chapter 5

Organic Analysis

Key Terms

chromatography

compound

electromagnetic spectrum

electrophoresis

element

enzyme

fluoresce

frequency

gas (vapor)

infrared

inorganic

ion

laser

liquid

matter

monochromatic light

monochromator

organic

periodic table

phase

photon

physical state

proteins

pyrolysis

solid

spectrophotometry

sublimation

ultraviolet

visible light

wavelength

X-ray

Learning Objectives

After studying this chapter you should be able to:

- Define and distinguish elements and compounds

- Contrast the differences between a solid, liquid, and gas
- Define and distinguish organic and inorganic compounds
- Understand the difference between qualitative and quantitative analysis
- Describe and explain the process of chromatography
- List and describe the parts of a gas chromatograph
- Explain the difference between thin-layer chromatography, gas chromatography, and electrophoresis
- Understand the differences between the wave and particle theories of light
- Describe the electromagnetic spectrum
- Name the parts of a simple absorption spectrophotometer
- Describe the utility of ultraviolet and infrared spectroscopy for the identification of organic compounds
- Describe the concept and utility of mass spectrometry for identification analysis

Death by Tylenol

In 1982, two firefighters from a Chicago suburb were casually discussing four bizarre deaths that had recently taken place in a neighboring area. As they discussed the circumstances of the deaths, they realized that each of the victims had taken Tylenol. Their suspicions were immediately reported to police investigators. Tragically, before the general public could be alerted, three more victims died after taking poison-laced Tylenol capsules. Seven individuals, all in the Chicago area, were the first victims to die from what has be-

come known as *product tampering.* A forensic chemical analysis of Tylenol capsules recovered from the victims' residences showed that the capsules were filled with potassium cyanide in a quantity ten thousand times what was needed to kill an average person. It was quickly determined that the cyanide was not introduced into the bottles at the factory. Instead, the perpetrator methodically emptied each of twenty to thirty capsules and then refilled them with potassium cyanide. The tampered capsules were rebottled, carefully repackaged, and placed on the shelves of six different stores. The case of the Tylenol murders remains unsolved, and the $100,000 reward offered by Tylenol's manufacturer remains unclaimed.

In the previous chapter, some physical properties were described and used to characterize glass and soil evidence. Before we can apply other physical properties, as well as chemical properties, to the identification and comparison of evidence, we need to gain an insight into the composition of matter. Beginning with knowledge of the fundamental building block of all substances—the element—it will be convenient for us to classify all evidence as either organic or inorganic. The procedures used to measure the properties associated with each class are distinctly different and merit separate chapters for their description. In later chapters, we will continually return to these procedures as we discuss the examination of the various kinds of physical evidence. This chapter will be devoted, in large part, to reviewing a variety of techniques and instruments that have become the indispensable tools of the forensic scientist for examining organic evidence.

ELEMENTS AND COMPOUNDS

Matter is anything that has mass and occupies space. As we examine the world that surrounds us and consider the countless variety of materials that we encounter, we must consider one of

humankind's most remarkable accomplishments the discovery of the concept of the atom to explain the composition of all matter. This search had its earliest contribution from the ancient Greek philosophers, who suggested air, water, fire, and earth as matter's fundamental building blocks. It culminated with the development of the atomic theory and the discovery of matter's simplest identity, the **element**.

An element is the simplest substance known and provides the building block from which all matter is composed. At present, 118 elements have been identified (see Table 5–1); of these, 89 occur naturally on the earth, and the remainder have been created in the laboratory. In Figure 5–1, all the elements are listed by name and symbol in a form that has become known as the **periodic table**. This table is most useful to chemists because it systematically arranges elements with similar chemical properties in the same vertical row or group.

Table 5–1 List of Elements with Their Symbols and Atomic Masses

Element	**Symbol**	**Atomic Mass[a] (amu)**
Actinum	Ac	(227)
Aluminum	Al	26.9815
Americium	Am	(243)
Antimony	Sb	121.75
Argon	Ar	39.948
Arsenic	As	74.9216
Astatine	At	(210)

Barium	Ba	137.34
Berkelium	Bk	(247)
Beryllium	Be	9.01218
Bismuth	Bi	208.9806
Bohrium	Bh	(262)
Boron	B	10.81
Bromine	Br	79.904
Cadmium	Cd	112.40
Calcium	Ca	40.08
Californium	Cf	(251)
Carbon	C	12.011
Cerium	Ce	140.12
Cesium	Cs	132.9055
Chlorine	Cl	35.453
Chromium	Cr	51.996
Cobalt	Co	58.9332
Copper	Cu	63.546
Curium	Cm	(247)
Darmstadtium	Ds	(271)

Dubnium	Db	(260)
Dysprosium	Dy	162.50
Einsteinium	Es	(254)
Erbium	Er	167.26
Europium	Eu	151.96
Fermium	Fm	(253)
Fluorine	F	18.9984
Francium	Fr	(223)
Gadolinium	Gd	157.25
Gallium	Ga	69.72
Germanium	Ge	72.59
Gold	Au	196.9665
Hafnium	Hf	178.49
Hassium	Hs	(265)
Helium	He	4.00260
Holmium	Ho	164.9303
Hydrogen	H	1.0080
Indium	In	114.82
Iodine	I	126.9045

Iridium	Ir	192.22
Iron	Fe	55.847
Krypton	Kr	83.80
Lanthanum	La	138.9055
Lawrencium	Lr	(257)
Lead	Pb	207.2
Lithium	Li	6.941
Lutetium	Lu	174.97
Magnesium	Mg	24.305
Manganese	Mn	54.9380
Meitnerium	Mt	(266)
Mendelevium	Md	(256)
Mercury	Hg	200.59
Molybdenum	Mo	95.94
Neodymium	Nd	144.24
Neon	Ne	20.179
Neptunium	Np	237.0482
Nickel	Ni	58.71
Niobium	Nb	92.9064

Nitrogen	N	14.0067
Nobelium	No	(254)
Osmium	Os	190.2
Oxygen	O	15.9994
Palladium	Pd	106.4
Phosphorus	P	30.9738
Platinum	Pt	195.09
Plutonium	Pu	(244)
Polonium	Po	(209)
Potassium	K	39.102
Praseodymium	Pr	140.9077
Promethium	Pm	(145)
Protactinium	Pa	231.0359
Radium	Ra	226.0254
Radon	Rn	(222)
Rhenium	Re	186.2
Rhodium	Rh	102.9055
Roentgenium	Rg	(272)
Rubidium	Rb	85.4678

Ruthenium	Ru	101.07
Rutherfordium	Rf	(257)
Samarium	Sm	105.4
Scandium	Sc	44.9559
Seaborgium	Sg	(263)
Selenium	Se	78.96
Silicon	Si	28.086
Silver	Ag	107.868
Sodium	Na	22.9898
Strontium	Sr	87.62
Sulfur	S	32.06
Tantalum	Ta	180.9479
Technetium	Tc	98.9062
Tellurium	Te	127.60
Terbium	Tb	158.9254
Thallium	Tl	204.37
Thorium	Th	232.0381
Thulium	Tm	168.9342
Tin	Sn	118.69

Titanium	Ti	47.90
Tungsten	W	183.85
Ununbium	Uub	(285)
Ununtrium	Uut	(284)
Ununquadium	Uuq	(289)
Ununpentium	Uup	(288)
Ununhexium	Uuh	(292)
Ununseptium	Uus	(?)
Ununoctium	Uuo	(?)
Uranium	U	238.029
Vanadium	V	50.9414
Xenon	Xe	131.3
Ytterbium	Yb	173.04
Yttrium	Y	88.9059
Zinc	Zn	65.57
Zirconium	Zr	91.22

[a]Based on the assigned relative atomic mass of C = exactly 12; parentheses denote the mass number of the isotope with the longest half-life.

For convenience, chemists have chosen letter symbols to represent the elements. Many of

these symbols come from the first letter of the element's English name—for example, carbon (C), hydrogen (H), and oxygen (O). Others are two-letter abbreviations of the English name—for example, calcium (Ca) and zinc (Zn). Some symbols are derived from the first letters of Latin or Greek names. Thus, the symbol for silver, Ag, comes from the Latin name *argentum;* copper, Cu, from the Latin *cuprum;* and helium, He, from the Greek name *helios.*

The smallest particle of an element that can exist and still retain its identity as that element is the atom. When we write the symbol C we mean one atom of carbon; the chemical symbol for carbon dioxide, CO_2, signifies one atom of carbon combined with two atoms of oxygen. When two or more elements are combined to form a substance, as with carbon dioxide, a new substance is created, different in its physical and chemical properties from its elemental components. This new material is called a **compound**. Compounds contain at least two elements. Considering that there are eighty-nine natural elements, it is easy to imagine the large number of possible elemental combinations that may form compounds. Not surprisingly, more than 16 million known compounds have already been identified.

Just as the atom is the basic particle of an element, the molecule is the smallest unit of a compound. Thus, a molecule of carbon dioxide is represented by the symbol CO_2, and a molecule of table salt is symbolized by NaCl, representing the combination of one atom of the element sodium (Na) with one atom of the element chlorine (Cl).

As we look around us and view the materials that make up the earth, it becomes an awesome task even to attempt to estimate the number of different kinds of matter that exist. A much more logical approach is to classify matter according to the physical form it takes. These forms are called **physical states**. There are three such states: **solid**, **liquid**, and **gas (vapor)**. A solid is rigid and therefore has a definite shape and volume. A liquid also occupies a specific volume, but its

fluidity causes it to take the shape of the container in which it is residing. A gas has neither a definite shape nor volume, and it will completely fill any container into which it is placed.

Substances can change from one state to another. For example, as water is heated, it is converted from a liquid form into a vapor. At a high enough temperature (100°C), water boils and rapidly changes into steam. Similarly, at 0°C, water solidifies or freezes into ice. Under certain conditions, some solids can be converted directly into a gaseous state. For instance, a piece of dry ice (solid carbon dioxide) left standing at room temperature quickly forms carbon dioxide vapor and disappears. This change of state from a solid to a gas is called **sublimation**.

In each of these examples, no new chemical species are formed; matter is simply being changed from one physical state to another. Water, whether in the form of liquid, ice, or steam, remains chemically H_2O. Simply, what has been altered are the attractive forces between the water molecules. In a solid, these forces are very strong, and the molecules are held closely together in a rigid state. In a liquid, the attractive forces are not as strong, and the molecules have more mobility. Finally, in the vapor state, appreciable attractive forces no longer exist among the molecules; thus, they may move in any direction at will.

Chemists are forever combining different substances, no matter whether they are in the solid, liquid, or gaseous states, hoping to create new and useful products. Our everyday observations should make it apparent that not all attempts at mixing matter can be productive. For instance, oil spills demonstrate that oil and water do not mix. **Whenever substances can be distinguished by a visible boundary, different phases are said to exist.** Thus, oil floating on water is an example of a two-phase system. The oil and water each constitute a separate liquid phase, clearly distinct from each other. Similarly, when sugar is first added to water, it does not dissolve, and two distinctly different phases exist: the solid sugar and the liquid water. However, after stirring,

all the sugar dissolves, leaving just one liquid phase.

SELECTING AN ANALYTICAL TECHNIQUE

Now that the basic components of matter have been defined, proper selection of analytical techniques that enable the forensic scientist to identify or compare matter can best be understood by categorizing all substances into one of two broad groups: **organics** and **inorganics**.

Organic substances contain carbon, commonly in combination with hydrogen, oxygen, nitrogen, chlorine, phosphorus, or other elements. Inorganic substances encompass all other known chemical substances. Each of these two broad groups has distinctive and characteristic properties. Thus, once the analyst has determined whether a material is organic or inorganic, the properties to be measured and the choice of analytical techniques to be used are generally the same for all materials in each group.

Another consideration in selecting an analytical technique is the need for either a *qualitative* or a *quantitative* determination. The former relates just to the identity of the material, whereas the latter refers to the percentage combination of the components of a mixture. Hence, a qualitative identification of a powder may reveal the presence of heroin and quinine, whereas a quantitative analysis may conclude the presence of 10 percent heroin and 90 percent quinine. Obviously, a qualitative identification must precede any attempt at quantitation, for little value is served by attempting to quantitate a material without first determining its identity. Essentially, a qualitative analysis of a material requires the determination of numerous properties using a variety of analytical techniques. On the other hand, a quantitative measurement is usually accomplished by the precise measurement of a single property of the material.

Most evidence received by crime laboratories requires identification of organic compounds.

These compounds may include substances such as commonly abused drugs (such as alcohol, marijuana, heroin, amphetamines, and barbiturates), synthetic fibers, petroleum products, paint binders, and high-order explosives. As we have already observed, organic compounds are composed of a combination of a relatively small number of elements that must include carbon; fortunately, the nature of the forces or bonds between these elements is such that the resultant compounds can readily be characterized by their absorption of light. The study of the absorption of light by chemical substances, known as **spectrophotometry**, is a basic tool for the characterization and identification of organic materials. Although spectrophotometry is most applicable to organic analysis, its optimal use requires that a material be in a relatively pure state. Because the purity of physical evidence is almost always beyond the control of the criminalist, this criterion often is not met. For this reason, the analytical technique of **chromatography** is widely applied for the analysis of physical evidence. **Chromatography is a means of separating and tentatively identifying the components of a mixture.** We will discuss both techniques in this chapter.

CHROMATOGRAPHY

Theory of Chromatography

Chromatography as a technique for purifying substances is particularly useful for analyzing the multicomponent specimens that are frequently received in the crime laboratory. For example, illicit drugs sold on the street are not manufactured to meet government labeling standards; instead, they may be diluted with practically any material at the disposal of the drug dealer to increase the quantity of product available to prospective customers. Hence, the task of identifying an illicit-drug preparation would be arduous without the aid of chromatographic methods to first

separate the mixture into its components.

The theory of chromatography is based on the observation that chemical substances tend to partially escape into the surrounding environment when dissolved in a liquid or when absorbed on a solid surface. This is best illustrated by a gas dissolved in a beaker of water kept at a constant temperature. It will be convenient for us to characterize the water in the beaker as the liquid phase and the air above it as the gas phase. If the beaker is covered with a bell jar, as shown in Figure 5–2, some of the gas molecules (represented by the dark circles) escape from the water into the surrounding enclosed air. The molecules that remain are said to be in the liquid phase; the molecules that have escaped into the air are said to be in the gas phase. As the gas molecules escape into the surrounding air, they accumulate above the water; here, random motion carries some of them back into the water. Eventually, a point is reached at which the number of molecules leaving the water is equal to the number returning. At this time, the liquid and gas phases are in *equilibrium.* If the temperature of the water is increased, the equilibrium state readjusts itself to a point at which more gas molecules move into the gas phase.

This behavior was first observed in 1803 by a British chemist, William Henry. His explanation of this phenomenon, known appropriately as Henry's law, may be stated as follows: *When a volatile chemical compound is dissolved in a liquid and is brought to equilibrium with air, there is a fixed ratio between the concentration of the volatile compound in air and its concentration in the liquid, and this ratio remains constant for a given temperature.*

The distribution or partitioning of a gas between the liquid and gas phases is determined by the solubility of the gas in the liquid. The higher its solubility, the greater the tendency of the gas molecules to remain in the liquid phase. If two different gases are simultaneously dissolved in the same liquid, each will reach a state of equilibrium with the surrounding air independently of

the other. For example, as shown in Figure 5–3, gas A (green balls) and gas B (blue balls) are both dissolved in water. At equilibrium, gas A has a greater number of molecules dissolved in the water than does gas B. This is so because gas A is more soluble in water than gas B.

Now return to the concept of chromatography. In Figures 5–2 and 5–3, both phases—liquid and gas—were kept stationary; that is, they were not moving. During a chromatographic process, this is not the case; instead, one phase is always made to move continuously in one direction over a stationary or fixed phase. For example, in Figure 5–3, showing the two gases represented by blue and green balls dissolved in water, chromatography will occur only when the air is forced to move continuously in one direction over the water. Because gas B has a greater percentage of its molecules in the moving gas phase than does gas A, its molecules will travel over the liquid at a faster pace than those of gas A. Eventually, when the moving phase has advanced a reasonable distance, gas B will become entirely separated from gas A and the chromatographic process will be complete. This process is illustrated in Figure 5–4.

Simply, we can think of chromatography as being analogous to a race between chemical compounds. At the starting line, all the participating substances are mixed together; however, as the race progresses, materials that prefer the moving phase slowly pull ahead of those that prefer to remain in the stationary phase. Finally, at the end of the race, all the participants are separated, each crossing the finish line at different times.

The different types of chromatographic systems are as varied as the number of stationary and moving-phase combinations that can be devised. However, three chromatographic processes—gas chromatography, high-performance liquid chromatography, and thin-layer chromatography—are most applicable for solving many analytical problems in the crime laboratory.

Gas Chromatography (GC)

Gas chromatography (GC) separates mixtures on the basis of their distribution between a stationary liquid phase and a moving gas phase. This technique is widely used because of its ability to resolve a highly complex mixture into its components, usually within minutes.

In gas chromatography, the moving phase is actually a gas called the *carrier gas*, which flows through a column constructed of stainless steel or glass. The stationary phase is a thin film of liquid within the column. Two types of columns are used: the *packed column* and the *capillary column.* With the packed column, the stationary phase is a thin film of liquid that is fixed onto small granular particles packed into the column. This column is usually constructed of stainless steel or glass and is 2 to 6 meters in length and about 3 millimeters in diameter. Capillary columns are composed of glass and are much longer than packed columns—15 to 60 meters in length. These types of columns are very narrow, ranging from 0.25 to 0.75 millimeter in diameter. Capillary columns can be made narrower than packed columns because their stationary liquid phase is actually coated as a very thin film directly onto the column's inner wall. In any case, as the carrier gas flows through the packed or capillary column, it carries with it the components of a mixture that have been injected into the column. Components with a greater affinity for the moving gas phase travel through the column more quickly than those with a greater affinity for the stationary liquid phase. Eventually, after the mixture has traversed the length of the column, it emerges separated into its components.

A simplified scheme of the gas chromatograph is shown in Figure 5–5. The operation of the instrument can be summed up briefly as follows: A gas stream, the so-called carrier gas, is fed into the column at a constant rate. The carrier gas is chemically inert and is generally nitrogen or

helium. The sample under investigation is injected as a liquid into a heated injection port with a syringe, where it is immediately vaporized and swept into the column by the carrier gas. The column itself is heated in an oven in order to keep the sample in a vapor state as it travels through the column. In the column, the components of the sample travel in the direction of the carrier gas flow at speeds that are determined by their distribution between the stationary and moving phases. If the analyst has selected the proper liquid phase and has made the column long enough, the components of the sample will be completely separated as they emerge from the column.

As each component emerges from the column, it enters a detector. One type of detector uses a flame to ionize the emerging chemical substance, thus generating an electrical signal. The signal is recorded onto a strip-chart recorder as a function of time. This written record of the separation is called a *chromatogram.* A gas chromatogram is a plot of the recorder response (vertical axis) versus time (horizontal axis). A typical chromatogram shows a series of peaks, each peak corresponding to one component of the mixture. The time required for a component to emerge from the column from the time of its injection into the column is known as the *retention time,* which is a useful identifying characteristic of a material. Figure 5–6(a) shows the chromatogram of two barbiturates; each barbiturate has tentatively been identified by comparing its retention time to those of known barbiturates, shown in Figure 5–6 (b). (See Appendix III for chromatographic conditions.) However, because other substances may have comparable retention times under similar chromatographic conditions, gas chromatography cannot be considered an absolute means of identification. Conclusions derived from this technique must be confirmed by other testing procedures.

An added advantage of gas chromatography is that it is extremely sensitive and can yield

quantitative results. The amount of substance passing through the GC detector is proportional to the peak area recorded; therefore, by chromatographing a known concentration of a material and comparing it to the unknown, the amount of the sample may be determined by proportion. Gas chromatography has sufficient sensitivity to detect and quantitate materials at the nanogram (0.000000001 gram or 1×10^{-29} gram) level.[1]

An important extension of the application of gas chromatography to forensic science is the technique of **pyrolysis** *gas chromatography.* Many solid materials commonly encountered as physical evidence—for example, paint chips, fibers, and plastics—cannot be readily dissolved in a solvent for injection into the gas chromatograph. Thus, under normal conditions these substances cannot be subjected to gas chromatographic analysis. However, materials such as these can be heated or pyrolyzed to high temperatures (500–1000°C) so that they will decompose into numerous gaseous products. Pyrolyzers permit these gaseous products to enter the carrier gas stream, where they flow into and through the GC column. The pyrolyzed material can then be characterized by the pattern produced by its chromatogram or *pyrogram.* Figure 5–7 illustrates the pyrogram of a paint chip. The complexity of the paint pyrogram serves as a "fingerprint" of the material and gives the examiner many points to compare with other paints that are analyzed in a similar fashion.

High-Performance Liquid Chromatography (HPLC)

Recall that a chromatographic system requires a moving phase and a stationary phase in contact with each other. The previous section described gas chromatography, in which the stationary phase is a thin film and the moving phase is a gas. However, by changing the nature of these phases, one can create different forms of chromatography. One form finding increasing utility in

crime laboratories is high-performance liquid chromatography (HPLC). Its moving phase is a liquid that is pumped through a column filled with fine solid particles. In one form of HPLC, the surfaces of these solid particles are chemically treated and act as the stationary phase. As the liquid moving phase is pumped through the column, a sample is injected into the column. As the liquid carries the sample through the column, different components are retarded to different degrees, depending on their interaction with the stationary phase. This leads to a separation of the different components making up the sample mixture.

The major advantage of HPLC is that the entire process takes place at room temperature. With GC, the sample must first be vaporized and made to travel through a heated column. Hence, any materials sensitive to high temperatures may not survive their passage through the column. In such situations, the analyst may turn to HPLC as the method of choice. Organic explosives are generally heat sensitive and therefore more readily separated by HPLC. Likewise, heat-sensitive drugs, such as LSD, lend themselves to analysis by HPLC.

Thin-Layer Chromatography (TLC)

The technique of thin-layer chromatography (TLC) uses a solid stationary phase and a moving liquid phase to separate the constituents of a mixture. A thin-layer plate is prepared by coating a glass plate with a thin film of a granular material, usually silica gel or aluminum oxide. This granular material serves as the solid stationary phase and is usually held in place on the plate with a binding agent such as plaster of Paris. If the sample to be analyzed is a solid, it must first be dissolved in a suitable solvent and a few microliters of the solution spotted with a capillary tube onto the granular surface near the lower edge of the plate. A liquid sample may be applied directly to the plate in the same manner. The plate is then placed upright into a closed chamber

that contains a selected liquid, with care that the liquid does not touch the sample spot.

The liquid slowly rises up the plate by capillary action. This rising liquid is the moving phase in thin-layer chromatography. As the liquid moves past the sample spot, the components of the sample become distributed between the stationary solid phase and the moving liquid phase. The components with the greatest affinity for the moving phase travel up the plate faster than those that have greater affinity for the stationary phase. When the liquid front has moved a sufficient distance (usually 10 cm), the development is complete, and the plate is removed from the chamber and dried (see Figure 5–8). An example of the chromatographic separation of ink is shown in Figure 5–9.

Because most compounds are colorless, no separation will be noticed after development unless the materials are *visualized*. To accomplish this, the plates are placed under ultraviolet light, revealing select materials that **fluoresce** as bright spots on a dark background. When a fluorescent dye has been incorporated into the solid phase, nonfluorescent substances appear as dark spots against a fluorescent background when exposed to the ultraviolet light. In a second method of visualization, the plate is sprayed with a chemical reagent that reacts with the separated substances and causes them to form colored spots. Figure 5–10 shows the chromatogram of a marijuana extract that has been separated into its components by TLC and visualized by having been sprayed with a chemical reagent.

Once the components of a sample have been separated, their identification must follow. For this, the questioned sample must be developed alongside an authentic or standard sample on the same TLC plate. If both the standard and the unknown travel the same distance up the plate from their origins, they can tentatively be identified as being the same. For example, suppose a sample suspected of containing heroin and quinine is chromatographed alongside known heroin and qui-

nine standards, as shown in Figure 5–11. The identity of the suspect material is confirmed by comparing the migration distances of the heroin and quinine standards against those of the components of the unknown material. If the distances are the same, a tentative identification can be made. However, such an identification cannot be considered definitive, for numerous other substances can migrate the same distance up the plate when chromatographed under similar conditions. Thus, thin-layer chromatography alone cannot provide an absolute identification; it must be used in conjunction with other testing procedures to prove absolute identity.

The distance a spot has traveled up a thin-layer plate can be assigned a numerical value known as the R_f value. This value is defined as the distance traveled by the component divided by the distance traveled by the moving liquid phase. For example, in Figure 5–11 the moving phase traveled 10 centimeters up the plate before the plate was removed from the tank. After visualization, the heroin spot moved 8 centimeters, which has an R_f value of 0.8; the quinine migrated 4 centimeters, for an R_f value of 0.4.

Thousands of possible combinations of liquid and solid phases can be chosen in thin-layer chromatography. Fortunately, years of research have produced much published data relating to the proper selection of TLC conditions for separating and identifying specific classes of substances—for example, drugs, dyes, and petroleum products. These references, along with the experience of the analyst, will aid in the proper selection of TLC conditions for specific problems.

Thin-layer chromatography is a powerful tool for solving many of the analytical problems presented to the forensic scientist. The method is both rapid and sensitive; moreover, less than 100 micrograms of suspect material are required for the analysis. In addition, the equipment necessary for TLC work has minimal cost and space requirements. Importantly, numerous samples can be analyzed simultaneously on one thin-layer plate. The principal application of this tech-

nique is in the detection and identification of components in complex mixtures.

Electrophoresis

Electrophoresis is somewhat related to thin-layer chromatography in that it separates materials according to their migration rates on a stationary solid phase. However, it does not use a moving liquid phase to move the material; instead, an electrical potential is placed across the stationary medium. The nature of this medium can vary; most forensic applications call for a starch or agar gel coated onto a glass plate. Under these conditions, only substances that possess an electrical charge migrate across the stationary phase (see Figure 5–12). The technique is particularly useful for separating and identifying complex biochemical mixtures. In forensic science, electrophoresis finds its most successful application in the characterization of **proteins** and DNA in dried blood (see Figure 5–13).

Because many substances in blood carry an electrical charge, they can be separated and identified by electrophoresis. Forensic serologists have developed several electrophoretic procedures for characterizing dried blood. Many **enzymes** present in blood are actually composed of distinct proteins that can be separated by electrophoresis on starch gel. These proteins migrate on the plate at speeds that vary according to their electrical charge and size. After completion of the electrophoresis run, the separated proteins are stained with a suitable developing agent for visual observation. In this manner, characteristic band patterns are obtained that are related to the enzyme type present in the blood. Likewise, as shown in Figure 5–12, mixtures of DNA fragments can be separated by gel electrophoresis by taking advantage of the fact that the rate of movement of DNA across a gel-coated plate depends on the molecule's size. Smaller DNA fragments move at a faster rate along the plate than larger DNA fragments. This technique will be discussed in

further detail in Chapters 12 and 13.

SPECTROPHOTOMETRY

Theory of Light

We have already seen that when white light passes through a glass prism, it is dispersed into a continuous spectrum of colors. This phenomenon demonstrates that white light is not homogeneous but is actually composed of a range of colors that extends from red through violet. Similarly, the observation that a substance has a color is also consistent with this description of white light. For example, when light passes through a red glass, the glass absorbs all the component colors of light except red, which passes through or is transmitted by the glass. Likewise, one can determine the color of an opaque object by observing its ability to absorb some of the component colors of light while reflecting others back to the eye. Color is thus a visual indication that objects absorb certain portions of **visible light** and transmit or reflect others. Scientists have long recognized this phenomenon and have learned to characterize different chemical substances by the type and quantity of light they absorb.

To understand why materials absorb light, one must first comprehend the nature of light. Two simple models explain light's behavior. **The first model describes light as a continuous wave; the second depicts it as a stream of discrete energy particles.** Together, these two very different descriptions explain all of the observed properties of light, but by itself, no one model can explain all the facets of the behavior of light.

The wave concept depicts light as having an up-and-down motion of a continuous wave, as shown in Figure 5–14. Several terms are used to describe such a wave. The distance between two consecutive crests (or one trough to the next trough) is called the **wavelength**; the Greek letter

lambda (λ) is used as its symbol, and the unit of nanometers is frequently used to express its value. The number of crests (or troughs) passing any one given point in a unit of time is defined as the **frequency** of the wave. Frequency is normally designated by the letter *f* and is expressed in cycles per second (cps). The speed of light in a vacuum is a universal constant at 300 million meters per second and is designated by the symbol *c*. Frequency and wavelength are inversely proportional to one another, as shown by the relationship expressed in Equation (5–1):

$$F = c/\lambda \qquad \textbf{(5–1)}$$

Actually, visible light is only a small part of a large family of radiation waves known as the **electromagnetic spectrum**. All electromagnetic waves travel at the speed of light (*c*) and are distinguishable from one another only by their different wavelengths or frequencies. (Figure 5–15 illustrates the various types of electromagnetic waves in order of decreasing frequency.) Hence, the only property that distinguishes **X-rays** from radio waves is the different frequencies the two types of waves possess. Similarly, the range of colors that make up the visible spectrum can be correlated with frequency. For instance, the lowest frequencies of visible light are red; waves with a lower frequency fall into the invisible infrared (IR) region. The highest frequencies of visible light are violet; waves with a higher frequency extend into the invisible ultraviolet (UV) region. No definite boundaries exist between any colors or regions of the electromagnetic spectrum; instead, each region is composed of a continuous range of frequencies, each blending into the other.

Ordinarily, light in any region of the electromagnetic spectrum is a collection of waves possessing a range of wavelengths. Under normal circumstances, this light comprises waves that are all out of step with each other (incoherent light). However, scientists can now produce light that

has all its waves pulsating in unison (see Figure 5–16). This is called **coherent light** or a **laser** (*l*ight *a*mplification by the *s*timulated *e*mission of *r*adiation) beam. Light in this form is very intense and can be focused on a very small area. Laser beams can be focused to pinpoints that are so intense that they can zap microscopic holes in a diamond.

As long as electromagnetic radiation is moving through space, its behavior can be described as that of a continuous wave; however, once radiation is absorbed by a substance, the model of light as a stream of discrete particles must be invoked to best describe its behavior. Here, light is depicted as consisting of energy particles that are known as **photons**. Each photon has a definite amount of energy associated with its behavior. This energy is related to the frequency of light, as shown by Equation (5–2):

$$E = hf \qquad \textbf{(5–2)}$$

where E specifies the energy of the photon, f is the frequency of radiation, and h is a universal constant called Planck's constant. As shown by Equation (5–2), the energy of a photon is directly proportional to its frequency. Therefore, the photons of ultraviolet light will be more energetic than the photons of visible or infrared light, and exposure to the more energetic photons of X-rays presents more danger to human health than exposure to the photons of radio waves.

Absorption of Electromagnetic Radiation

Just as a substance can absorb visible light to produce color, many of the invisible radiations of the electromagnetic spectrum are likewise absorbed. This absorption phenomenon is the basis for spectrophotometry, an important analytical technique in chemical identification. Spectrophotometry measures the quantity of radiation that a particular material absorbs as a function of wavelength or frequency.

We have already observed in the description of color that an object does not absorb all the visible light it is exposed to; instead, it selectively absorbs some frequencies and reflects or transmits others. Similarly, the absorption of other types of electromagnetic radiation by chemical substances is also selective. These key questions must be asked: Why does a particular substance absorb only at certain frequencies and not at others? And are these frequencies predictable? The answers are not simple. Scientists find it difficult to predict with certainty all the frequencies at which any one substance will absorb in a particular region of the electromagnetic spectrum. What is known, however, is that a chemical substance absorbs photons of radiation with a frequency that corresponds to an energy requirement of the substance, as defined by Equation (5–2). Different materials have different energy requirements and therefore absorb at different frequencies. Most important to the analyst is that these absorbed frequencies are measurable and can be used to characterize a material.

The selective absorption of a substance is measured by an instrument called a *spectrophotometer,* which produces a graph or *absorption spectrum* that depicts the absorption of light as a function of wavelength or frequency. The absorption of ultraviolet (UV), visible, and infrared (IR) radiation is particularly applicable for obtaining qualitative data pertaining to the identification of organic substances.

Absorption at a single wavelength or frequency of light is not 100 percent complete—some radiation is transmitted or reflected by the material. Just how much radiation a substance absorbs is defined by a fundamental relationship known as Beer's law, shown in Equation (5–3):

$$A = kc \qquad \textbf{(5–3)}$$

Here, A symbolizes the absorption or the quantity of light taken up at a single frequency, c is the

concentration of the absorbing material, and *k* is a proportionality constant. This relationship shows that the quantity of light absorbed at any frequency is directly proportional to the concentration of the absorbing species; the more material you have, the more radiation it will absorb. By defining the relationship between absorbance and concentration, Beer's law permits spectrophotometry to be used as a technique for quantification.

The Spectrophotometer

The spectrophotometer measures and records the absorption spectrum of a chemical substance. The basic components of a simple spectrophotometer are the same regardless of whether it is designed to measure the absorption of UV, visible, or IR radiation. These components are illustrated in Figure 5–17. They include (1) a radiation source, (2) a monochromator or frequency selector, (3) a sample holder, (4) a detector to convert electromagnetic radiation into an electrical signal, and (5) a recorder to produce a record of the signal.

The choice of source will vary with the type of radiation desired. For visible radiation, an ordinary tungsten bulb provides a convenient source of radiation. In the UV region, a hydrogen or deuterium discharge lamp is normally used, and a heated molded rod containing a mixture of rare-earth oxides is a good source of IR light.

The function of the **monochromator** is to select a single wavelength or frequency of light from the source—**monochromatic light**. Some inexpensive spectrophotometers pass the light through colored glass filters to remove all radiation from the beam except for a desired range of wavelengths. More precise spectrophotometers use a prism or diffraction grating to disperse radiation into its component wavelengths or frequencies.[2] The desired wavelength is obtained when the dispersed radiation is focused onto a narrow slit that permits only selected wavelengths

to pass through.

Most laboratory infrared spectrophotometers use Fourier transform analysis to measure the wavelengths of light at which a material will absorb in the infrared spectrum. This approach does not use any dispersive elements that select single wavelengths or frequencies of light emitted from a source; instead, the heart of a *Fourier transform infrared* (FT-IR) *spectrometer* is the Michelson interferometer. The interferometer uses a beam-splitting prism and two mirrors, one movable and one stationary, to direct light toward a sample. As the wavelengths pass through the sample and reach a detector, they are all measured simultaneously. A mathematical operation, the Fourier transform method, is used to decode the measured signals and record the wavelength data. These Fourier calculations are rapidly carried out by a computer. In a matter of seconds, a computer-operated FT-IR instrument can produce an infrared absorption pattern compatible to one generated by a prism instrument.

Sample preparation varies with the type of radiation being studied. Absorption spectra in the UV and visible regions are usually obtained from samples that have been dissolved in an appropriate solvent. Because the cells holding the solution must be transparent to the light being measured, glass cells are used in the visible region and quartz cells in the ultraviolet region. Practically all substances absorb in some region of the IR spectrum, so sampling techniques must be modified to measure absorption in this spectral region; special cells made out of sodium chloride or potassium bromide are commonly used because they will not absorb light over a wide range of the IR portion of the electromagnetic spectrum.

The detector measures the quantity of radiation that passes through the sample by converting it to an electrical signal. UV and visible spectrophotometers employ photoelectric tube detectors. A signal is generated when the photons strike the tube surface to produce a current that is di-

rectly proportional to the intensity of the light transmitted through the sample. When this signal is compared to the intensity of light that is transmitted to the detector in the absence of an absorbing material, the absorbance of a substance can be determined at each wavelength or frequency of light selected. The signal from the detection system is then fed into a recorder, which plots absorbance as a function of wavelength or frequency. Modern spectrophotometers are designed to trace an entire absorption spectrum automatically.

Ultraviolet, Visible, and Infrared Spectrophotometry

Ultraviolet and visible spectrophotometry measure the absorbance of UV and visible light as a function of wavelength or frequency. For example, the UV absorption spectrum of heroin shows a maximum absorption band at a wavelength of 278 nanometers (see Figure 5–18). This shows that the simplicity of a UV spectrum facilitates its use as a tool for determining a material's probable identity. For instance, a white powder may have a UV spectrum comparable to heroin and therefore may be tentatively identified as such. (Fortunately, sugar and starch, common diluents of heroin, do not absorb UV light.) However, this technique will not provide a definitive result; other drugs or materials may have a UV absorption spectrum similar to that of heroin. But this lack of specificity does not diminish the value of the technique, for the analyst has quickly eliminated thousands of other possible drugs from consideration and can now proceed to conduct other confirmatory tests, such as thin-layer or gas chromatography, to complete the identification.

In contrast to the simplicity of a UV spectrum, absorption in the **infrared** region provides a far more complex pattern. Figure 5–19 depicts the IR spectra of heroin and secobarbital. Here, the absorption bands are so numerous that each spectrum can provide enough characteristics to

identify a substance specifically. **Different materials always have distinctively different infrared spectra; each IR spectrum is therefore equivalent to a "fingerprint" of that substance and no other.** This technique is one of the few tests available to the forensic scientist that can be considered specific in itself for identification. The IR spectra of thousands of organic compounds have been collected, indexed, and cataloged to serve as invaluable references for identifying organic substances.

MASS SPECTROMETRY

A previous section discussed the operation of the gas chromatograph. This instrument is one of the most important tools in a crime laboratory. Its ability to separate the components of a complex mixture is unsurpassed. However, gas chromatography (GC) does have one important drawback—its inability to produce specific identification. A forensic chemist cannot unequivocally state the identification of a substance based solely on a retention time as determined by the gas chromatograph. Fortunately, by coupling the gas chromatograph to a mass spectrometer this problem has largely been overcome.

The separation of a mixture's components is first accomplished on the gas chromatograph. A direct connection between the GC column and the mass spectrometer then allows each component to flow into the spectrometer as it emerges from the gas chromatograph. In the mass spectrometer, the material enters a high-vacuum chamber where a beam of high-energy electrons is aimed at the sample molecules. The electrons collide with the molecules, causing them to lose electrons and to acquire a positive charge (commonly called **ions**). These positively charged molecules or ions are very unstable or are formed with excess energy and almost instantaneously decompose into numerous smaller fragments. The fragments then pass through an electric or

magnetic field, where they are separated according to their masses. **The unique feature of mass spectrometry is that under carefully controlled conditions, no two substances produce the same fragmentation pattern.** In essence, one can think of this pattern as a "fingerprint" of the substance being examined (see Figure 5–20).

The technique thus provides a specific means for identifying a chemical structure. It is also sensitive to minute concentrations. At present, mass spectrometry finds its widest application in the identification of drugs; however, further research is expected to yield significant applications for identifying other types of physical evidence. Figure 5–21 illustrates the mass spectra of heroin and cocaine; each line represents a fragment of a different mass (actually the ratio of mass to charge), and the line height reflects the relative abundance of each fragment. Note how different the fragmentation patterns of heroin and cocaine are. Each mass spectrum is unique to each drug and therefore serves as a specific test for identifying it.

The combination of the gas chromatograph and mass spectrometer is further enhanced when a computer is added to the system. The integrated gas chromatograph/mass spectrometer/computer system provides the ultimate in speed, accuracy, and sensitivity. With the ability to record and store in its memory several hundred mass spectra, such a system can detect and identify substances present in only one-millionth-of-a-gram quantities. Furthermore, the computer can be programmed to compare an unknown spectrum against a comprehensive library of mass spectra stored in its memory. The advent of personal computers and microcircuitry has made it possible to design mass spectrometer systems that can fit on a small table. Such a unit is pictured in Figure 5–22. Research-grade mass spectrometers are found in laboratories as larger floor-model units (see Figure 5–23).

Chapter Summary

The proper selection of analytical techniques that will allow the forensic scientist to identify or compare matter can best be understood by categorizing all substances into one of two broad groups: organics and inorganics. In general, organic substances contain carbon. Inorganic materials encompass all other known chemical substances. Another consideration in selecting an analytical technique is the need for either a qualitative or a quantitative determination. The former relates just to the identity of the material, whereas the latter requires the determination of the percent composition of the components of a mixture.

Chromatography, spectrophotometry, and mass spectrometry are all readily used by a forensic scientist to identify or compare organic materials. Chromatography is a means of separating and tentatively identifying the components of a mixture. Spectrophotometry is the study of the absorption of light by chemical substances. Mass spectrometry characterizes organic molecules by observing their fragmentation pattern after their collision with a beam of high-energy electrons. Gas chromatography (GC) separates mixtures on the basis of their distribution between a stationary liquid phase and a mobile gas phase. In GC, the moving phase is actually a gas called the carrier gas, which flows through a column. The stationary phase is a thin film of liquid contained within the column. After a mixture has traversed the length of the column, it emerges separated into its components. The written record of this separation is called a chromatogram. A direct connection between the GC column and the mass spectrometer allows each component to flow into the mass spectrometer as it emerges from the GC. Fragmentation of each component by high-energy electrons produces a “fingerprint” pattern of the substance being examined.

Other forms of chromatography applicable to forensic science are high-performance liquid

chromatography (HPLC) and thin-layer chromatography (TLC). HPLC separates compounds using a stationary phase and a mobile liquid phase and is used with temperature-sensitive compounds. TLC uses a solid stationary phase, usually coated onto a glass plate, and a mobile liquid phase to separate the components of the mixture. A technique analogous to TLC is electrophoresis, in which materials are forced to move across a gel-coated plate under the influence of an electrical potential. In this manner, substances such as proteins and DNA can be separated and characterized.

Most forensic laboratories use ultraviolet (UV) and infrared (IR) spectrophotometers to characterize chemical compounds. In contrast to the simplicity of a UV spectrum, absorption in the infrared region provides a far more complex pattern. Different materials always have distinctively different infrared spectra; each IR spectrum is therefore equivalent to a "fingerprint" of that substance.

Review Questions

1. Anything that has mass and occupies space is defined as _____.
2. The basic building blocks of all substances are the _____.
3. The number of elements known today is _____.
4. An arrangement of elements by similar chemical properties is accomplished in the _____ table.
5. A(n) _____ is the smallest particle of an element that can exist.
6. Substances composed of two or more elements are called _____.
7. A(n) _____ is the smallest unit of a compound formed by the union of two or more

atoms.

8. The physical state that retains a definite shape and volume is a(n) _____.

9. A gas (has, has no) definite shape or volume.

10. During the process of _____, solids go directly to the gaseous state, bypassing the liquid state.

11. The attraction forces between the molecules of a liquid are (more, less) than those in a solid.

12. Different _____ are separated by definite visible boundaries.

13. Carbon-containing substances are classified as _____.

14. _____ substances encompass all non-carbon-containing materials.

15. A(n) _____ analysis describes the identity of a material, and a(n) _____ analysis relates to a determination of the quantity of a substance.

16. The study of the absorption of light by chemical substances is known as _____.

17. A mixture's components can be separated by the technique of _____.

18. True or False: Henry's law describes the distribution of a volatile chemical compound between its liquid and gas phases. _____

19. The (higher, lower) the solubility of a gas in a liquid, the greater its tendency to remain dissolved in that liquid.

20. True or False: In order for chromatography to occur, one phase must move continuously in one direction over a stationary phase. _____

21. A technique that separates mixtures on the basis of their distribution between a stationary

liquid phase and a moving gas phase is _____.

22. The time required for a substance to travel through the gas chromatographic column is a useful identifying characteristic known as _____.

23. Solid materials that are not readily dissolved in solvents for injection into the gas chromatograph can be _____ into numerous gaseous products prior to entering the gas chromatograph.

24. A major advantage of high-performance liquid chromatography is that the entire process takes place at _____ temperature.

25. A technique that uses a moving liquid phase and a stationary solid phase to separate mixtures is _____.

26. Because most chemical compounds are colorless, the final step of the thin-layer development usually requires that they be _____ by spraying with a chemical reagent.

27. The distance a spot has traveled up a thin-layer plate can be assigned a numerical value known as the _____ value.

28. True or False: Thin-layer chromatography yields the positive identification of a material.

_____

29. The migration of materials along a stationary phase under the influence of an electrical potential describes the technique of _____.

30. True or False: Color is a usual indication that substances selectively absorb light.

_____

31. The distance between two successive identical points on a wave is known as _____.

32. True or False: Frequency and wavelength are directly proportional to one another.

_____

33. Light, X-rays, and radio waves are all members of the _____ spectrum.

34. Red light is (higher, lower) in frequency than violet light.

35. Light that has all its waves pulsating in unison is called a(n) _____.

36. One model of light depicts it as consisting of energy particles known as _____.

37. True or False: The energy of a light particle (photon) is directly proportional to its frequency.

_____

38. Red light is (more, less) energetic than violet light.

39. The selective absorption of electromagnetic radiation by materials (can, cannot) be used as an aid for identification.

40. The amount of radiation a substance will absorb is directly proportional to its concentration as defined by _____ law.

41. The _____ is the instrument used to measure and record the absorption spectrum of a chemical substance.

42. The function of the _____ is to select a single frequency of light emanating from the spectrophotometer's source.

43. An (ultraviolet, infrared) absorption spectrum provides a unique "fingerprint" of a chemical substance.

44. The technique of _____ exposes molecules to a beam of high-energy electrons in order to fragment them.

45. True or False: A mass spectrum is normally considered a specific means for identifying a chemical substance. _____

Further References

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Matter

All things of substance. Matter is composed of atoms or molecules.

Element

A fundamental particle of matter. An element cannot be broken down into simpler substances by chemical means.

Periodic Table

A chart of elements arranged in a systematic fashion. Vertical rows are called groups or families; horizontal rows are called series. Elements in a given row have similar properties.

Compound

A pure substance composed of two or more elements.

Physical State

A condition or stage in the form of matter; a solid, liquid, or gas.

Solid

A state of matter in which the molecules are held closely together in a rigid state.

Liquid

A state of matter in which molecules are in contact with one another but are not rigidly held in place.

Gas (Vapor)

A state of matter in which the attractive forces between molecules are small enough to permit them to move with complete freedom.

Sublimation

A physical change from the solid state directly into the gaseous state.

Phase

A uniform body of matter; different phases are separated by definite visible boundaries.

Organic

Describes a substance composed of carbon and often smaller amounts of hydrogen, oxygen, nitrogen, chlorine, phosphorus, or other elements.

Inorganic

Describes a chemical compound not based on carbon.

Spectrophotometry

An analytical method for identifying a substance by its selective absorption of different wavelengths of light.

Chromatography

Any of several analytical techniques for separating organic mixtures into their components by attraction to a stationary phase while being propelled by a moving phase.

Pyrolysis

The decomposition of organic matter by heat.

WebExtra 5.1

Watch the Gas Chromatograph at Work

www.prenhall.com/Saferstein

Fluoresce

To emit visible light when exposed to light of a shorter wavelength—that is, ultraviolet light.

WebExtra 5.2

Watch Animated Depictions of Thin-Layer Chromatography and Gas Chromatography

www.prenhall.com/Saferstein

Electrophoresis

A technique for separating molecules through migration on a support medium while under the influence of an electrical potential.

Proteins

Polymers of amino acids that play basic roles in the structures and functions of living things.

Enzyme

A type of protein that acts as a catalyst for certain specific reactions.

WebExtra 5.3

An Animated Demonstration of Gel Electrophoresis

www.prenhall.com/Saferstein

Visible Light

Colored light ranging from red to violet in the electromagnetic spectrum.

Wavelength

The distance between crests of adjacent waves.

Frequency

The number of waves that pass a given point per second.

Electromagnetic Spectrum

The entire range of radiation energy from the most energetic cosmic rays to the least energetic radio waves.

X-ray

A high-energy, short-wavelength form of electromagnetic radiation.

Laser

An acronym for light amplification by stimulated emission of radiation; light that has all its waves pulsating in unison.

Photon

A small packet of electromagnetic radiation energy. Each photon contains a unit of energy equal to the product of Planck's constant and the frequency of radiation: $E = hf$.

Monochromator

A device for isolating individual wavelengths or frequencies of light.

Monochromatic Light

Light having a single wavelength or frequency.

WebExtra 5.4

See How a Spectrophotometer Works

www.prenhall.com/Saferstein

Ultraviolet

Invisible long frequencies of light beyond violet in the visible spectrum.

Infrared

Invisible short frequencies of light before red in the visible spectrum.

Ion

An atom or molecule bearing a positive or negative charge.

WebExtra 5.5

Watch the Gas Chromatograph/Mass Spectrometer at Work

www.prenhall.com/Saferstein

Figure 5–1 The periodic table.

Figure 5–2 Evaporation of a liquid.

Figure 5–3 At equilibrium, there are more gas A molecules (green balls) than gas B molecules (blue balls) in the liquid phase.

Figure 5–4 In this illustration of chromatography, the molecules represented by the blue balls have a greater affinity for the upper phase and hence will be pushed along at a faster rate by the moving air. Eventually, the two sets of molecules will separate from each other, completing the chromatographic process.

Figure 5–5 Basic gas chromatography. Gas chromatography permits rapid separation of complex mixtures into individual compounds and allows identification and quantitative determination of each compound. As shown, a sample is introduced by a syringe (1) into a heated injection chamber (2). A constant stream of nitrogen gas (3) flows through the injector, carrying the sample into the column (4), which contains a thin film of liquid. The sample is separated in the column, and the carrier gas and separated components emerge from the column and enter the detector (5). Signals developed by the detector activate the recorder (7), which makes a permanent record of the separation by tracing a series of peaks on the chromatograph (8). The time of elution identifies the component present, and the peak area identifies the concentration. *Courtesy Varian Inc., Palo Alto, Calif.*

Figure 5–6 (a) An unknown mixture of barbiturates is identified by comparing its retention times to (b), a known mixture of barbiturates. *Courtesy Varian Inc., Palo Alto, Calif.*

Figure 5–7 Pyrogram of a GM automobile paint. *Courtesy Varian Inc., Palo Alto, Calif.*

Figure 5–8 (a) In thin-layer chromatography, a liquid sample is spotted onto the granular surface of a gel-coated plate. (b) The plate is placed into a closed chamber that contains a liquid. As the liquid rises up the plate, the components of the sample distribute themselves between the coating and the moving liquid. The mixture is separated, with substances with a greater affinity for the moving liquid traveling up the plate at a faster speed.

Figure 5–9 (a) The liquid phase begins to move up the stationary phase. (b) Liquid moves past the ink spot carrying the ink components up the stationary phase. (c) The moving liquid has separated the ink into its several components. *Courtesy Fundamental Photographs, NYC*

Figure 5–10 Thin-layer chromatogram of a marijuana extract. *Courtesy Sirchie Finger Print Laboratories, Inc., Youngsville, N.C., www.sirchie.com*

Figure 5–11 Chromatograms of known heroin (1) and quinine (2) standards alongside suspect sample (3).

Figure 5–12 Electrophoresis separates mixtures of DNA by forcing them to migrate across a gel-coated plate under the influence of an electrical potential. Due to variations in size, DNA fragments move across the plate at different speeds.

Figure 5–13 DNA fragments separated by gel electrophoresis are visualized under a UV light. *Courtesy Cytographics, Visuals Unlimited*

Figure 5–14 The frequency of the lower wave is twice that of the upper wave.

Figure 5–15 The electromagnetic spectrum.

Figure 5–16 Coherent and incoherent radiation.

Figure 5–17 Parts of a simple spectrophotometer.

Figure 5–18 The ultraviolet spectrum of heroin.

Figure 5–19 (a) Infrared spectrum of heroin. (b) Infrared spectrum of secobarbital.

Figure 5–20 How GC/MS works. Left to right, the sample is separated into its components by the gas chromatograph, and then the components are ionized and identified by characteristic fragmentation patterns of the spectra produced by the mass spectrometer. *Courtesy Agilent Technologies, Inc., Palo Alto, Calif.*

Figure 5–21 (a) Mass spectrum of heroin. (b) Mass spectrum of cocaine.

Figure 5–22 A tabletop mass spectrometer. (1) The sample is injected into a heated inlet port, and a carrier gas sweeps it into the column. (2) The GC column separates the mixture into its components. (3) In the ion source, a filament wire emits electrons that strike the sample molecules, causing them to fragment as they leave the GC column. (4) The quadrupole, consisting of four rods, separates the fragments according to their mass. (5) The detector counts the fragments passing through the quadrupole. The signal is small and must be amplified. (6) The data system is responsible for total control of the entire GC/MS system. It detects and measures the abundance of each fragment and displays the mass spectrum. *Courtesy Agilent Technologies, Inc., Palo Alto, Calif.*

Figure 5–23 A scientist injecting a sample into a research-grade mass spectrometer. *Courtesy Geoff Tompkinson/Science Photo Library, Photo Researchers, Inc.*

[1] Powers of 10 are quite useful and simple for handling large or small numbers. The exponent expresses the number of places the decimal point must be moved. If the exponent is positive, the decimal point is moved to the right; if it is negative, the decimal point is moved to the left. Thus, to express 1×10^{-9} as a number, the decimal point is simply moved nine places to the left of 1.

[2] A diffraction grating is made by scratching thousands of parallel lines on a transparent surface such as glass. As light passes through the narrow spacings between the lines, it spreads out and produces a spectrum similar to that formed by a prism.