alcohols | Amines | Phosphines

TABLE 4 Mechanisms: S_N1: Substitution, Nucleophilic, Unimolecular

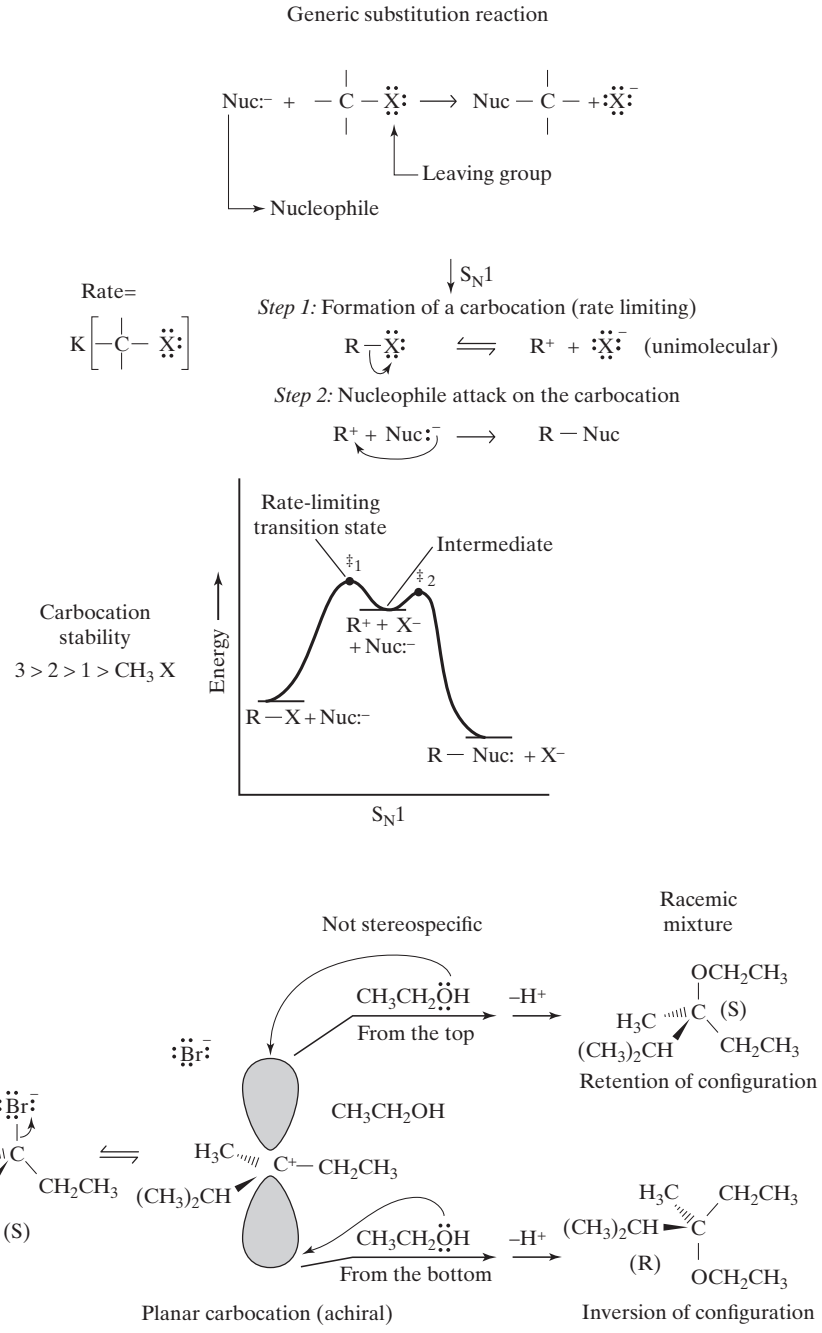
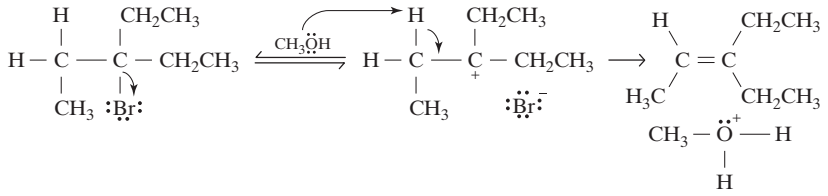
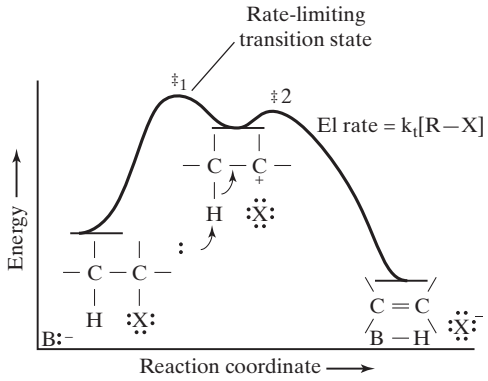


TABLE 5 Elimination Mechanisms: E1 (Elimination, Unimolecular) and E2 (Elimination, Bimolecular)

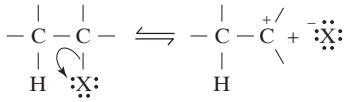
Elimination reactions

E1

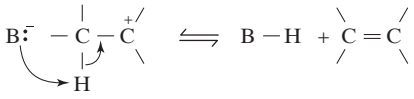




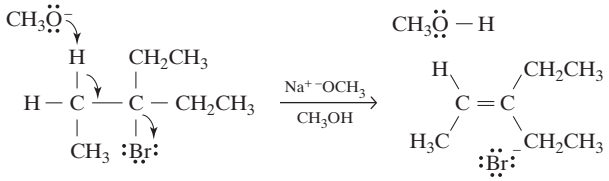
Step 1: Formation of a carbocation (rate limiting).



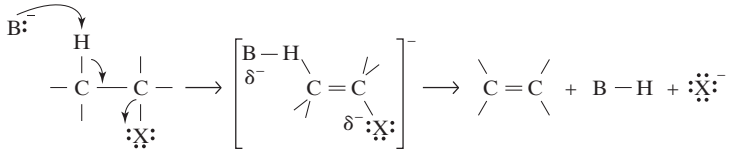
Step 2: A base abstracts a proton (fast).



E2



The concerted E2 reaction takes place in a single step.



E2 rate = k_r[R—X][B⁻] Transition state

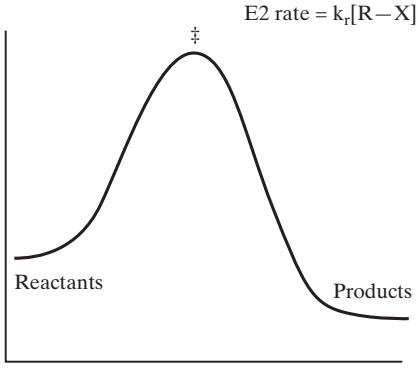


TABLE 6 Summary of Substitution and Elimination Mechanisms

Nucleophillic substitutions—Summary

	S_N1	S_N2
Promoting factors		
Nucleophilic	Weak nucleophiles are OK	Strong nucleophile needed
Substrate (RX)	3 > 2	$CH_3X > 1 > 2$
Solvent	Good ionizing solvent needed	Wide variety of solvents
Leaving group	Good one required	Good one required
Other	$AgNO_3$ forces ionization	
Characteristics		
Kinetics	First order, $k_t[RX]$	Second order, $k_t[RX][Nuc:^-]$
Steriochemistry	Mixture of inversion and retention	Complete inversion
Rearrangements	Common	Impossible

Eliminations—Summary

	E_1	E_2
Promoting factors		
Base	Weak bases work	Strong base required
Solvent	Good ionizing solvent	Wide variety of solvents
Substrate	3 > 2	3 > 2 > 1
Leaving group	Good one required	Good one required
Characteristics		
Kinetics	First order, $k_t[RX]$	Second order, $k_t[RX][B:^-]$
Orientation	Most highly substituted alkene	Most highly substituted alkene
Stereochemistry	No special geometry	Coplanar transition state required
Rearrangements	Common	Impossible

Thermodynamic Quantities

Substance	ΔH_f (kJ/mol)	ΔG_f (kJ/mol)	S (J/mol-K)
Aluminum			
Al(*s*)	0	0	28.32
$AlCl_3$(*s*)	−705.6	−630.0	109.3
Al_2O_3(*s*)	−1669.8	−1576.5	51.00
Barium			
Ba(*s*)	0	0	63.2
$BaCO_3$(*s*)	−1216.3	−1137.6	112.1
BaO(*s*)	−553.5	−525.1	70.42
Carbon			
C(*g*)	718.4	672.9	158.0
C(*s*, diamond)	1.88	2.84	2.43
C(*s*, graphite)	0	0	5.69
CCl_4(*g*)	−106.7	−64.0	309.4
CCl_4(*l*)	−139.3	−68.6	214.4
CF_4(*g*)	−679.9	−635.1	262.3
CH_4(*g*)	−74.8	−50.8	186.3
C_2H_2(*g*)	226.77	209.2	200.8
C_2H_4(*g*)	52.30	68.11	219.4
C_2H_6(*g*)	−84.68	−32.89	229.5
C_3H_8(*g*)	−103.85	−23.47	269.9
C_4H_{10}(*g*)	−124.73	−15.71	310.0
C_4H_{10}(*l*)	−147.6	−15.0	231.0
C_6H_6(*g*)	82.9	129.7	269.2
C_6H_6(*l*)	49.0	124.5	172.8
CH_3OH(*g*)	−201.2	−161.9	237.6
CH_3OH(*l*)	−238.6	−166.23	126.8
C_2H_5OH(*g*)	−235.1	−168.5	282.7
C_2H_5OH(*l*)	−277.7	−174.76	160.7
$C_6H_{12}O_6$(*s*)	−1273.02	−910.4	212.1
CO(*g*)	−110.5	−137.2	197.9
CO_2(*g*)	−393.5	−394.4	213.6

Substance	ΔH_f (kJ/mol)	ΔG_f (kJ/mol)	S (J/mol-K)
Hydrogen			
H(*g*)	217.94	203.26	114.60
H^+(*aq*)	0	0	0
H^+(*g*)	1536.2	1517.0	108.9
H_2(*g*)	0	0	130.58
Lead			
Pb(*s*)	0	0	68.85
$PbBr_2$(*s*)	−277.4	−260.7	161
$PbCO_3$(*s*)	−699.1	−625.5	131.0
$Pb(NO_3)_2$(*aq*)	−421.3	−246.9	303.3
$Pb(NO_3)_2$(*s*)	−451.9	—	—
PbO(*s*)	−217.3	−187.9	68.70
Nitrogen			
N(*g*)	472.7	455.5	153.3
N_2(*g*)	0	0	191.50
NH_3(*aq*)	−80.29	−26.50	111.3
NH_3(*g*)	−46.19	−16.66	192.5
NH_4^+(*aq*)	−132.5	−79.31	113.4
N_2H_4(*g*)	95.40	159.4	238.5
NH_4CN(*s*)	0.0	—	—
NH_4Cl(*s*)	−314.4	−203.0	94.6
NH_4NO_3(*s*)	−365.6	−184.0	151
NO(*g*)	90.37	86.71	210.62
NO_2(*g*)	33.84	51.84	240.45
N_2O(*g*)	81.6	103.59	220.0
N_2O_4(*g*)	9.66	98.28	304.3
NOCl(*g*)	52.6	66.3	264
HNO_3(*aq*)	−206.6	−110.5	146
HNO_3(*g*)	−134.3	−73.94	266.4
Oxygen			
O(*g*)	247.5	230.1	161.0
O_2(*g*)	0	0	205.0

(*continued*)

Thermodynamic Quantities

Substance	ΔH_f (kJ/mol)	ΔG_f (kJ/mol)	S (J/mol-K)
$O_3(g)$	142.3	163.4	237.6
$OH^-(aq)$	−230.0	−157.3	−10.7
$H_2O(g)$	−241.82	−228.57	188.83
$H_2O(l)$	−285.83	−237.13	69.91
$H_2O_2(g)$	−136.10	−105.48	232.9
$H_2O_2(l)$	−187.8	−120.4	109.6
Potassium			
$K(g)$	89.99	61.17	160.2
$K(s)$	0	0	64.67
$KCl(s)$	−435.9	−408.3	82.7
$KClO_3(s)$	−391.2	−289.9	143.0
$KClO_3(aq)$	−349.5	−284.9	265.7
$K_2CO_3(s)$	−1150.18	−1064.58	155.44
$KNO_3(s)$	−492.70	−393.13	132.9
$K_2O(s)$	−363.2	−322.1	94.14
$KO_2(s)$	−284.5	−240.6	122.5
$K_2O_2(s)$	−495.8	−429.8	113.0
$KOH(s)$	−424.7	−378.9	78.91
$KOH(aq)$	−482.4	−440.5	91.6

Apothecary and Other Units in Forensic Chemistry

The apothecary system is an older system of weights and measures that can be traced to antiquity. It was widely used in trade, medicine, and pharmacy through the Victorian era. The system deals with weight and volume but not length. The similarity of the names between weight and volume units can be confusing. The fundamental unit of weight is the *grain* and the *minim* is the fundamental unit of volume. Sixty minims makes up one fluid dram (also called drachm outside the United States). The origin of the dram dates to Ancient Greece, where the drachma coin weighed 1 dram.

Common apothecary units and equivalents (rounded as appropriate):

dram (fluid, fl dr)	60 minims or 3.697 mL or 1/8 fluid ounce
ounce (fluid)	8 drams or 480 minim or 29.57 mL
pint (fluid)	16 ounces or 128 drams or 7680 minim or 473.18 mL
gallon (U.S., fluid)	8 pints or 128 ounces or 1,024 drams or 61,440 minim or 3.785 L
grain (weight)	1/480th of a Troy ounce or 64.8 mg
scruple (weight)	20 grains or 1.296 g
dram (weight, dr)	3 scruples or 60 grains or 3.89 g
ounce (weight)	8 drams or 24 scruples or 480 grains or 31.1 g
pound	12 ounces or 96 drams or 288 scruples or 5760 grains or 0.373 kg

Example: Convert a dosage of a drug prescribed as 2.80 grains to milligrams:

$$2.80 \text{ grains} \times \frac{0.0648 \text{ g}}{1 \text{ grain}} \times \frac{1000 \text{ mg}}{\text{g}} = 181 \text{ mg}$$

The weight unit of **grain** is also associated with firearms, where bullet weights and powder loads in ammunition are typically reported in grains.

Concentration units used by forensic scientists are typical of chemistry and pharmacology. Interestingly, the concentration unit most familiar to chemists, molarity (M) is not widely used although millimolar (mm) and micromolar (μm) are seen. Other concentration units encountered:

$$\text{Weight percent (\%w/w)} = \frac{\text{weight solute}}{\text{total weight of the solution}} * 100$$

$$\text{Volume percent (\%v/v)} = \frac{\text{volume solute}}{\text{total volume of the solution}} * 100$$

$$\text{Weight percent (\%w/v)} = \frac{\text{weight solute (g)}}{\text{volume of the solution, mL}} * 100$$

$$\text{Parts-per-thousand (ppt)} = \frac{\text{weight solute (g)}}{\text{volume of the solution, L}} = \text{g/L}$$

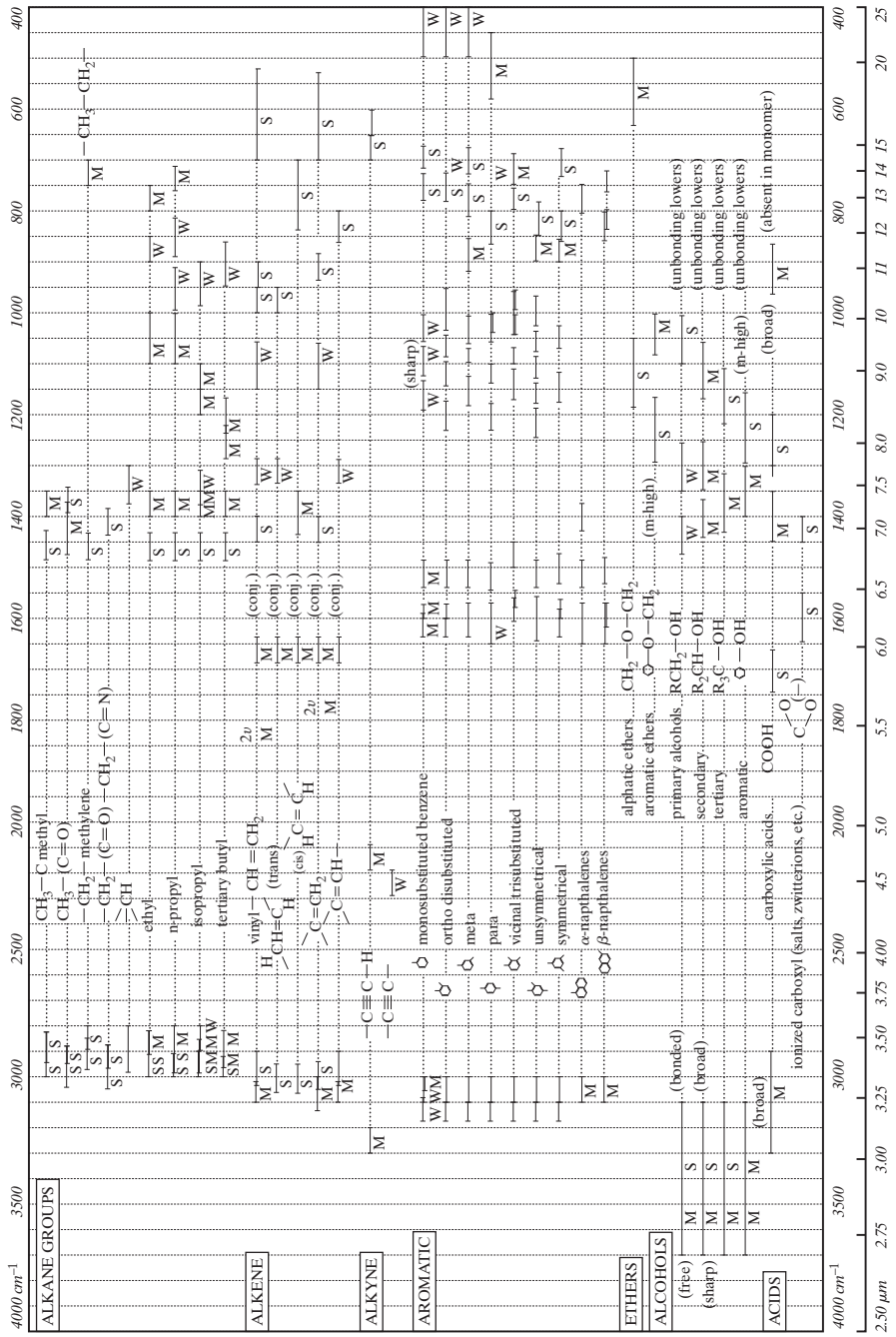
$$\text{Parts-per-million (ppm)} = \frac{\text{weight solute (mg)}}{\text{volume of the solution, L}} = \text{mg/L}$$

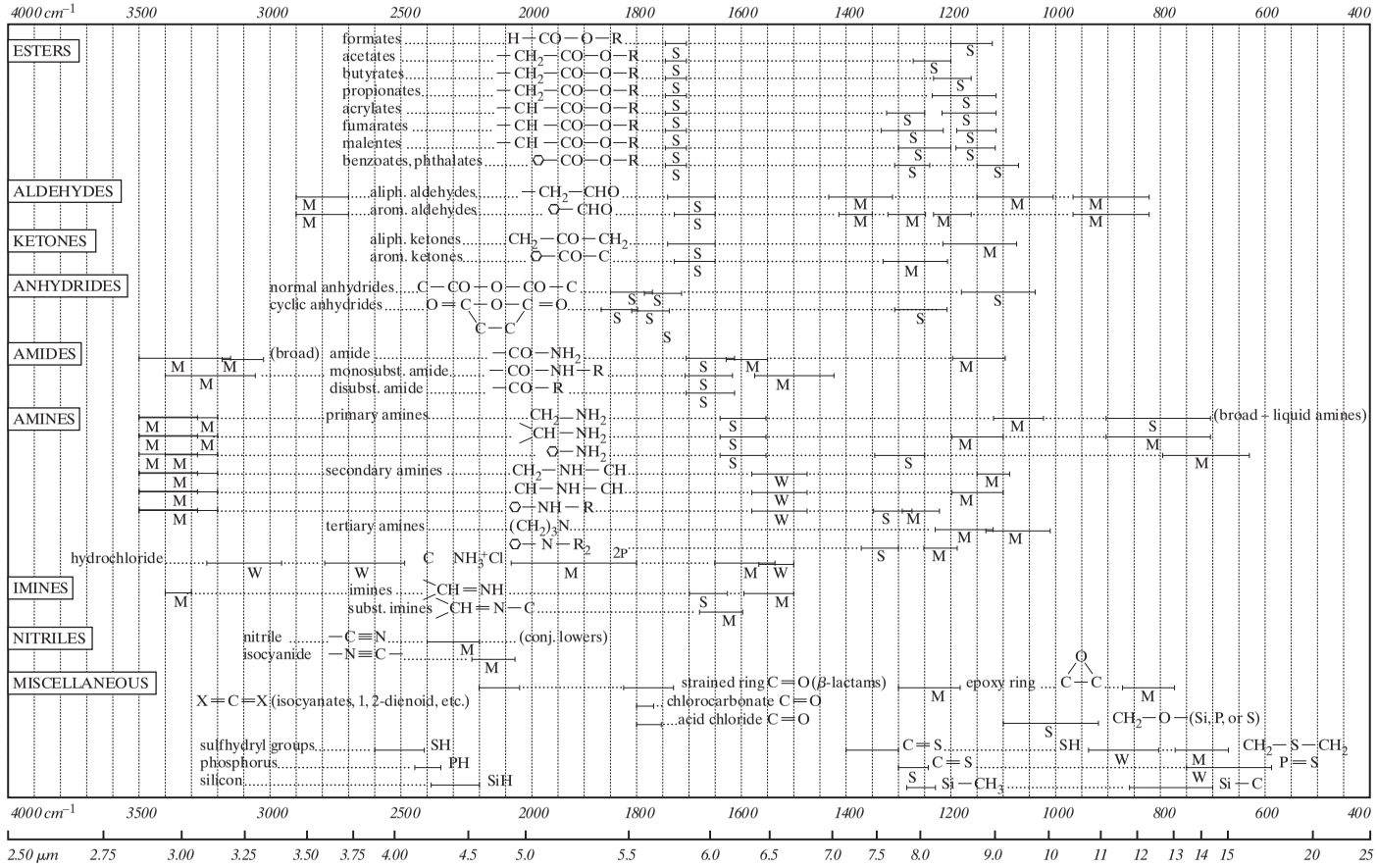
$$\text{Parts-per-billion (ppb)} = \frac{\text{weight solute } (\mu\text{g})}{\text{volume of the solution, L}} = \mu\text{g/L}$$

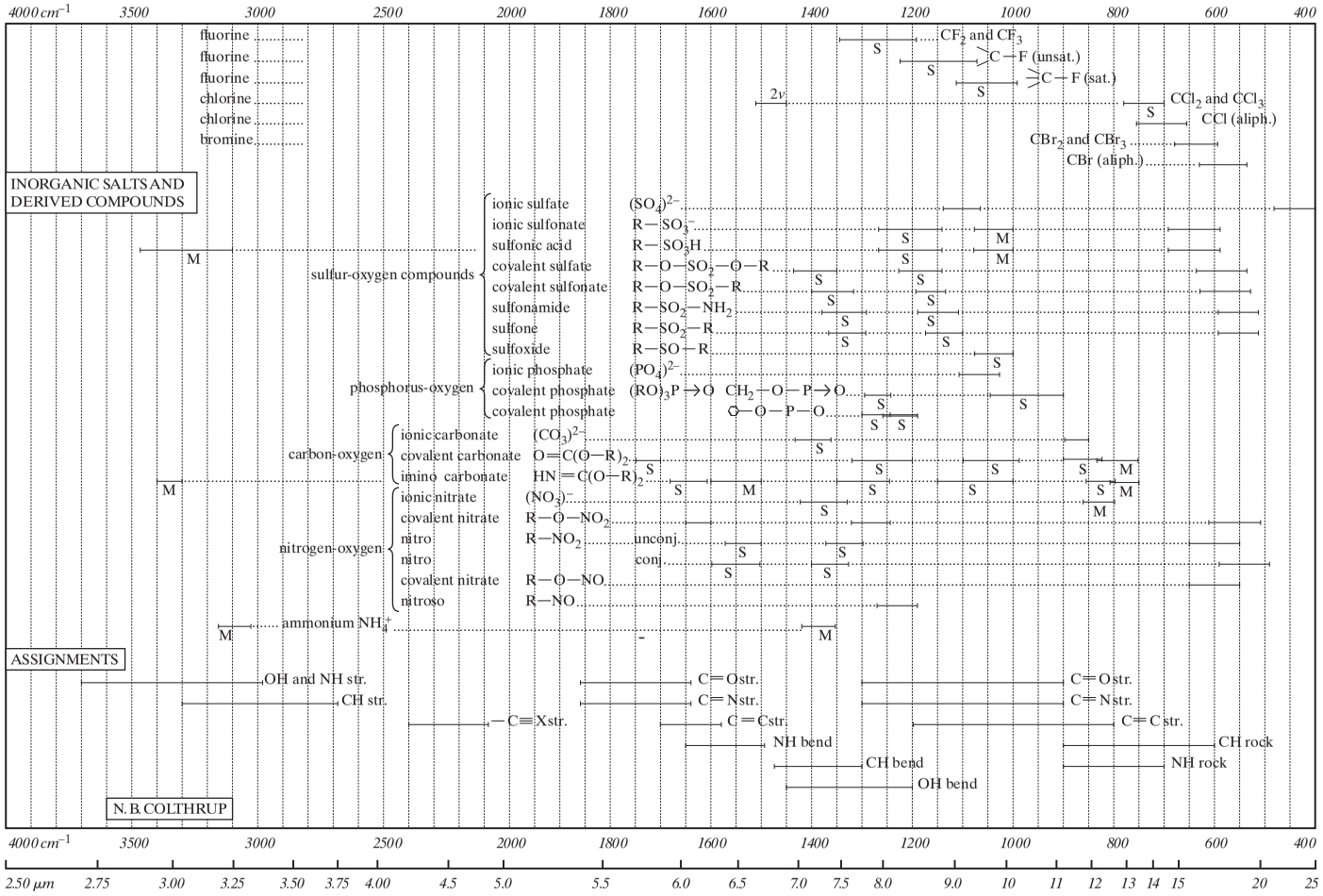
The last three units (ppt, ppm, and ppb) are concentrations for aqueous solutions. For example, assuming that water has a density of 1.0, a milliliter weighs one gram. A part-per-thousand would correspond to one gram per thousand grams or one gram per liter. If other solvents or solids are involved, a part-per-thousand would be reported as grams per kilogram (g/kg), a ppm as mg/kg, and a ppb as μg/kg. Note that for concentrations in the gas phase, ppt, ppm, and ppb may be defined differently; this should be made clear in the associated text.

Sources: How Many? A Dictionary of Units and Measures. Last accessed July 2011. www.unc.edu/~rowlett/units/index.html.
Jerrad, H. G. and D. B. McNeill, *Dictionary of Scientific Units*, 4th ed. London: Chapman and Hall, 1980.
Oxford English Dictionary (On-Line version OED). Oxford University Press.

Characteristic Infrared Group Frequencies




4000 cm⁻¹ 3500 3000 2500 2000 1800 1600 1400 1200 1000 800 600 400
ESTERS
formates H—CO—O—R
acetates —CH2—CO—O—R
butyrates —CH2—CO—O—R
propionates —CH2—CO—O—R
acrylates —CH —CO—O—R
fumarates —CH —CO—O—R
malentes —CH —CO—O—R
benzoates, phthalates O—CO—O—R
ALDEHYDES
aliph. aldehydes —CH2—CHO
arom. aldehydes O— CHO
KETONES
aliph. ketones CH2—CO—CH2
arom. ketones O—CO—C
ANHYDRIDES
normal anhydrides C—CO—O—CO—C
cyclic anhydrides O=C—O—C=O
AMIDES
(broad) amide —CO—NH2
monosubst. amide —CO—NH—R
disubst. amide —CO—R
AMINES
primary amines CH2—NH2
CH—NH2
O—NH2
(broad – liquid amines)
secondary amines CH2—NH—CH
CH—NH—CH
O—NH—R
tertiary amines (CH2)3N
O—N—R2
hydrochloride C NH3+Cl 2P'
IMINES
imines CH=NH
subst. imines CH=N—C
NITRILES
nitrile —C≡N (conj. lowers)
isocyanide —N≡C—
MISCELLANEOUS
strained ring C=O (β-lactams)
epoxy ring
X=C=X (isocyanates, 1, 2-dienoid, etc.)
chlorocarbonate C=O
acid chloride C=O
CH2—O—(Si, P, or S)
sulfhydryl groups SH
phosphorus PH
silicon SiH
C=S
SH
CH2—S—CH2
C=S
P=S
Si—CH3
Si—C
4000 cm⁻¹ 3500 3000 2500 2000 1800 1600 1400 1200 1000 800 600 400
2.50 μm 2.75 3.00 3.25 3.50 3.75 4.00 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 9.0 10 11 12 13 14 15 20 25


4000 cm⁻¹
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
600
400
fluorine
fluorine
fluorine
chlorine
chlorine
bromine
CF2 and CF3
C—F (unsat.)
C—F (sat.)
2ν
CCl2 and CCl3
CCl (aliph.)
CBr2 and CBr3
CBr (aliph.)
INORGANIC SALTS AND DERIVED COMPOUNDS
sulfur-oxygen compounds
ionic sulfate (SO4)2−
ionic sulfonate R—SO3−
sulfonic acid R—SO3H
covalent sulfate R—O—SO2—O—R
covalent sulfonate R—O—SO2—R
sulfonamide R—SO2—NH2
sulfone R—SO2—R
sulfoxide R—SO—R
phosphorus-oxygen
ionic phosphate (PO4)2−
covalent phosphate (RO)3P→O
covalent phosphate CH2—O—P→O
covalent phosphate O—O—P—O
carbon-oxygen
ionic carbonate (CO3)2−
covalent carbonate O=C(O—R)2
imino carbonate HN=C(O—R)2
nitrogen-oxygen
ionic nitrate (NO3)−
covalent nitrate R—O—NO2
nitro R—NO2 unconj.
nitro conj.
covalent nitrate R—O—NO
nitroso R—NO
ammonium NH4+
ASSIGNMENTS
OH and NH str.
CH str.
—C≡X str.
C=O str.
C=N str.
C=C str.
NH bend
CH bend
OH bend
CH rock
NH rock
N. B. COLTHRUP
2.50 μm
2.75
3.00
3.25
3.50
3.75
4.00
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
9.0
10
11
12
13
14
15
20
25

Reagents for Color Test Reagents

Benedict: Dissolve 1.73 g copper sulfate in 10 mL distilled water. Dissolve 17.3 g trisodium citrate and 10 g anhydrous sodium carbonate in 80 mL distilled water with heating. Cool and pour into copper sulfate solution and dilute to 100 mL.

Cobalt thiocyanate: Dissolve 2.0 g of $Co(SCN)_2$ in 100 mL distilled water.

Dilli-Koppanyi: A) Dissolve 0.1 g cobalt acetate in 100 mL methanol. Add 0.2 mL glacial acetic acid. B) Add 5 mL isopropylamine to 95 mL methanol. Do not combine A and B in bulk. To use, add 2 drops A to the sample followed by 1 drop of B.

Dragendorff: Dissolve 1 g bismuth subnitrate in 3 mL 10M HCl with heating. Dilute to 20 mL and add 1 g KI. A black precipitate indicates formation of BiI_3, which can be dissolved by adding 2M HCl and KI.

Duquenois: A) Add 2.5 mL acetaldehyde and 2.0 g vanillin to 100 mL 95% ethanol. B) Concentrated HCl (12M). C) Chloroform. To use, add a few drops of A to dried plant extract and shake the test tube. Add a drop of B and shake, allow color to form. Add enough C to form a distinct layer about the same depth as the combined A + B layer. Shake.

Erlich: Add 2.0 g of p-dimethylaminobenzaldehyde (p-DMAB) to 50 mL of 95% ethanol and 50 mL concentrated HCl (12M).

Ferric chloride: Dissolve 2.0 g anhydrous ferric chloride or the equivalent weight of a hydrate in 100 mL distilled water.

Froedhe: Dissolve 0.5 g molybdic acid or sodium molybdate in 100 mL hot concentrated H_2SO_4 (18M).

Iodoplatinate: Add 2 mL of a 5% (w/v) solution of platinic chloride and 5 g KI to 98 mL distilled water.

Liebermann: Dissolve 1 g KNO_2 in 10 mL concentrated H_2SO_4 (18M).

Mandelin: Dissolve 1.0 g ammonium vanadate in 100 mL concentrated sulfuric acid (18M).

Marquis: Cautiously add 100 mL concentrated H_2SO_4 (18M) to 5 mL 37–40% formaldehyde solution.

Simon: A) Dissolve 1 g sodium nitroprusside $Na_2(Fe(CN)_5NO)$ in 50 mL distilled water and add 2 mL acetaldehyde. B) 2% Na_2CO_3. To use, add 1 drop A to sample and then 1 drop B.

Zwikker: A) Dissolve 0.5 g $CuSO_4 \cdot 5\ H_2O$ in 100 mL distilled water. B) Add 5 mL pyridine to 95 mL chloroform.

Sources: Cole, M. D., *The Analysis of Controlled Substances*. Chichester, England: Wiley, 2003.
Galichet, L. Y., et al., ed., *Clarke's Analysis of Drugs and Poisons*, Vol. 1. London: Pharmaceutical Press, 2004.
O'Neal, C. L., et al., "Validation of Twelve Chemical Spot Tests for the Detection of Drugs of Abuse." *Forensic Science International*, 109 (2000): 189–201.
Seigel, J. A., "Forensic Identification of Controlled Substances," in *Forensic Science Handbook, Volume II*, R. Saferstein, ed. Upper Saddle River, NJ: Prentice Hall, 1988.

Tables for Statistical Testing

These are abbreviated tables for tests mentioned in the text. An excellent on-line reference for detailed tables can be found at the NIST website, Engineering Statistics (www.itl.nist.gov/div898/handbook/index.htm). This site includes a brief overview of each test, links to tables, and example programs to execute the test.

TABLE 1 Dixon Values, Single Outlier

		Significance Level (One-Sided Test)		
n	**Criterion**	**10 percent**	**5 percent**	**1 percent**
3	$r_{10} = (x_2 - x_1)/(x_n - x_1)$ if smallest value is suspected;	0.886	0.941	0.988
4	$= (x_n - x_{n-1})/(x_n - x_1)$ if largest value is suspected.	0.679	0.765	0.889
5		0.557	0.642	0.780
6		0.482	0.560	0.698
7		0.434	0.507	0.637
8	$r_{11} = (x_2 - x_1)/(x_{n-1} - x_1)$ if smallest value is suspected;	0.479	0.554	0.683
9	$= (x_n - x_{n-1})/(x_n - x_2)$ if largest value is suspected.	0.441	0.512	0.635
10		0.409	0.477	0.597
11	$r_{21} = (x_3 - x_1)/(x_{n-1} - x_1)$ if smallest value is suspected;	0.517	0.576	0.679
12	$= (x_n - x_{n-2})/(x_n - x_2)$ if largest value is suspected.	0.490	0.546	0.642
13		0.467	0.521	0.615
14	$r_{22} = (x_3 - x_1)/(x_{n-2} - x_1)$ if smallest value is suspected;	0.492	0.546	0.641
15	$= (x_n - x_{n-2})/(x_n - x_3)$ if largest value is suspected.	0.472	0.525	0.616
16		0.454	0.507	0.595
17		0.438	0.490	0.577
18		0.424	0.475	0.561
19		0.412	0.462	0.547
20		0.401	0.450	0.535

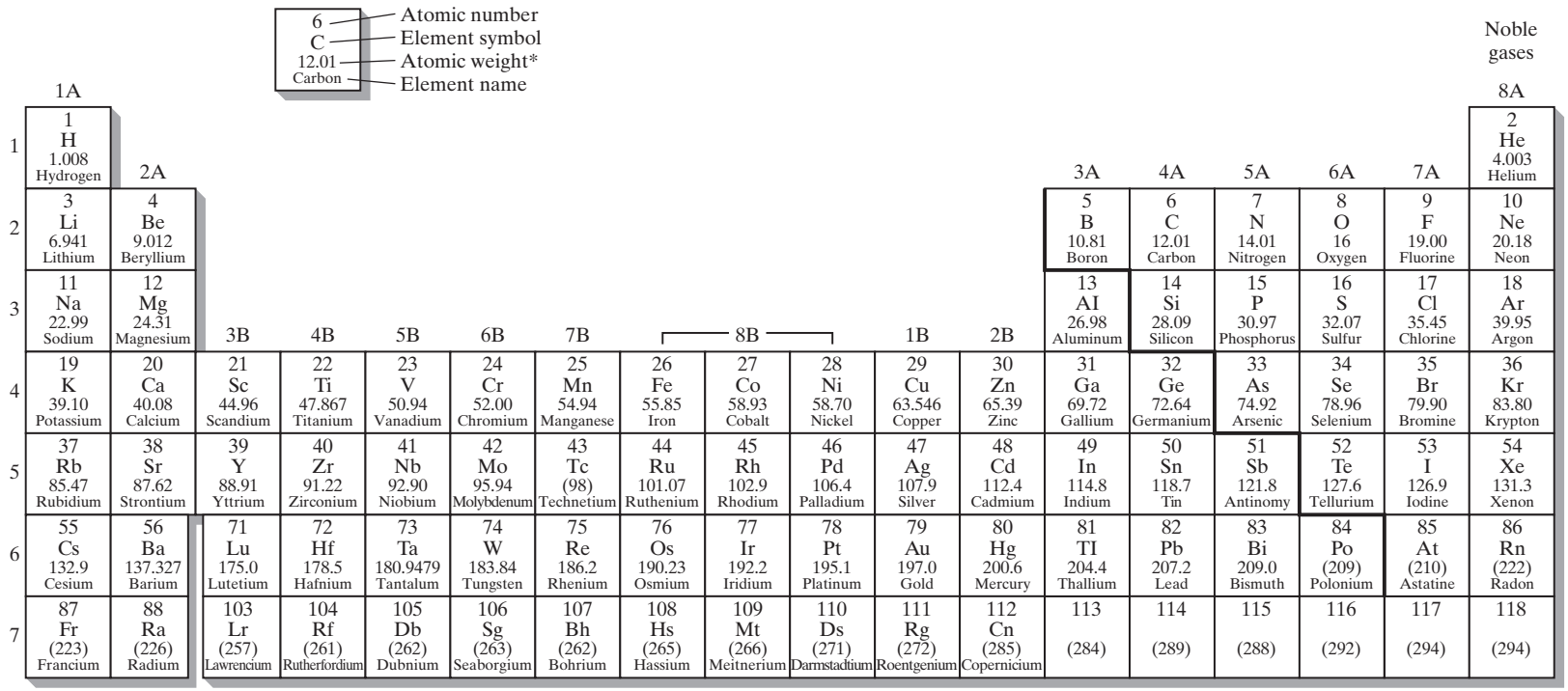
Source: "Standard Practice for Dealing With Outlying Observations," in *ASTM Standard E 178-02* ASTM International, 2004.

TABLE 2 Critical Value for Grubbs Test (Student's t distribution, upper critical values)

Degrees of Freedom	0.10	0.05	0.025	0.01	0.005	0.001
1	3.078	6.314	12.706	31.821	63.657	318.313
2	1.886	2.920	4.303	6.965	9.925	22.327
3	1.638	2.353	3.182	4.541	5.841	10.215
4	1.533	2.132	2.776	3.747	4.604	7.173
5	1.476	2.015	2.571	3.365	4.032	5.893
6	1.440	1.943	2.447	3.143	3.707	5.208
7	1.415	1.895	2.365	2.998	3.499	4.782
8	1.397	1.860	2.306	2.896	3.355	4.499
9	1.383	1.833	2.262	2.821	3.250	4.296
10	1.372	1.812	2.228	2.764	3.169	4.143
11	1.363	1.796	2.201	2.718	3.106	4.024
12	1.356	1.782	2.179	2.681	3.055	3.929
13	1.350	1.771	2.160	2.650	3.012	3.852
14	1.345	1.761	2.145	2.624	2.977	3.787
15	1.341	1.753	2.131	2.602	2.947	3.733
16	1.337	1.746	2.120	2.583	2.921	3.686
17	1.333	1.740	2.110	2.567	2.898	3.646
18	1.330	1.734	2.101	2.552	2.878	3.610
19	1.328	1.729	2.093	2.539	2.861	3.579
20	1.325	1.725	2.086	2.528	2.845	3.552

Source: NIST website, cited above.

Periodic Table of the Elements



* Numbers in parentheses are mass numbers of the most stable or best-known isotope of radioactive elements.

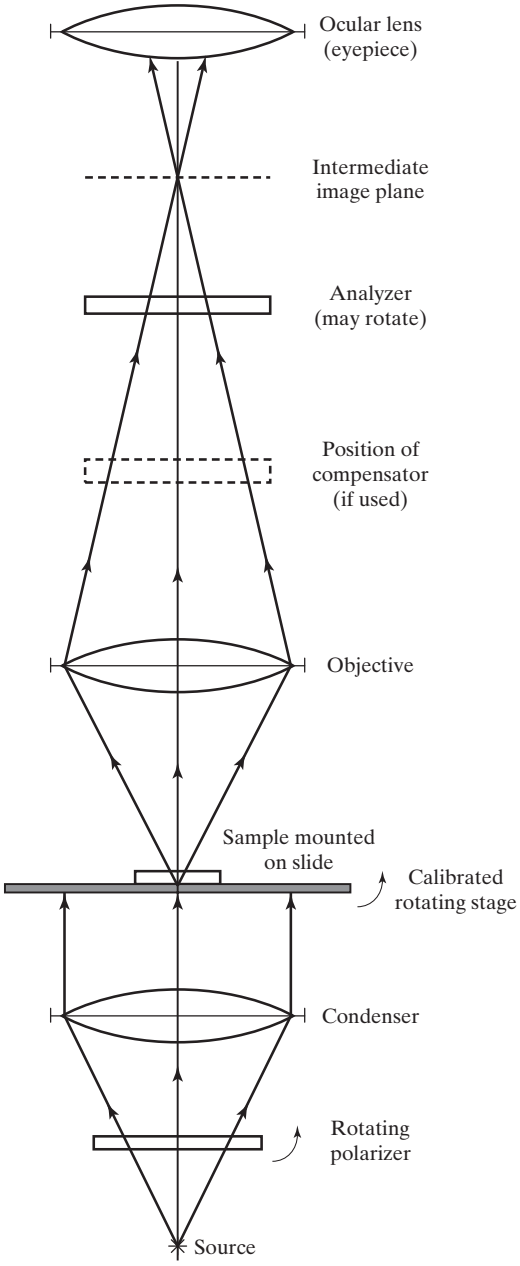
Concentrated Acids and Bases

	Approximate weight percent in reagent grade	Molarity corresponding to wt %	mL of reagent needed to prepare 1.0 1 of 1.0-M solution
Acids			
Acetic	99.8	17.4	57.5
Hydrochloric	37.2	12.1	82.6
Hydrofluoric	49.0	28.9	34.6
Nitric	70.4	15.9	62.9
Perchloric	70.5	11.7	85.5
Phosphoric	85.5	14.8	67.6
Sulfuric	96.0	18.0	55.6
Bases			
Ammonia*	28.0	14.5	69.0
Sodium hydroxide	50.5†	19.3	51.8
Potassium hydroxide	52.0	14.2	70.4

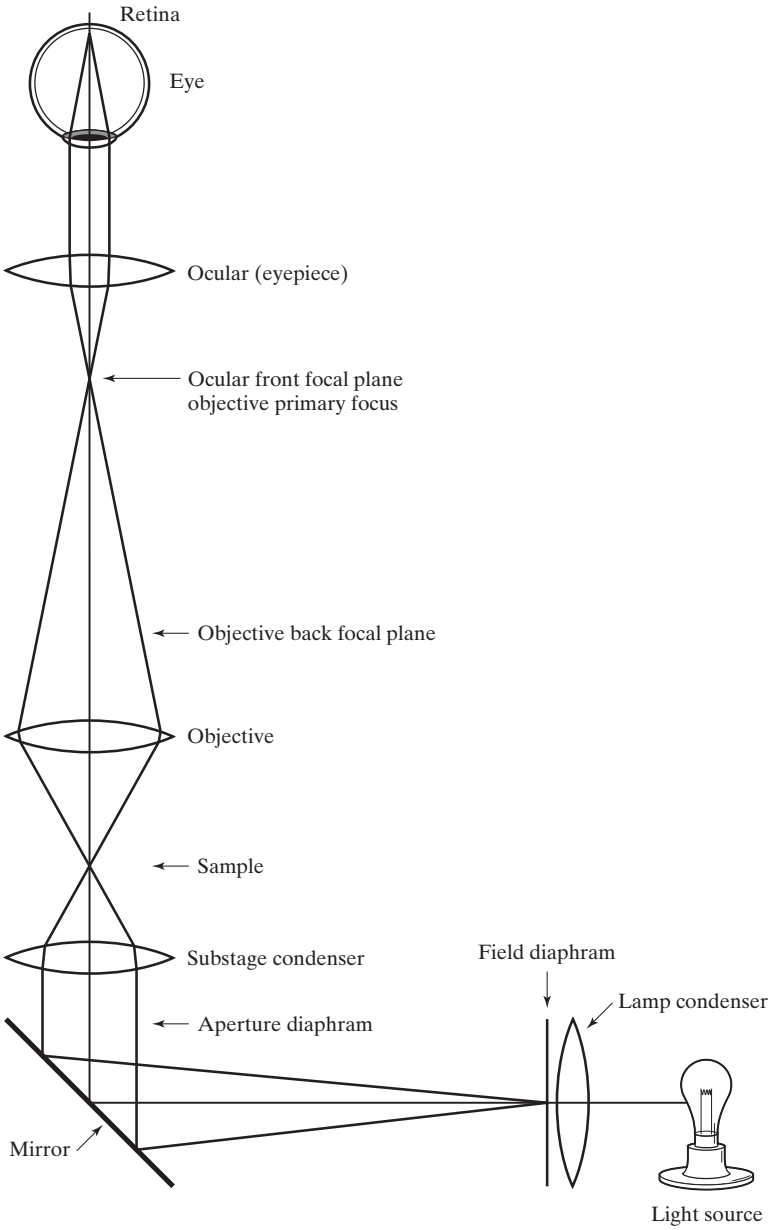
*28.0% ammonia is the same as 56.6% ammonium hydroxide.
†Saturated solution at 20°C.

Prefix Notation

Exponential	Prefix name	Symbol
0^9	giga-	G-
10^6	mega-	M-
10^3	kilo-	k-
10^{-1}	deci-	d-
10^{-2}	centi-	c-
10^{-3}	milli-	m-
10^{-6}	micro-	μ-
10^{-9}	nano-	n-
10^{-12}	pico-	p-
10^{-15}	femto-	f-
10^{-18}	atto-	a-



Optical path of a generic polarizing light microscope



Optical path of a generic light microscope

Regions of the Infrared Spectrum for Preliminary Analysis

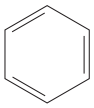
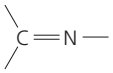
Region	Group	Possible compounds present (or absent)
3700–3100	—OH	Alcohols, aldehydes, carboxylic acids
	—NH	Amides, amines
	≡C—H	Alkynes
3100–3000	=CH	Aromatic compounds
—	$-CH_2-$ or —CH=CH—	Alkenes or unsaturated rings
3000–2800	—CH, $-CH_2-$, $-CH_3$	Aliphatic groups
2800–2600	—CHO	Aldehydes (Fermi doublet)
2700–2400	—POH	Phosphorus compounds
	—SH	Mercaptans and thiols
	—PH	Phosphines
2400–2000	—C≡N	Nitriles
	$-N=\overset{+}{N}=\overset{-}{N}$	Azides
	—C≡C—	Alkynes
1870–1650	C=O	Acid halides, aldehydes, amides, amino acids, anhydrides, carboxylic acids, esters, ketones, lactams, lactones, quinones
1650–1550	C=C, C=N, NH	Unsaturated aliphatics, aromatics, unsaturated heterocycles, amides, amines, amino acids
1550–1300	NO_2	Nitro compounds
	CH_3 and CH_2	Alkanes, alkenes, etc.
1300–1000	C—O—C and C—OH	Ethers, alcohols, sugars
	S=O, P=O, C—F	Sulfur, phosphorus, and fluorine compounds
1100–800	Si—O and P—O	Organosilicon and phosphorus compounds
1000–650	=C—H	Alkenes and aromatic compounds
	—NH	Aliphatic amines
800–400	C—halogen	Halogen compounds
	Aromatic rings	Aromatic compounds

Some Characteristic Fragment Ions in EI Mass Spectrometry (MS)*

Mass	Ion	Possible Functionality	Mass	Ion	Possible Functionality
15	CH_3^+	Methyl, alkane	50	$C_4H_2^+$	Aryl
29	$C_2H_5^+$, HCO^+	Alkane, aldehyde	51	$C_4H_3^+$	Aryl
30	$CH_2{=}NH_2^+$	Amine	77	$C_6H_5^+$	Phenyl
31	$CH_2{=}OH^+$	Ether or alcohol	83	$C_6H_{11}^+$	Cyclohexyl
39	$C_3H_3^+$	Aryl	91	$C_7H_7^+$	Benzyl
43	$C_3H_7^+$, CH_3CO^+	Alkane, ketone	105	$C_6H_5C_2H_4^+$	Substituted benzene
45	CO_2H^+, CHS^+	Carboxylic acid, thiophene		$CH_3C_6H_4CH_2^+$	Disubstituted benzene
47	CH_3S^+	Thioether		$C_6H_5CO^+$	Benzoyl

*Lowest number of homologous series is listed.

Electronic Absorption Data for Isolated Chromophores

Chromophore	Example	Solvent	λ_{max} (nm)	ϵ (liter mol $^{-1}$ cm $^{-1}$)
C═C	1-Hexene	Heptane	180	12,500
—C≡C—	1-Butyne	Vapor	172	4,500
	Benzene	Water	254	205
			203.5	7,400
	Toluene	Water	261	225
			206.5	7,000
C═O	Acetaldehyde	Vapor	298	12.5
			182	10,000
	Acetone	Cyclohexane	275	22
			190	1,000
	Camphor	Hexane	295	14
—COOH	Acetic acid	Ethanol	204	41
—COCl	Acetyl chloride	Heptane	240	34
—COOR	Ethyl acetate	Water	204	60
—$CONH_2$	Acetamide	Methanol	205	160
—NO_2	Nitromethane	Hexane	279	15.8
			202	4,400
$=\overset{+}{N}=\overset{-}{N}$	Diazomethane	Diethyl ether	417	7
—N═N—	*trans*-Azomethane	Water	343	25
	C_2H_5CH—NC_4H_9	Isooctane	238	200

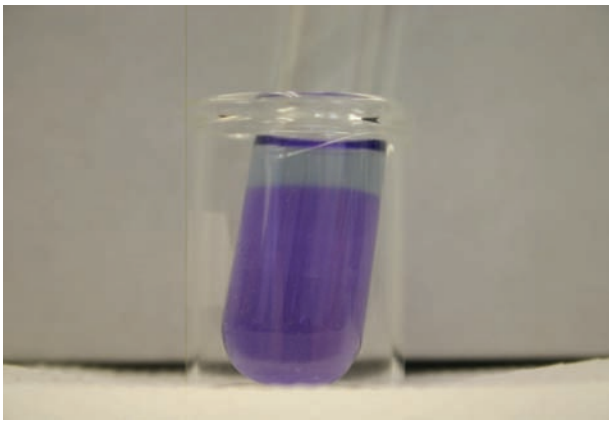
From J.B. Lambert, H.F. Shurvell, L. Verbit, R.G. Cooks, and G.H. Stout, *Organic Structural Analysis*, Macmillan Publishing, New York, 1976.

Some Characteristic Neutral Losses in EI Mass Spectrometry (MS)

Mass	Composition	Possible Functionality	Mass	Composition	Possible Functionality
14	Impurity, homologue		29	C_2H_5	Alkyl
			30	CH_2O	Methoxy
15	CH_3	Methyl		NO	Aromatic nitro
16	CH_4	Methyl		C_2H_6	Alkyl (Cl)
	O (rarely)	Amine oxide	31	CH_3O	Methoxy
	NH_2	Amide	32	CH_3OH	Methyl ester
17	NH_3	Amine (Cl)	33	$H_2O^+CH_3$	Alcohol
	OH	Acid, tertiary alcohol		HS	Mercaptan
18	H_2O	Alcohol, aldehyde, acid (Cl)	35	Cl	Chloro compound
19	F	Fluoride	36	HCl	Chloro compound
20	HF	Fluoride	42	CH_2CO	Acetate
26	C_2H_2	Aromatic	43	C_3H_7	Propyl
27	HCN	Nitrile,	44	CO_2	Anhydride
28	CO	heteroaromatic	46	NO_2	Aromatic nitro
	C_2H_4	Phenol	50	CF_2	Fluoride
	N_2	Ether			
		Azo			

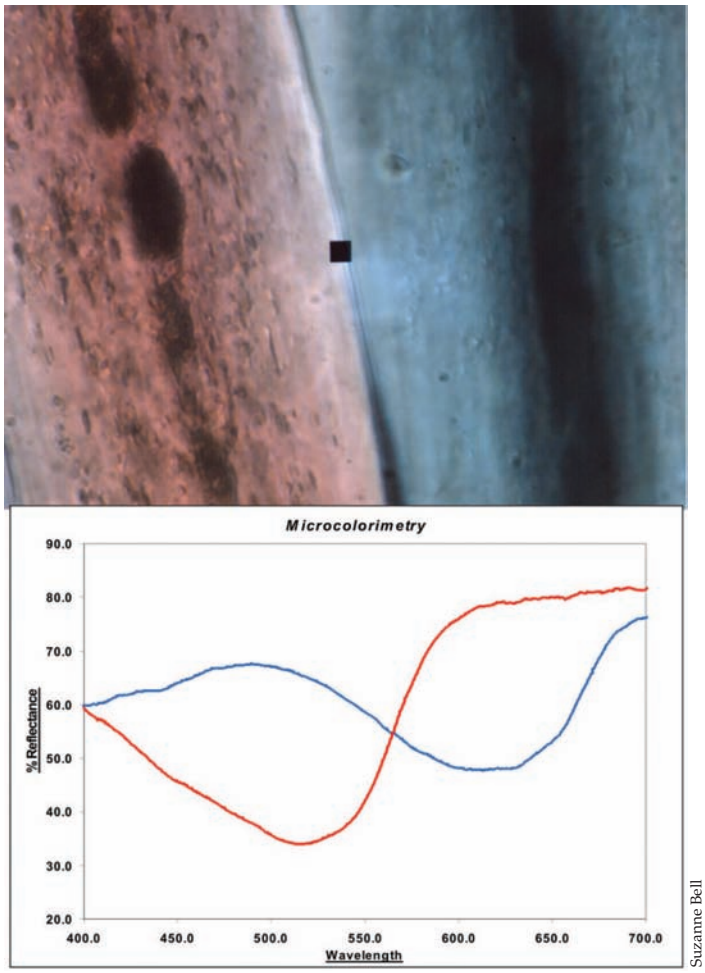
Suzanne Bell

FIGURE 1 A solution of Δ^9-THC to which a few drops of the Duquenois color test reagent has been added.



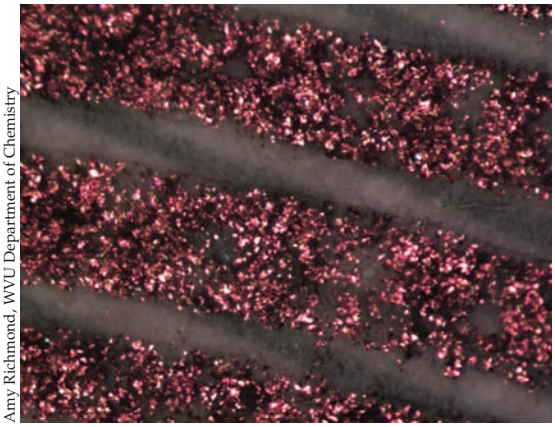
Suzanne Bell

FIGURE 2 The full Duquenois-Levine color test. Note the purple compound that has extracted into the lower chloroform layer.



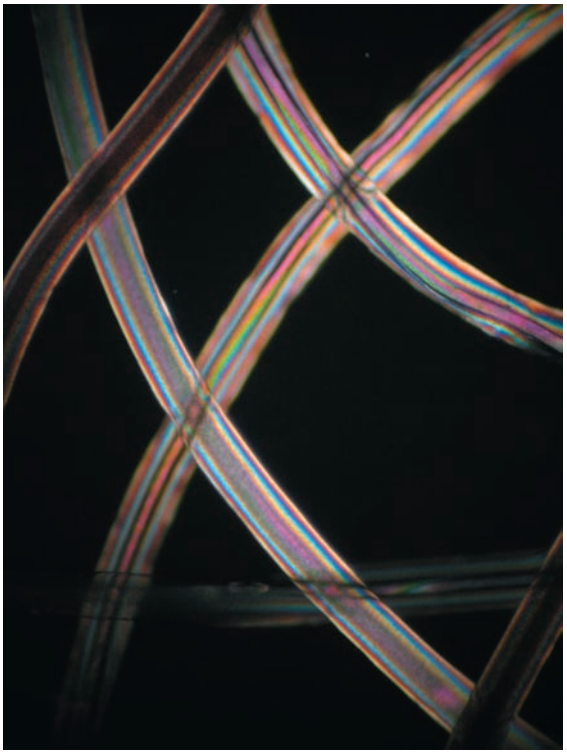
Suzanne Bell

FIGURE 3 Two fibers viewed side-by-side under a UV/VIS microspectrophotometer. The spectra for each in the visible range are shown below. The small box in the upper frame is the aperture of the microspectrophotometer.



Amy Richmond, WVU Department of Chemistry

FIGURE 4 An example of intaglio printing in which there is depth and texture in the surface of the currency shown here.



Suzanne Bell

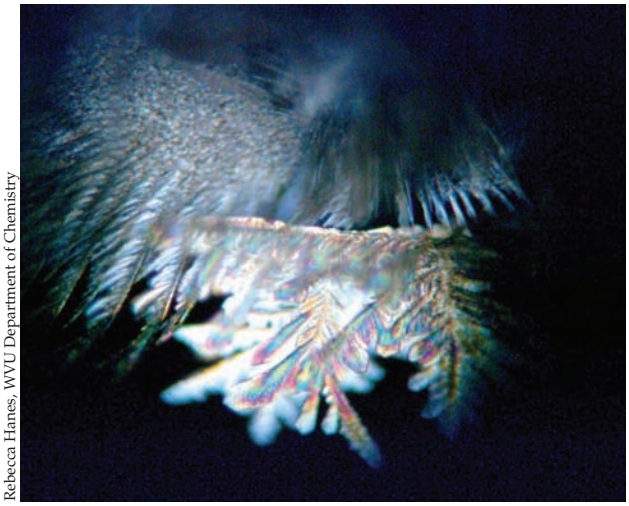
FIGURE 5 Synthetic fibers under crossed polars. Note that the intensity of the colors depends on the angle; there is a fiber towards the bottom of the image that is horizontal and nearly invisible. The colors, along with the Michel-Levy chart, can be used to gauge thickness.

Suzanne Bell

FIGURE 6 Examples of presumptive color tests for drugs. The pink reagent is cobalt thiocyanate and in the lower right is this reagent combined with cocaine. Note the shiny solid that forms, an ion/pair complex. The upper tier of tests consist of the Marquis reagent added to methamphetamine (right) and amphetamine (left). The middle well in the right column contains oxycodone and Marquis reagent.

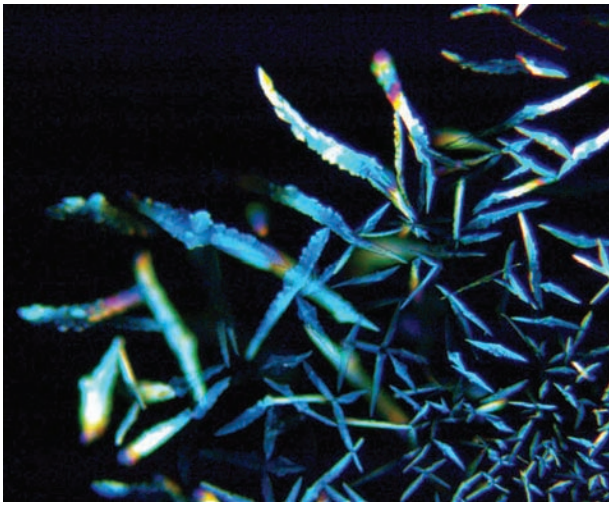
Suzanne Bell

FIGURE 7 A bullet hole treated with sodium rhodizonate. The pink color indicates the presence of lead.



Rebecca Hanes, WVU Department of Chemistry

FIGURE 8 An example of a microcrystal test for drugs; here produced by cocaine and gold chloride in an agar matrix. The image was taken under polarized light and interference colors are visible in the blades.



Rebecca Hanes, WVU Department of Chemistry

FIGURE 9 A second type of drug crystal showing a serrated blade appearance under polarized light.

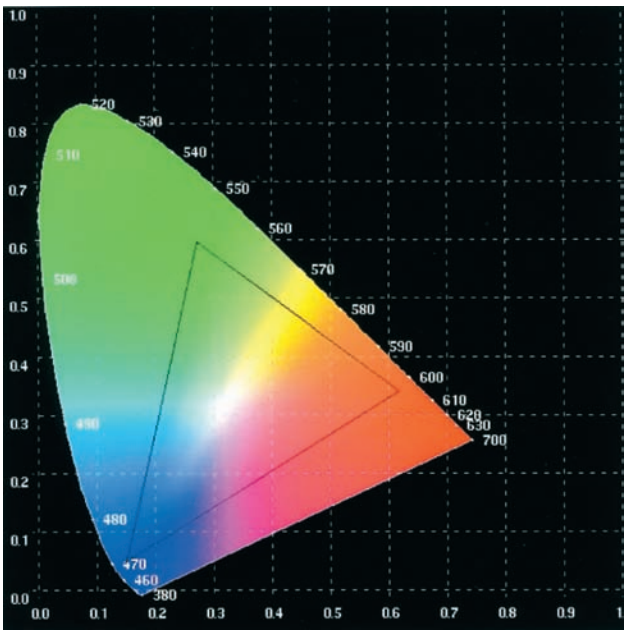


FIGURE 10 A plot of the chromaticity parabola showing locations of colors.

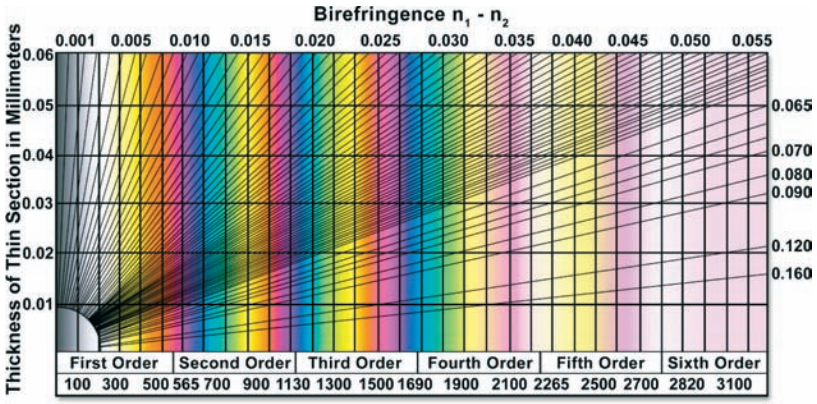
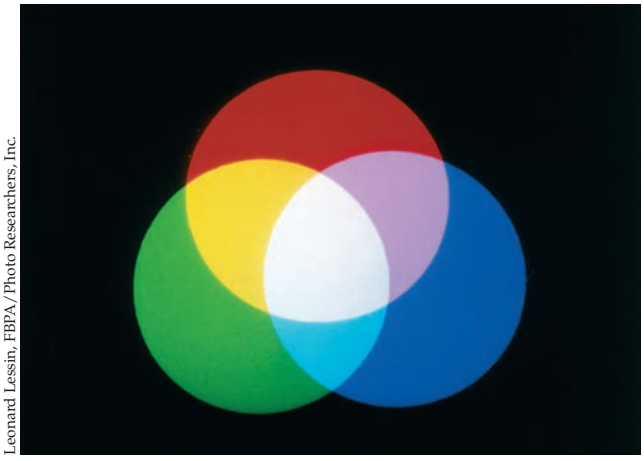


FIGURE 11 The Michel-Levy chart used in polarized light microscopy.



Leonard Lessin, FBPA/Photo Researchers, Inc.

FIGURE 12 Additive colors. Where all three colors combine, white is perceived. This is also called the RGB system.

Andy Crawford/Dorling Kindersley

FIGURE 13 Subtractive colors where the combination of all three produce black. This is also called the CMY system or CMYK system.

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