Chapter 10

Spectroscopic Methods

Chapter Overview

An early example of a colorimetric analysis is Nessler's method for ammonia, which was introduced in 1856. Nessler found that adding an alkaline solution of HgI_2 and KI to a dilute solution of ammonia produced a yellow-to-reddish brown colloid, in which the colloid's color depended on the concentration of ammonia. By visually comparing the color of a sample to the colors of a series of standards, Nessler was able to determine the concentration of ammonia.

Colorimetry, in which a sample absorbs visible light, is one example of a spectroscopic method of analysis. At the end of the nineteenth century, spectroscopy was limited to the absorption, emission, and scattering of visible, ultraviolet, and infrared electromagnetic radiation. Since then, spectroscopy has expanded to include other forms of electromagnetic radiation—such as X-rays, microwaves, and radio waves—and other energetic particles—such as electrons and ions.

10A   Overview of Spectroscopy

The focus of this chapter is on the interaction of ultraviolet, visible, and infrared radiation with matter. Because these techniques use optical materials to disperse and focus the radiation, they often are identified as optical spectroscopies. For convenience we will use the simpler term SPECTROSCOPY in place of optical spectroscopy; however, you should understand we will consider only a limited piece of what is a much broader area of analytical techniques.

Despite the difference in instrumentation, all spectroscopic techniques share several common features. Before we consider individual examples in greater detail, let's take a moment to consider some of these similarities. As you work through the chapter, this overview will help you focus on the similarities between different spectroscopic methods of analysis. You will find it easier to understand a new analytical method when you can see its relationship to other similar methods.
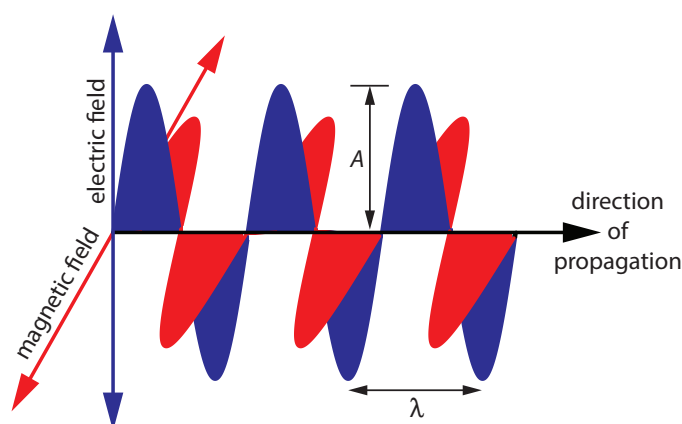
10A.1   What is Electromagnetic Radiation

ELECTROMAGNETIC RADIATION—light—is a form of energy whose behavior is described by the properties of both waves and particles. Some properties of electromagnetic radiation, such as its refraction when it passes from one medium to another (Figure 10.1), are explained best when we describe light as a wave. Other properties, such as absorption and emission, are better described by treating light as a particle. The exact nature of electromagnetic radiation remains unclear, as it has since the development of quantum mechanics in the first quarter of the 20th century.[1] Nevertheless, this dual model of wave and particle behavior provide a useful description for electromagnetic radiation.

WAVE PROPERTIES OF ELECTROMAGNETIC RADIATION

Electromagnetic radiation consists of oscillating electric and magnetic fields that propagate through space along a linear path and with a constant velocity. In a vacuum, electromagnetic radiation travels at the speed of light, c, which is 2.99792×10^8 m/s. When electromagnetic radiation moves through a medium other than a vacuum, its velocity, v, is less than the speed of light in a vacuum. The difference between v and c is sufficiently small ($<0.1\%$) that the speed of light to three significant figures, 3.00×10^8 m/s, is accurate enough for most purposes.

The oscillations in the electric field and the magnetic field are perpendicular to each other and to the direction of the wave's propagation. Figure 10.2 shows an example of plane-polarized electromagnetic radiation, which consists of a single oscillating electric field and a single oscillating magnetic field.

Figure 10.1 The Golden Gate bridge as seen through rain drops. Refraction of light by the rain drops produces the distorted images. Source: Mila Zinkova (commons.wikipedia.org).

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1   Home, D.; Gribbin, J. *New Scientist* 1991, 2 Nov. 30–33.

Figure 10.2 Plane-polarized electromagnetic radiation showing the oscillating electric field in **blue** and the oscillating magnetic field in **red**. The radiation's amplitude, A, and its wavelength, λ, are shown. Normally, electromagnetic radiation is unpolarized, with oscillating electric and magnetic fields present in all possible planes perpendicular to the direction of propagation.

An electromagnetic wave is characterized by several fundamental properties, including its velocity, amplitude, frequency, phase angle, polarization, and direction of propagation.[2] For example, the amplitude of the oscillating electric field at any point along the propagating wave is

$$A_t = A_e \sin(2\pi\nu t + \Phi)$$

where A_t is the magnitude of the electric field at time t, A_e is the electric field's maximum AMPLITUDE, ν is the wave's FREQUENCY—the number of oscillations in the electric field per unit time—and Φ is a PHASE ANGLE that accounts for the fact that A_t need not have a value of zero at $t=0$. The identical equation for the magnetic field is

$$A_t = A_m \sin(2\pi\nu t + \Phi)$$

where A_m is the magnetic field's maximum amplitude.

Other properties also are useful for characterizing the wave behavior of electromagnetic radiation. The WAVELENGTH, λ, is defined as the distance between successive maxima (see Figure 10.2). For ultraviolet and visible electromagnetic radiation the wavelength usually is expressed in nanometers (1 nm $= 10^{-9}$ m), and for infrared radiation it is expressed in microns (1 μm $= 10^{-6}$ m). The relationship between wavelength and frequency is

$$\lambda = \frac{c}{\nu}$$

Another unit useful unit is the WAVENUMBER, $\overline{\nu}$, which is the reciprocal of wavelength

$$\overline{\nu} = \frac{1}{\lambda}$$

Wavenumbers frequently are used to characterize infrared radiation, with the units given in cm^{-1}.

When electromagnetic radiation moves between different media—for example, when it moves from air into water—its frequency, ν, remains constant. Because its velocity depends upon the medium in which it is traveling, the electromagnetic radiation's wavelength, λ, changes. If we replace the speed of light in a vacuum, c, with its speed in the medium, v, then the wavelength is

$$\lambda = \frac{v}{\nu}$$

This change in wavelength as light passes between two media explains the refraction of electromagnetic radiation shown in Figure 10.1.

2   Ball, D. W. *Spectroscopy* **1994**, *9(5)*, 24–25.

Example 10.1

In 1817, Josef Fraunhofer studied the spectrum of solar radiation, observing a continuous spectrum with numerous dark lines. Fraunhofer labeled the most prominent of the dark lines with letters. In 1859, Gustav Kirchhoff showed that the D line in the sun's spectrum was due to the absorption of solar radiation by sodium atoms. The wavelength of the sodium D line is 589 nm. What are the frequency and the wavenumber for this line?

SOLUTION

The frequency and wavenumber of the sodium D line are

$$\nu = \frac{c}{\lambda} = \frac{3.00 \times 10^8 \text{ m/s}}{589 \times 10^{-9} \text{ m}} = 5.09 \times 10^{14} \text{ s}^{-1}$$

$$\overline{\nu} = \frac{1}{\lambda} = \frac{1}{589 \times 10^{-9} \text{ m}} \times \frac{1 \text{ m}}{100 \text{ cm}} = 1.70 \times 10^4 \text{ cm}^{-1}$$

Practice Exercise 10.1

Another historically important series of spectral lines is the Balmer series of emission lines from hydrogen. One of its lines has a wavelength of 656.3 nm. What are the frequency and the wavenumber for this line?

Click here to review your answer to this exercise.

PARTICLE PROPERTIES OF ELECTROMAGNETIC RADIATION

When matter absorbs electromagnetic radiation it undergoes a change in energy. The interaction between matter and electromagnetic radiation is easiest to understand if we assume that radiation consists of a beam of energetic particles called photons. When a PHOTON is absorbed by a sample it is "destroyed" and its energy acquired by the sample.[3] The energy of a photon, in joules, is related to its frequency, wavelength, and wavenumber by the following equalities

$$E = h\nu = \frac{hc}{\lambda} = hc\overline{\nu}$$

where h is Planck's constant, which has a value of 6.626×10^{-34} Js.

Practice Exercise 10.2

What is the energy of a photon for the Balmer line at a wavelength of 656.3 nm?

Click here to review your answer to this exercise.

Example 10.2

What is the energy of a photon from the sodium D line at 589 nm?

SOLUTION

The photon's energy is

$$E = \frac{hc}{\lambda} = \frac{(6.626 \times 10^{-34} \text{ Js})(3.00 \times 10^8 \text{ m/s})}{589 \times 10^{-9} \text{ m}} = 3.37 \times 10^{-19} \text{ J}$$

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3   Ball, D. W. *Spectroscopy* **1994**, *9(6)* 20–21.
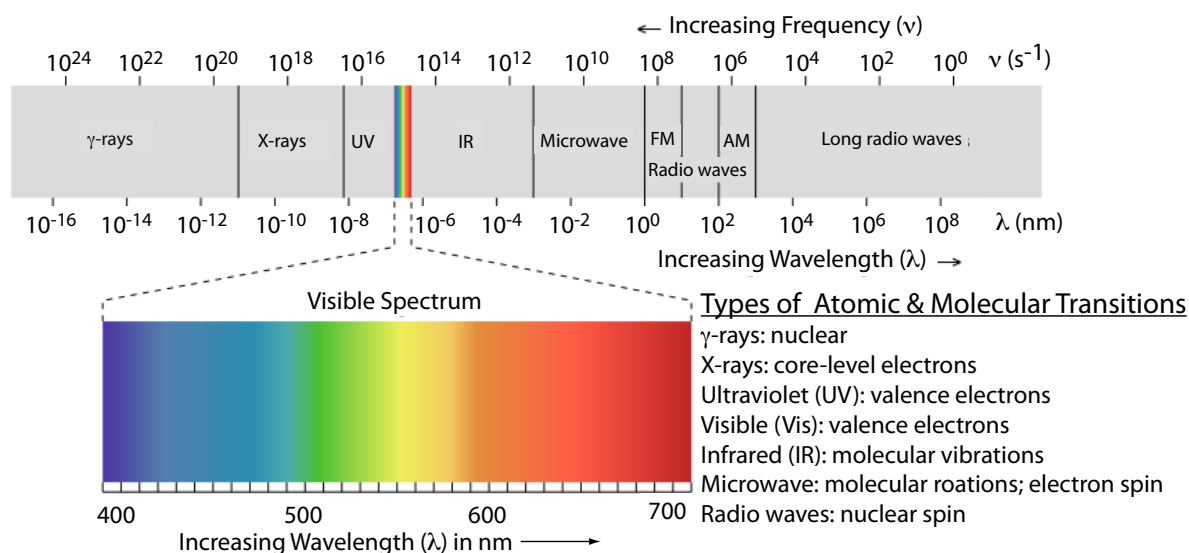
THE ELECTROMAGNETIC SPECTRUM

The frequency and the wavelength of electromagnetic radiation vary over many orders of magnitude. For convenience, we divide electromagnetic radiation into different regions—the ELECTROMAGNETIC SPECTRUM—based on the type of atomic or molecular transitions that gives rise to the absorption or emission of photons (Figure 10.3). The boundaries between the regions of the electromagnetic spectrum are not rigid and overlap between spectral regions is possible.

10A.2   Photons as a Signal Source

In the previous section we defined several characteristic properties of electromagnetic radiation, including its energy, velocity, amplitude, frequency, phase angle, polarization, and direction of propagation. A spectroscopic measurement is possible only if the photon's interaction with the sample leads to a change in one or more of these characteristic properties.

We will divide spectroscopy into two broad classes of techniques. In one class of techniques there is a transfer of energy between the photon and the sample. Table 10.1 provides a list of several representative examples.

In absorption spectroscopy a photon is absorbed by an atom or molecule, which undergoes a transition from a lower-energy state to a higher-energy, or excited state (Figure 10.4). The type of transition depends on the photon's energy. The electromagnetic spectrum in Figure 10.3, for example, shows that absorbing a photon of visible light promotes one of the atom's or molecule's valence electrons to a higher-energy level. When an molecule

Figure 10.3 The electromagnetic spectrum showing the boundaries between different regions and the type of atomic or molecular transitions responsible for the change in energy. The colored inset shows the visible spectrum. Source: modified from Zedh (www.commons.wikipedia.org).

## Table 10.1 Examples of Spectroscopic Techniques That Involve an Exchange of Energy Between a Photon and the Sample

| Type of Energy Transfer	Region of Electromagnetic Spectrum	Spectroscopic Technique[a]
absorption	γ-ray	Mossbauer spectroscopy
	X-ray	X-ray absorption spectroscopy
	UV/Vis	*UV/Vis spectroscopy* *atomic absorption spectroscopy*
	IR	*infrared spectroscopy* raman spectroscopy
	Microwave	microwave spectroscopy
	Radio wave	electron spin resonance spectroscopy nuclear magnetic resonance spectroscopy
emission (thermal excitation)	UV/Vis	*atomic emission spectroscopy*
photoluminescence	X-ray	X-ray fluorescence
	UV/Vis	*fluorescence spectroscopy* *phosphorescence spectroscopy* atomic fluorescence spectroscopy
chemiluminescence	UV/Vis	chemiluminescence spectroscopy

[a] Techniques discussed in this text are shown in *italics*.

absorbs infrared radiation, on the other hand, one of its chemical bonds experiences a change in vibrational energy.

When it absorbs electromagnetic radiation the number of photons passing through a sample decreases. The measurement of this decrease in photons, which we call ABSORBANCE, is a useful analytical signal. Note that each energy level in Figure 10.4 has a well-defined value because each is quantized. Absorption occurs only when the photon's energy, $h\nu$, matches the difference in energy, ΔE, between two energy levels. A plot of absorbance as a function of the photon's energy is called an ABSORBANCE SPECTRUM. Figure 10.5, for example, shows the absorbance spectrum of cranberry juice.

Figure 10.4 A simplified energy diagram that shows the absorption and emission of a photon by an atom or a molecule. When a photon of energy $h\nu$ strikes the atom or molecule, absorption may occur if the difference in energy, ΔE, between the ground state and the excited state is equal to the photon's energy. An atom or molecule in an excited state may emit a photon and return to the ground state. The photon's energy, $h\nu$, equals the difference in energy, ΔE, between the two states.
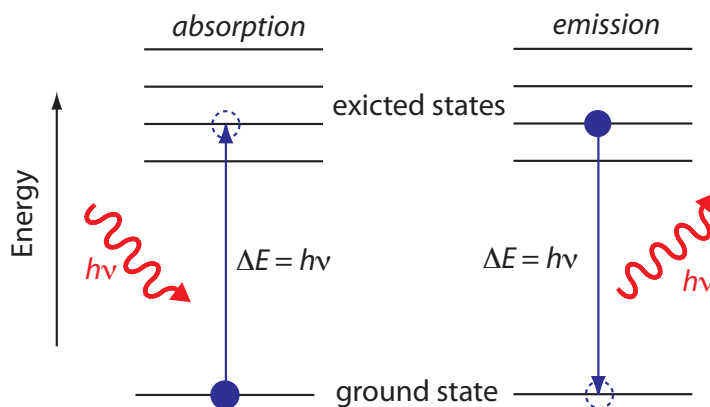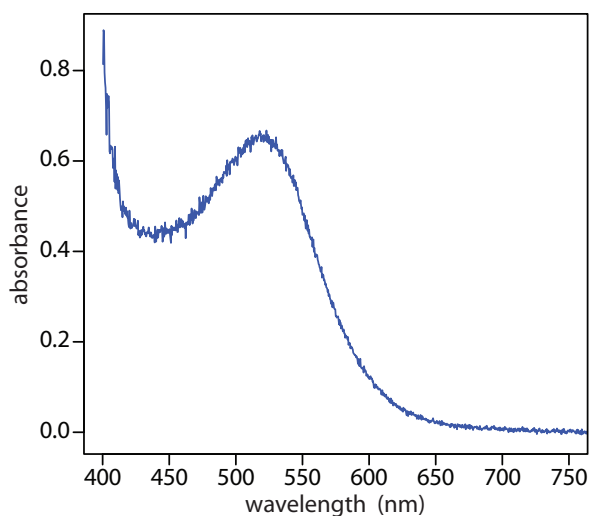
Figure 10.5 Visible absorbance spectrum for cranberry juice. The anthocyanin dyes in cranberry juice absorb visible light with blue, green, and yellow wavelengths (see Figure 10.3). As a result, the juice appears red.

When an atom or molecule in an excited state returns to a lower energy state, the excess energy often is released as a photon, a process we call EMISSION (Figure 10.4). There are several ways in which an atom or a molecule may end up in an excited state, including thermal energy, absorption of a photon, or as the result of a chemical reaction. Emission following the absorption of a photon is also called PHOTOLUMINESCENCE, and that following a chemical reaction is called CHEMILUMINESCENCE. A typical emission spectrum is shown in Figure 10.6.

In the second broad class of spectroscopic techniques, the electromagnetic radiation undergoes a change in amplitude, phase angle, polarization, or direction of propagation as a result of its refraction, reflection, scattering, diffraction, or dispersion by the sample. Several representative spectroscopic techniques are listed in Table 10.2.

Molecules also can release energy in the form of heat. We will return to this point later in the chapter.

10A.3 Basic Components of Spectroscopic Instruments

The spectroscopic techniques in Table 10.1 and Table 10.2 use instruments that share several common basic components, including a source of energy,
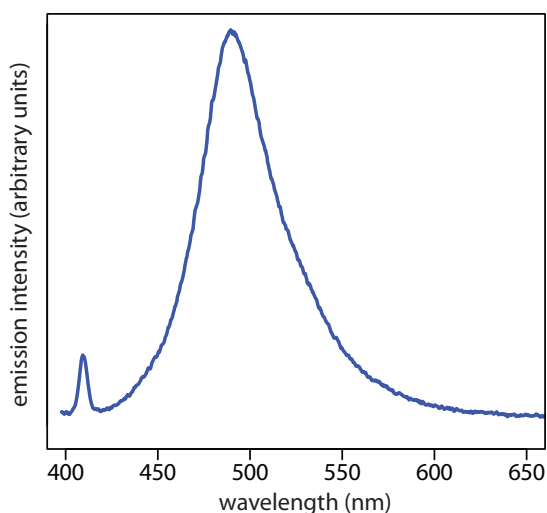
Figure 10.6 Photoluminescence spectrum of the dye coumarin 343, which is incorporated in a reverse micelle suspended in cyclohexanol. The dye's absorbance spectrum (not shown) has a broad peak around 400 nm. The sharp peak at 409 nm is from the laser source used to excite coumarin 343. The broad band centered at approximately 500 nm is the dye's emission band. Because the dye absorbs blue light, a solution of coumarin 343 appears yellow in the absence of photoluminescence. Its photoluminescent emission is blue-green. Source: data from Bridget Gourley, Department of Chemistry & Biochemistry, DePauw University.

Table 10.2 Examples of Spectroscopic Techniques That Do Not Involve an Exchange of Energy Between a Photon and the Sample		
Region of Electromagnetic Spectrum	Type of Interaction	Spectroscopic Technique[a]
X-ray	diffraction	X-ray diffraction
UV/Vis	refraction	refractometry
	scattering	*nephelometry* *turbidimetry*
	dispersion	optical rotary dispersion

[a] Techniques discussed in this text are shown in *italics*.

a means for isolating a narrow range of wavelengths, a detector for measuring the signal, and a signal processor that displays the signal in a form convenient for the analyst. In this section we introduce these basic components. Specific instrument designs are considered in later sections.

SOURCES OF ENERGY

All forms of spectroscopy require a source of energy. In absorption and scattering spectroscopy this energy is supplied by photons. Emission and photoluminescence spectroscopy use thermal, radiant (photon), or chemical energy to promote the analyte to a suitable excited state.

Sources of Electromagnetic Radiation. A source of electromagnetic radiation must provide an output that is both intense and stable. Sources of electromagnetic radiation are classified as either continuum or line sources. A CONTINUUM SOURCE emits radiation over a broad range of wavelengths, with a relatively smooth variation in intensity (Figure 10.7). A LINE SOURCE, on the other hand, emits radiation at selected wavelengths (Figure 10.8). Table 10.3 provides a list of the most common sources of electromagnetic radiation.

Sources of Thermal Energy. The most common sources of thermal energy are flames and plasmas. A flame source uses a combustion of a fuel and an oxidant to achieve temperatures of 2000–3400 K. Plasmas, which are hot, ionized gases, provide temperatures of 6000–10 000 K.

Chemical Sources of Energy Exothermic reactions also may serve as a source of energy. In chemiluminescence the analyte is raised to a higher-energy state by means of a chemical reaction, emitting characteristic radiation when it returns to a lower-energy state. When the chemical reaction results from a biological or enzymatic reaction, the emission of radiation is called bioluminescence. Commercially available "light sticks" and the flash of light from a firefly are examples of chemiluminescence and bioluminescence.
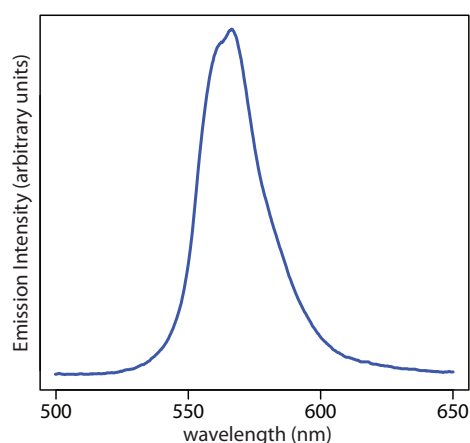
Figure 10.7 Spectrum showing the emission from a green LED, which provides continuous emission over a wavelength range of approximately 530–640 nm.

Table 10.3	Common Sources of Electromagnetic Radiation	
Source	Wavelength Region	Useful for...
H_2 and D_2 lamp	continuum source from 160–380 nm	molecular absorption
tungsten lamp	continuum source from 320–2400 nm	molecular absorption
Xe arc lamp	continuum source from 200–1000 nm	molecular fluorescence
nernst glower	continuum source from 0.4–20 μm	molecular absorption
globar	continuum source from 1–40 μm	molecular absorption
nichrome wire	continuum source from 0.75–20 μm	molecular absorption
hollow cathode lamp	line source in UV/Visible	atomic absorption
Hg vapor lamp	line source in UV/Visible	molecular fluorescence
laser	line source in UV/Visible/IR	atomic and molecular absorption, fluorescence, and scattering

## WAVELENGTH SELECTION

In Nessler's original colorimetric method for ammonia, which was described at the beginning of the chapter, the sample and several standard solutions of ammonia are placed in separate tall, flat-bottomed tubes. As shown in Figure 10.9, after adding the reagents and allowing the color to develop, the analyst evaluates the color by passing ambient light through the bottom of the tubes and looking down through the solutions. By matching the sample's color to that of a standard, the analyst is able to determine the concentration of ammonia in the sample.

In Figure 10.9 every wavelength of light from the source passes through the sample. This is not a problem if there is only one absorbing species in the sample. If the sample contains two components, then a quantitative analysis using Nessler's original method is impossible unless the standards
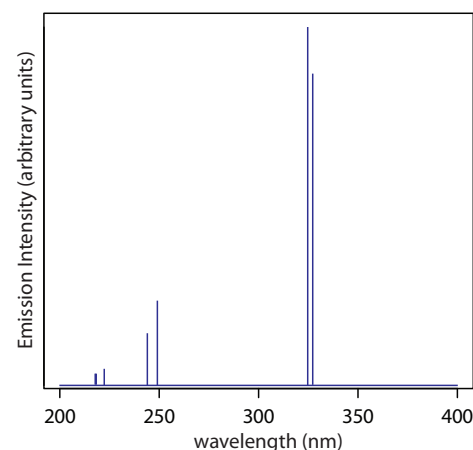
Figure 10.8 Emission spectrum from a Cu hollow cathode lamp. This spectrum consists of seven distinct emission lines (the first two differ by only 0.4 nm and are not resolved at the scale shown in this spectrum). Each emission line has a width of approximately 0.01 nm at ½ of its maximum intensity.
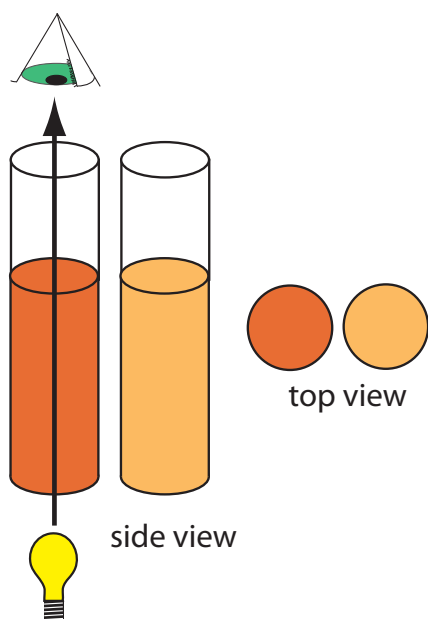
Figure 10.9 Nessler's original method for comparing the color of two solutions. Natural light passes upwards through the samples and standards and the analyst views the solutions by looking down toward the light source. The top view, shown on the right, is what the analyst sees. To determine the analyte's concentration, the analyst exchanges standards until the two colors match.
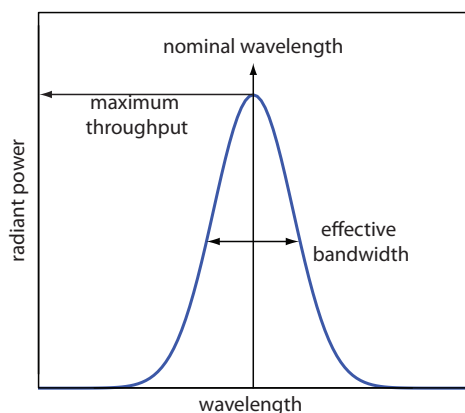
Figure 10.10 Radiation exiting a wavelength selector showing the band's nominal wavelength and its effective bandwidth.

contains the second component at the same concentration it has in the sample.

To overcome this problem, we want to select a wavelength that only the analyte absorbs. Unfortunately, we can not isolate a single wavelength of radiation from a continuum source, although we can narrow the range of wavelengths that reach the sample. As seen in Figure 10.10, a wavelength selector always passes a narrow band of radiation characterized by a NOMINAL WAVELENGTH, an EFFECTIVE BANDWIDTH, and a maximum throughput of radiation. The effective bandwidth is defined as the width of the radiation at half of its maximum throughput.

The ideal wavelength selector has a high throughput of radiation and a narrow effective bandwidth. A high throughput is desirable because the more photons that pass through the wavelength selector, the stronger the signal and the smaller the background noise. A narrow effective bandwidth provides a higher RESOLUTION, with spectral features separated by more than twice the effective bandwidth being resolved. As shown in Figure 10.11, these two features of a wavelength selector often are in opposition. A larger effective bandwidth favors a higher throughput of radiation, but provide less resolution. Decreasing the effective bandwidth improves resolution, but at the cost of a noisier signal.[4] For a qualitative analysis, resolution usually is more important than noise and a smaller effective bandwidth is desirable; however, in a quantitative analysis less noise usually is desirable.

Wavelength Selection Using Filters. The simplest method for isolating a narrow band of radiation is to use an absorption or interference FILTER. Absorption filters work by selectively absorbing radiation from a narrow region of the electromagnetic spectrum. Interference filters use constructive and destructive interference to isolate a narrow range of wavelengths. A simple example of an absorption filter is a piece of colored glass. A purple filter, for example, removes the complementary color green from 500–560 nm.

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4 Jiang, S.; Parker, G. A. *Am. Lab.* **1981**, October, 38–43.
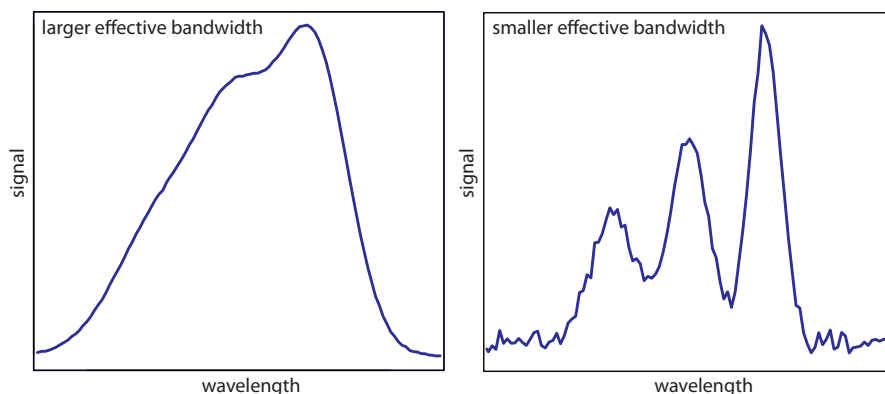
Figure 10.11 Example showing the effect of the wavelength selector's effective bandwidth on resolution and noise. The spectrum with the smaller effective bandwidth (on the right) has a better resolution, allowing us to see the presence of three peaks, but at the expense of a noisier signal. The spectrum with the larger effective bandwidth (on the left) has less noise, but at the expense of less resolution between the three peaks.

Commercially available absorption filters provide effective bandwidths of 30–250 nm, although the throughput at the low end of this range often is only 10% of the source's emission intensity. Interference filters are more expensive than absorption filters, but have narrower effective bandwidths, typically 10–20 nm, with maximum throughputs of at least 40%.
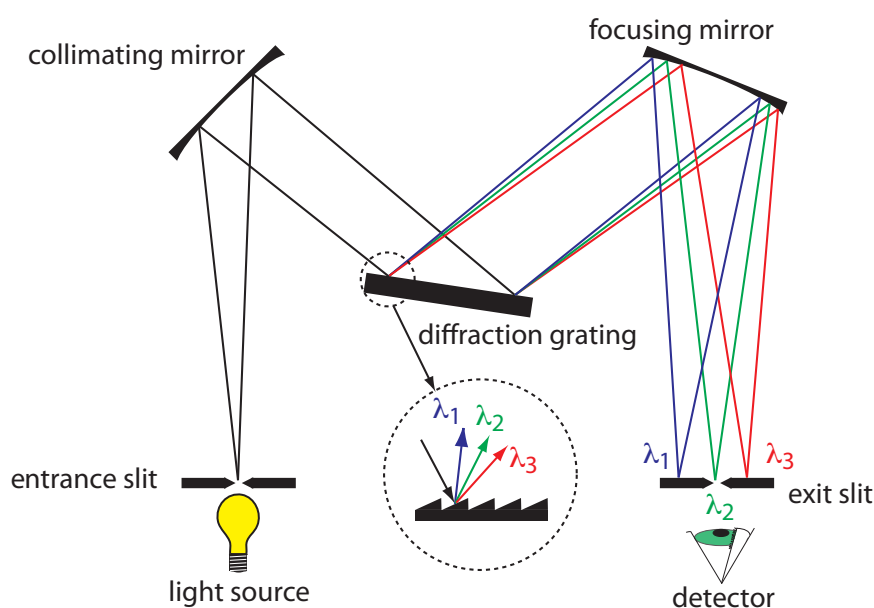
Wavelength Selection Using Monochromators. A filter has one significant limitation—because a filter has a fixed nominal wavelength, if we need to make measurements at two different wavelengths, then we must use two different filters. A MONOCHROMATOR is an alternative method for selecting a narrow band of radiation that also allows us to continuously adjust the band's nominal wavelength.

The construction of a typical monochromator is shown in Figure 10.12. Radiation from the source enters the monochromator through an entrance slit. The radiation is collected by a collimating mirror, which reflects a parallel beam of radiation to a diffraction grating. The diffraction grating is an optically reflecting surface with a large number of parallel grooves (see insert to Figure 10.12). The diffraction grating disperses the radiation and a second mirror focuses the radiation onto a planar surface that contains an exit slit. In some monochromators a prism is used in place of the diffraction grating.

Radiation exits the monochromator and passes to the detector. As shown in Figure 10.12, a monochromator converts a POLYCHROMATIC source of radiation at the entrance slit to a MONOCHROMATIC source of finite effective bandwidth at the exit slit. The choice of which wavelength exits the monochromator is determined by rotating the diffraction grating. A narrower exit slit provides a smaller effective bandwidth and better resolution than does a wider exit slit, but at the cost of a smaller throughput of radiation.

Polychromatic means many colored. Polychromatic radiation contains many different wavelengths of light.

Monochromatic means one color, or one wavelength. Although the light exiting a monochromator is not strictly of a single wavelength, its narrow effective bandwidth allows us to think of it as monochromatic.
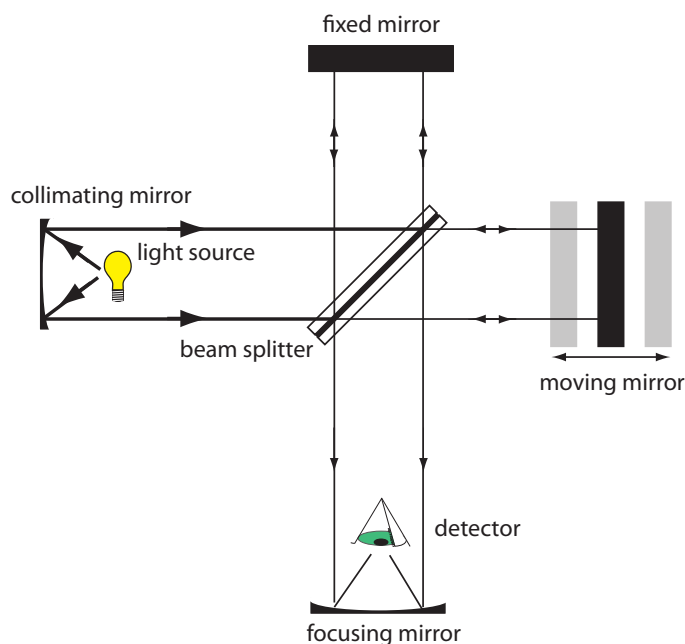

Figure 10.12 Schematic diagram of a monochromator that uses a diffraction grating to disperse the radiation.

Monochromators are classified as either fixed-wavelength or scanning. In a fixed-wavelength monochromator we manually select the wavelength by rotating the grating. Normally a fixed-wavelength monochromator is used for a quantitative analysis where measurements are made at one or two wavelengths. A scanning monochromator includes a drive mechanism that continuously rotates the grating, which allows successive wavelengths of light to exit from the monochromator. A scanning monochromator is used to acquire spectra, and, when operated in a fixed-wavelength mode, for a quantitative analysis.

Interferometers. An INTERFEROMETER provides an alternative approach for wavelength selection. Instead of filtering or dispersing the electromagnetic radiation, an interferometer allows source radiation of all wavelengths to reach the detector simultaneously (Figure 10.13). Radiation from the source is focused on a beam splitter that reflects half of the radiation to a fixed mirror and transmits the other half to a moving mirror. The radiation recombines at the beam splitter, where constructive and destructive interference determines, for each wavelength, the intensity of light that reaches the detector. As the moving mirror changes position, the wavelength of light that experiences maximum constructive interference and maximum destructive interference also changes. The signal at the detector shows intensity as a function of the moving mirror's position, expressed in units of distance or time. The result is called an INTERFEROGRAM or a time domain spectrum. The time domain spectrum is converted mathematically, by a process called a Fourier transform, to a spectrum (a frequency domain spectrum) that shows intensity as a function of the radiation's energy.

In comparison to a monochromator, an interferometer has two significant advantages. The first advantage, which is termed JACQUINOT'S AD-

The mathematical details of the Fourier transform are beyond the level of this textbook. You can consult the chapter's additional resources for additional information.

Figure 10.13 Schematic diagram of an interferometers.

VANTAGE, is the greater throughput of source radiation. Because an interferometer does not use slits and has fewer optical components from which radiation is scattered and lost, the throughput of radiation reaching the detector is 80–200× greater than that for a monochromator. The result is less noise. The second advantage, which is called FELLGETT'S ADVANTAGE, is a savings in the time needed to obtain a spectrum. Because the detector monitors all frequencies simultaneously, a spectrum takes approximately one second to record, as compared to 10–15 minutes when using a scanning monochromator.

DETECTORS

In Nessler's original method for determining ammonia (Figure 10.9) the analyst's eye serves as the detector, matching the sample's color to that of a standard. The human eye, of course, has a poor range—it responds only to visible light—and it is not particularly sensitive or accurate. Modern detectors use a sensitive TRANSDUCER to convert a signal consisting of photons into an easily measured electrical signal. Ideally the detector's signal, S, is a linear function of the electromagnetic radiation's power, P,

$$S = kP + D$$

where k is the detector's sensitivity, and D is the detector's DARK CURRENT, or the background current when we prevent the source's radiation from reaching the detector.

There are two broad classes of spectroscopic transducers: thermal transducers and photon transducers. Table 10.4 provides several representative examples of each class of transducers.

Photon Transducers. Phototubes and photomultipliers use a photosensitive surface that absorbs radiation in the ultraviolet, visible, or near IR to produce an electrical current that is proportional to the number of photons reaching the transducer (Figure 10.14). Other photon detectors use a semiconductor as the photosensitive surface. When the semiconductor absorbs

Transducer is a general term that refers to any device that converts a chemical or a physical property into an easily measured electrical signal. The retina in your eye, for example, is a transducer that converts photons into an electrical nerve impulse; your eardrum is a transducer that converts sound waves into a different electrical nerve impulse.

Table 10.4	Examples of Transducers for Spectroscopy		
Transducer	Class	Wavelength Range	Output Signal
phototube	photon	200–1000 nm	current
photomultiplier	photon	110–1000 nm	current
Si photodiode	photon	250–1100 nm	current
photoconductor	photon	750–6000 nm	change in resistance
photovoltaic cell	photon	400–5000 nm	current or voltage
thermocouple	thermal	0.8–40 μm	voltage
thermistor	thermal	0.8–40 μm	change in resistance
pneumatic	thermal	0.8–1000 μm	membrane displacement
pyroelectric	thermal	0.3–1000 μm	current
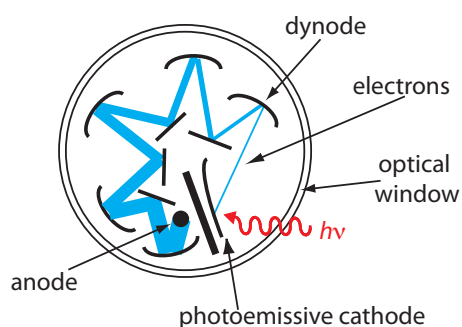
Figure 10.14 Schematic of a photomultiplier. A **photon** strikes the photoemissive cathode producing **electrons**, which accelerate toward a positively charged dynode. Collision of these electrons with the dynode generates additional electrons, which accelerate toward the next dynode. A total of 10^6–10^7 electrons per photon eventually reach the anode, generating an electrical current.

If the retina in your eye and the eardrum in your ear are transducers, then your brain is the signal processor.

Figure 10.3 provides a list of the types of atomic and molecular transitions associated with different types of electromagnetic radiation.

photons, valence electrons move to the semiconductor's conduction band, producing a measurable current. One advantage of the Si photodiode is that it is easy to miniaturize. Groups of photodiodes are gathered together in a linear array that contains 64–4096 individual photodiodes. With a width of 25 μm per diode, a linear array of 2048 photodiodes requires only 51.2 mm of linear space. By placing a PHOTODIODE ARRAY along the monochromator's focal plane, it is possible to monitor simultaneously an entire range of wavelengths.

Thermal Transducers. Infrared photons do not have enough energy to produce a measurable current with a photon transducer. A thermal transducer, therefore, is used for infrared spectroscopy. The absorption of infrared photons increases a thermal transducer's temperature, changing one or more of its characteristic properties. A pneumatic transducer, for example, is a small tube of xenon gas with an IR transparent window at one end and a flexible membrane at the other end. Photons enter the tube and are absorbed by a blackened surface, increasing the temperature of the gas. As the temperature inside the tube fluctuates, the gas expands and contracts and the flexible membrane moves in and out. Monitoring the membrane's displacement produces an electrical signal.

Signal Processors

A transducer's electrical signal is sent to a SIGNAL PROCESSOR where it is displayed in a form that is more convenient for the analyst. Examples of signal processors include analog or digital meters, recorders, and computers equipped with digital acquisition boards. A signal processor also is used to calibrate the detector's response, to amplify the transducer's signal, to remove noise by filtering, or to mathematically transform the signal.

10B   Spectroscopy Based on Absorption

In absorption spectroscopy a beam of electromagnetic radiation passes through a sample. Much of the radiation passes through the sample without a loss in intensity. At selected wavelengths, however, the radiation's intensity is attenuated. This process of attenuation is called absorption.

10B.1   Absorbance Spectra

There are two general requirements for an analyte's absorption of electromagnetic radiation. First, there must be a mechanism by which the radiation's electric field or magnetic field interacts with the analyte. For ultraviolet and visible radiation, absorption of a photon changes the energy of the analyte's valence electrons. A bond's vibrational energy is altered by the absorption of infrared radiation.

The second requirement is that the photon's energy, $h\nu$, must exactly equal the difference in energy, ΔE, between two of the analyte's quantized

energy states. Figure 10.4 shows a simplified view of a photon's absorption, which is useful because it emphasizes that the photon's energy must match the difference in energy between a lower-energy state and a higher-energy state. What is missing, however, is information about what types of energy states are involved, which transitions between energy states are likely to occur, and the appearance of the resulting spectrum.

We can use the energy level diagram in Figure 10.15 to explain an absorbance spectrum. The lines labeled E_0 and E_1 represent the analyte's ground (lowest) electronic state and its first electronic excited state. Superimposed on each electronic energy level is a series of lines representing vibrational energy levels.

INFRARED SPECTRA FOR MOLECULES AND POLYATOMIC IONS

The energy of infrared radiation produces a change in a molecule's or a polyatomic ion's vibrational energy, but is not sufficient to effect a change in its electronic energy. As shown in Figure 10.15, vibrational energy levels are quantized; that is, a molecule or polyatomic ion has only certain, discrete vibrational energies. The energy for an allowed vibrational mode, E_ν, is

$$E_\nu = \nu + \frac{1}{2} h\nu_0$$

where ν is the vibrational quantum number, which has values of 0, 1, 2, ..., and ν_0 is the bond's fundamental vibrational frequency. The value of ν_0, which is determined by the bond's strength and by the mass at each end of the bond, is a characteristic property of a bond. For example, a carbon-carbon single bond (C–C) absorbs infrared radiation at a lower energy than
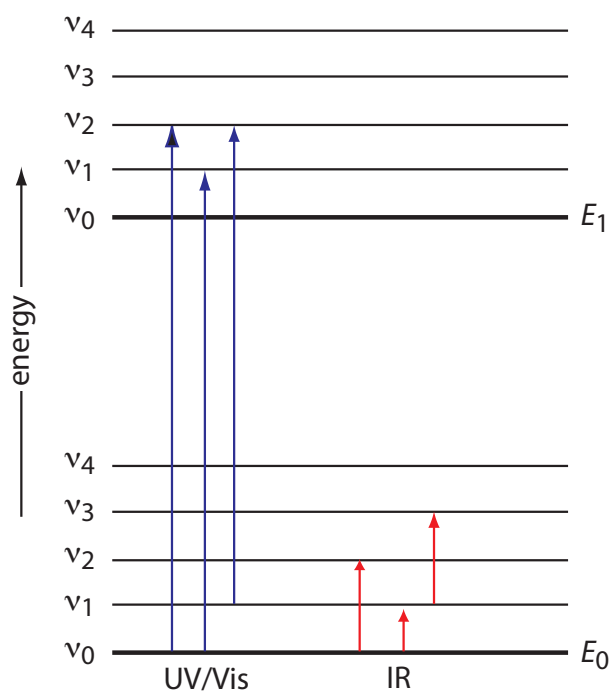
Figure 10.15 Diagram showing two electronic energy levels (E_0 and E_1), each with five vibrational energy levels (ν_0–ν_4). Absorption of ultraviolet and visible radiation (shown by the **blue** arrows) leads to a change in the analyte's electronic energy levels and, possibly, a change in vibrational energy as well. A change in vibrational energy without a change in electronic energy levels occurs with the absorption of infrared radiation (shown by the **red** arrows).

a carbon-carbon double bond (C=C) because a single bond is weaker than a double bond.

At room temperature most molecules are in their ground vibrational state ($\nu = 0$). A transition from the ground vibrational state to the first vibrational excited state ($\nu = 1$) requires absorption of a photon with an energy of $h\nu_0$. Transitions in which $\Delta\nu$ is ± 1 give rise to the fundamental absorption lines. Weaker absorption lines, called overtones, result from transitions in which $\Delta\nu$ is ± 2 or ± 3. The number of possible normal vibrational modes for a linear molecule is $3N-5$, and for a non-linear molecule is $3N-6$, where N is the number of atoms in the molecule. Not surprisingly, infrared spectra often show a considerable number of absorption bands. Even a relatively simple molecule, such as ethanol (C_2H_6O), for example, has $3 \times 9 - 6$, or 21 possible normal modes of vibration, although not all of these vibrational modes give rise to an absorption. The IR spectrum for ethanol is shown in Figure 10.16.

UV/Vis Spectra for Molecules and Ions

The valence electrons in organic molecules and polyatomic ions, such as CO_3^{2-}, occupy quantized sigma bonding (σ), pi bonding (π), and non-bonding (n) molecular orbitals (MOs). Unoccupied sigma antibonding (σ^*) and pi antibonding (π^*) molecular orbitals are slightly higher in energy. Because the difference in energy between the highest-energy occupied MOs and the lowest-energy unoccupied MOs corresponds to ultraviolet and visible radiation, absorption of a photon is possible.

Four types of transitions between quantized energy levels account for most molecular UV/Vis spectra. Table 10.5 lists the approximate wavelength ranges for these transitions, as well as a partial list of bonds, func-
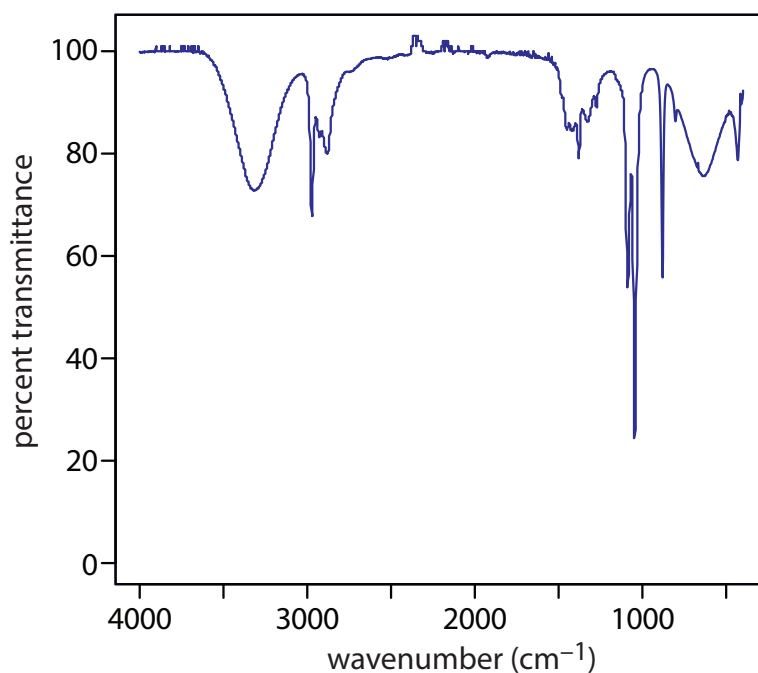
**Figure 10.16** Infrared spectrum of ethanol.

Table 10.5 Electronic Transitions Involving n, σ, and π Molecular Orbitals		
Transition	Wavelength Range	Examples
$\sigma \rightarrow \sigma^*$	<200 nm	C–C, C–H
$n \rightarrow \sigma^*$	160–260 nm	H_2O, CH_3OH, CH_3Cl
$\pi \rightarrow \pi^*$	200–500 nm	C=C, C=O, C=N, C≡C
$n \rightarrow \pi^*$	250–600 nm	C=O, C=N, N=N, N=O

tional groups, or molecules responsible for these transitions. Of these transitions, the most important are $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ because they involve important functional groups that are characteristic of many analytes and because the wavelengths are easily accessible. The bonds and functional groups that give rise to the absorption of ultraviolet and visible radiation are called CHROMOPHORES.

Many transition metal ions, such as Cu^{2+} and Co^{2+}, form colorful solutions because the metal ion absorbs visible light. The transitions that give rise to this absorption are valence electrons in the metal ion's d-orbitals. For a free metal ion, the five d-orbitals are of equal energy. In the presence of a complexing ligand or solvent molecule, however, the d-orbitals split into two or more groups that differ in energy. For example, in an octahedral complex of $Cu(H_2O)_6^{2+}$ the six water molecules perturb the d-orbitals into the two groups shown in Figure 10.17. The resulting $d \rightarrow d$ transitions for transition metal ions are relatively weak.

A more important source of UV/Vis absorption for inorganic metal–ligand complexes is charge transfer, in which absorption of a photon produces an excited state in which there is transfer of an electron from the metal, M, to the ligand, L.

$$M\text{–}L + h\nu \rightarrow (M^+\text{–}L^-)^*$$
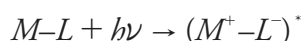
Charge-transfer absorption is important because it produces very large absorbances. One important example of a charge-transfer complex is that of o-phenanthroline with Fe^{2+}, the UV/Vis spectrum for which is shown in Figure 10.18. Charge-transfer absorption in which an electron moves from the ligand to the metal also is possible.

Comparing the IR spectrum in Figure 10.16 to the UV/Vis spectrum in Figure 10.18 shows us that UV/Vis absorption bands are often significantly broader than those for IR absorption. We can use Figure 10.15 to explain why this is true. When a species absorbs UV/Vis radiation, the transition between electronic energy levels may also include a transition between vibrational energy levels. The result is a number of closely spaced absorption bands that merge together to form a single broad absorption band.
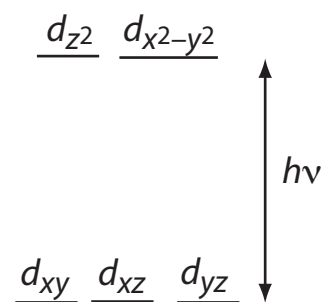
Figure 10.17 Splitting of the d-orbitals in an octahedral field.

Why is a larger absorbance desirable? An analytical method is more sensitive if a smaller concentration of analyte gives a larger signal.
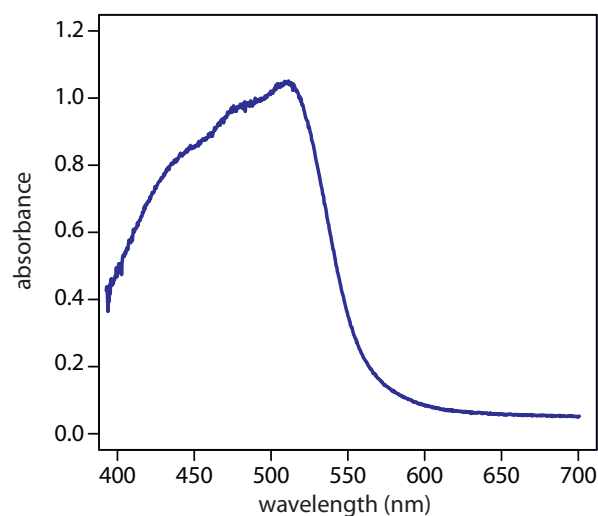
Figure 10.18 UV/Vis spectrum for the metal–ligand complex $Fe(phen)_3^{2+}$, where phen is the ligand o-phenanthroline.

UV/Vis Spectra for Atoms

The valence shell energy level diagram in Figure 10.19 might strike you as odd because it shows that the $3p$ orbitals are split into two groups of slightly different energy. The reasons for this splitting are unimportant in the context of our treatment of atomic absorption. For further information about the reasons for this splitting, consult the chapter's additional resources.

The energy of ultraviolet and visible electromagnetic radiation is sufficient to cause a change in an atom's valence electron configuration. Sodium, for example, has a single valence electron in its $3s$ atomic orbital. As shown in Figure 10.19, unoccupied, higher energy atomic orbitals also exist.

Absorption of a photon is accompanied by the excitation of an electron from a lower-energy atomic orbital to an atomic orbital of higher energy. Not all possible transitions between atomic orbitals are allowed. For sodium the only allowed transitions are those in which there is a change of ± 1 in the orbital quantum number (l); thus transitions from $s \rightarrow p$ orbitals are allowed, but transitions from $s \rightarrow s$ and from $s \rightarrow d$ orbitals are forbidden.

The atomic absorption spectrum for Na is shown in Figure 10.20, and is typical of that found for most atoms. The most obvious feature of this spectrum is that it consists of a small number of discrete absorption lines that correspond to transitions between the ground state (the $3s$ atomic orbital)
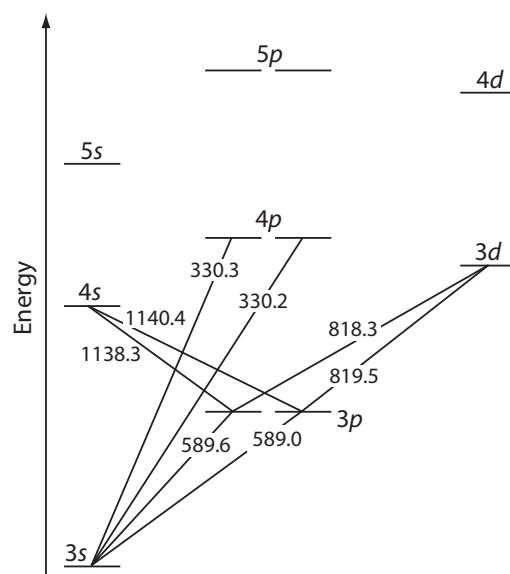
Figure 10.19 Valence shell energy level diagram for sodium. The wavelengths (in wavenumbers) corresponding to several transitions are shown.
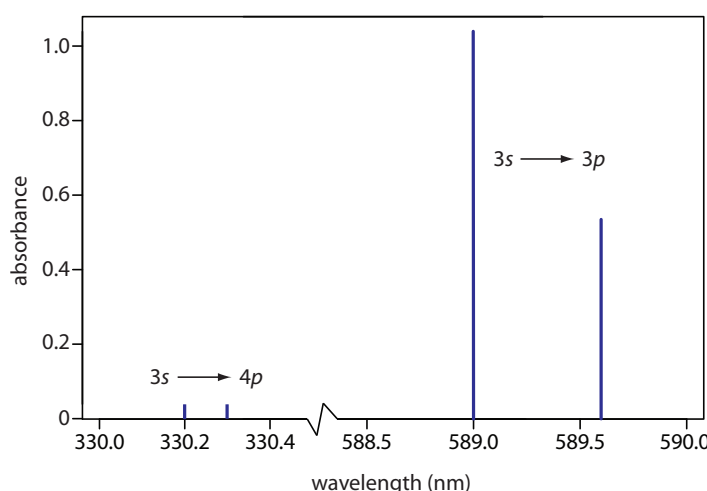
Figure 10.20 Atomic absorption spectrum for sodium. Note that the scale on the x-axis includes a break.

and the $3p$ and the $4p$ atomic orbitals. Absorption from excited states, such as the $3p \rightarrow 4s$ and the $3p \rightarrow 3d$ transitions included in Figure 10.19, are too weak to detect. Because an excited state's lifetime is short—an excited state atom typically returns to a lower energy state in 10^{-7} to 10^{-8} seconds—an atom in the exited state is likely to return to the ground state before it has an opportunity to absorb a photon.

Another feature of the atomic absorption spectrum in Figure 10.20 is the narrow width of the absorption lines, which is a consequence of the fixed difference in energy between the ground state and the excited state, and the lack of vibrational and rotational energy levels. Natural line widths for atomic absorption, which are governed by the uncertainty principle, are approximately 10^{-5} nm. Other contributions to broadening increase this line width to approximately 10^{-3} nm.

10B.2   Transmittance and Absorbance

As light passes through a sample, its power decreases as some of it is absorbed. This attenuation of radiation is described quantitatively by two separate, but related terms: transmittance and absorbance. As shown in Figure 10.21a, TRANSMITTANCE is the ratio of the source radiation's power as it exits the sample, P_T, to that incident on the sample, P_0.

$$T = \frac{P_T}{P_0} \qquad\qquad 10.1$$

Multiplying the transmittance by 100 gives the percent transmittance, $\%T$, which varies between 100% (no absorption) and 0% (complete absorption). All methods of detecting photons—including the human eye and modern photoelectric transducers—measure the transmittance of electromagnetic radiation.

Equation 10.1 does not distinguish between different mechanisms that prevent a photon emitted by the source from reaching the detector. In addition to absorption by the analyte, several additional phenomena contribute to the attenuation of radiation, including reflection and absorption by the
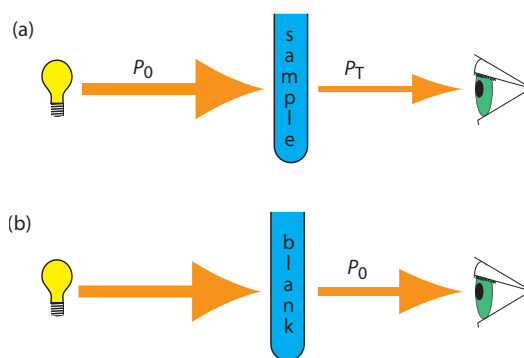
Figure 10.21 (a) Schematic diagram showing the attenuation of radiation passing through a sample; P_0 is the source's radiant power and P_T is the radiant power transmitted by the sample. (b) Schematic diagram showing how we redefine P_0 as the radiant power transmitted by the blank. Redefining P_0 in this way corrects the transmittance in (a) for the loss of radiation due to scattering, reflection, absorption by the sample's container, and absorption by the sample's matrix.

sample's container, absorption by other components in the sample's matrix, and the scattering of radiation. To compensate for this loss of the radiation's power, we use a method blank. As shown in Figure 10.21b, we redefine P_0 as the power exiting the method blank.

An alternative method for expressing the attenuation of electromagnetic radiation is absorbance, A, which we define as

$$A = -\log T = -\log \frac{P_T}{P_0} \qquad 10.2$$

Absorbance is the more common unit for expressing the attenuation of radiation because it is a linear function of the analyte's concentration.

Practice Exercise 10.3

What is the $\%T$ for a sample if its absorbance is 1.27?

Click here to review your answer to this exercise.

Example 10.3

A sample has a percent transmittance of 50%. What is its absorbance?

SOLUTION

A percent transmittance of 50.0% is the same as a transmittance of 0.500 Substituting into equation 10.2 gives

$$A = -\log T = -\log(0.500) = 0.301$$

Equation 10.1 has an important consequence for atomic absorption. As we learned from Figure 10.20, atomic absorption lines are very narrow. Even with a high quality monochromator, the effective bandwidth for a continuum source is 100–1000× greater than the width of an atomic absorption line. As a result, little radiation from a continuum source is absorbed when it passes through a sample of atoms; because $P_0 \approx P_T$ the measured absorbance effectively is zero. For this reason, atomic absorption requires that we use a line source instead of a continuum source.

10B.3   Absorbance and Concentration: Beer's Law

When monochromatic electromagnetic radiation passes through an infinitesimally thin layer of sample of thickness dx, it experiences a decrease in its power of dP (Figure 10.22). This fractional decrease in power is proportional to the sample's thickness and to the analyte's concentration, C; thus

$$-\frac{dP}{P} = \alpha C dx \qquad 10.3$$

where P is the power incident on the thin layer of sample and α is a proportionality constant. Integrating the left side of equation 10.3 over the sample's full thickness

$$-\int_{P=P_o}^{P=P_T} \frac{dP}{P} = \alpha C \int_{x=0}^{x=b} dx$$

$$\ln\frac{P_0}{P_T} = \alpha b C$$

converting from ln to log, and substituting into equation 10.2, gives

$$A = abC \qquad 10.4$$

where a is the analyte's ABSORPTIVITY with units of cm^{-1} $conc^{-1}$. If we express the concentration using molarity, then we replace a with the MOLAR ABSORPTIVITY, ε, which has units of cm^{-1} M^{-1}.

$$A = \varepsilon bC \qquad 10.5$$

The absorptivity and the molar absorptivity are proportional to the probability that the analyte absorbs a photon of a given energy. As a result, values for both a and ε depend on the wavelength of the absorbed photon.

Example 10.4

A 5.00×10^{-4} M solution of analyte is placed in a sample cell that has a pathlength of 1.00 cm. At a wavelength of 490 nm, the solution's absorbance is 0.338. What is the analyte's molar absorptivity at this wavelength?

SOLUTION

Solving equation 10.5 for ε and making appropriate substitutions gives

$$\varepsilon = \frac{A}{bC} = \frac{0.338}{(1.00 \text{ cm})(5.00 \times 10^{-4} \text{ M})} = 676 \text{ cm}^{-1}\text{M}^{-1}$$

Practice Exercise 10.4

A solution of the analyte from Example 10.4 has an absorbance of 0.228 in a 1.00-cm sample cell. What is the analyte's concentration?

Click here to review your answer to this exercise.
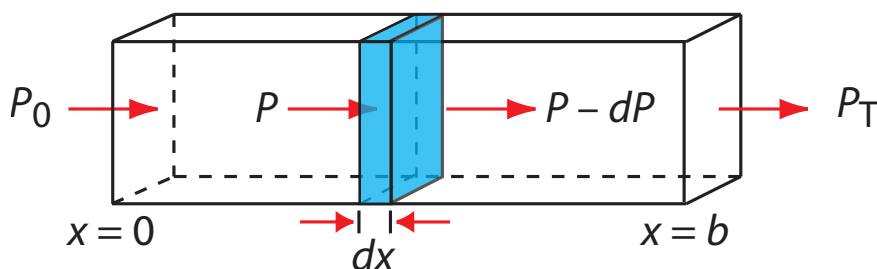
Figure 10.22 Factors used to derive the Beer's law.

Equation 10.4 and equation 10.5, which establish the linear relationship between absorbance and concentration, are known as BEER'S LAW. Calibration curves based on Beer's law are common in quantitative analyses.

10B.4   Beer's Law and Multicomponent Samples

We can extend Beer's law to a sample that contains several absorbing components. If there are no interactions between the components, then the individual absorbances, A_i, are additive. For a two-component mixture of analyte's X and Y, the total absorbance, A_{tot}, is

$$A_{tot} = A_X + A_Y = \varepsilon_X b C_X + \varepsilon_Y b C_Y$$

Generalizing, the absorbance for a mixture of n components, A_{mix}, is

$$A_{mix} = \sum_{i=1}^{n} A_i = \sum_{i=1}^{n} \varepsilon_i b C_i \qquad 10.6$$

10B.5   Limitations to Beer's Law

Beer's law suggests that a plot of absorbance vs. concentration—we will call this a Beer's law plot—is a straight line with a y-intercept of zero and a slope of ab or εb. In some cases a Beer's law plot deviates from this ideal behavior (see Figure 10.23), and such deviations from linearity are divided into three categories: fundamental, chemical, and instrumental.

FUNDAMENTAL LIMITATIONS TO BEER'S LAW

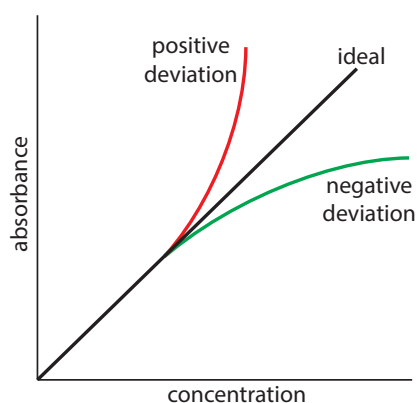Beer's law is a limiting law that is valid only for low concentrations of analyte. There are two contributions to this fundamental limitation to Beer's law. At higher concentrations the individual particles of analyte no longer are independent of each other. The resulting interaction between particles of analyte may change the analyte's absorptivity. A second contribution is that an analyte's absorptivity depends on the solution's refractive index. Because a solution's refractive index varies with the analyte's concentration, values of a and ε may change. For sufficiently low concentrations of analyte, the refractive index essentially is constant and a Beer's law plot is linear.

Figure 10.23 Plots of absorbance vs. concentration showing **positive** and **negative** deviations from the **ideal** Beer's law relationship, which is a straight line.

CHEMICAL LIMITATIONS TO BEER'S LAW

A chemical deviation from Beer's law may occur if the analyte is involved in an equilibrium reaction. Consider, for example, the weak acid, HA. To construct a Beer's law plot we prepare a series of standard solutions—each of which contains a known total concentration of HA—and then measure each solution's absorbance at the same wavelength. Because HA is a weak acid, it is in equilibrium with its conjugate weak base, A^-.

$$\text{HA}(aq) + \text{H}_2\text{O}(l) \rightleftharpoons \text{H}_3\text{O}^+(aq) + \text{A}^-(aq)$$
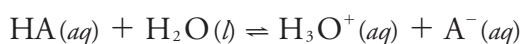
If both HA and A^- absorb at the selected wavelength, then Beer's law is

$$A = \varepsilon_{HA}bC_{HA} + \varepsilon_{A^-}bC_{A^-} \qquad 10.7$$

Because the weak acid's total concentration, C_{total}, is

$$C_{total} = C_{HA} + C_{A^-}$$

we can write the concentrations of HA and A^- as

$$C_{HA} = \alpha_{HA}C_{total} \qquad 10.8$$

$$C_{A^-} = (1 - \alpha_{HA})C_{total} \qquad 10.9$$

where α_{HA} is the fraction of weak acid present as HA. Substituting equation 10.8 and equation 10.9 into equation 10.7 and rearranging, gives

$$A = (\varepsilon_{HA}\alpha_{HA} + \varepsilon_{A^-} - \varepsilon_{A^-}\alpha_{A^-})bC_{total} \qquad 10.10$$

To obtain a linear Beer's law plot, we must satisfy one of two conditions. If ε_{HA} and ε_{A^-} have the same value at the selected wavelength, then equation 10.10 simplifies to

$$A = \varepsilon_{A^-}bC_{total} = \varepsilon_{HA}bC_{total}$$

Alternatively, if α_{HA} has the same value for all standard solutions, then each term within the parentheses of equation 10.10 is constant—which we re-place with k—and a linear calibration curve is obtained at any wavelength.

$$A = kbC_{total}$$

Because HA is a weak acid, the value of α_{HA} varies with pH. To hold α_{HA} constant we buffer each standard solution to the same pH. Depending on the relative values of α_{HA} and α_A, the calibration curve has a positive or a negative deviation from Beer's law if we do not buffer the standards to the same pH.

For a monoprotic weak acid, the equation for α_{HA} is

$$\alpha_{HA} = \frac{[H_3O^+]}{[H_3O^+] + K_a}$$

Problem 10.6 in the end of chapter problems asks you to explore this chemical limitation to Beer's law.

INSTRUMENTAL LIMITATIONS TO BEER'S LAW

There are two principal instrumental limitations to Beer's law. The first limitation is that Beer's law assumes that radiation reaching the sample is of a single wavelength—that is, it assumes a purely monochromatic source of radiation. As shown in Figure 10.10, even the best wavelength selector passes radiation with a small, but finite effective bandwidth. Polychromatic radiation always gives a negative deviation from Beer's law, but the effect is smaller if the value of ε essentially is constant over the wavelength range passed by the wavelength selector. For this reason, as shown in Figure 10.24, it is better to make absorbance measurements at the top of a broad absorption peak. In addition, the deviation from Beer's law is less serious if the source's effective bandwidth is less than one-tenth of the absorbing species' natural bandwidth.[5] When measurements must be made on a slope, linearity is improved by using a narrower effective bandwidth.

Problem 10.7 in the end of chapter problems ask you to explore the effect of polychromatic radiation on the linearity of Beer's law.

5   (a) Strong, F. C., III *Anal. Chem.* **1984**, *56*, 16A–34A; Gilbert, D. D. *J. Chem. Educ.* **1991**, *68*, A278–A281.
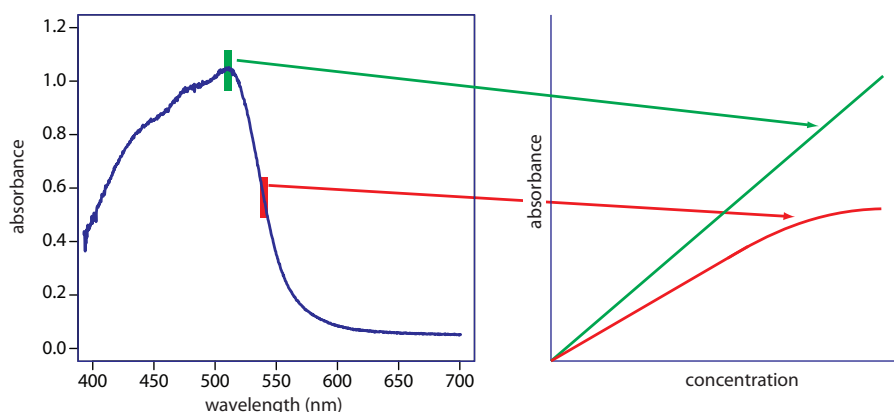
Figure 10.24 Effect of wavelength selection on the linearity of a Beer's law plot.

STRAY RADIATION is the second contribution to instrumental deviations from Beer's law. Stray radiation arises from imperfections in the wavelength selector that allow light to enter the instrument and to reach the detector without passing through the sample. Stray radiation adds an additional contribution, P_{stray}, to the radiant power that reaches the detector; thus

$$A = -\log \frac{P_T + P_{stray}}{P_0 + P_{stray}}$$

For a small concentration of analyte, P_{stray} is significantly smaller than P_0 and P_T, and the absorbance is unaffected by the stray radiation. For higher concentrations of analyte, less light passes through the sample and P_T and P_{stray} become similar in magnitude. This results is an absorbance that is smaller than expected, and a negative deviation from Beer's law.

10C   UV/Vis and IR Spectroscopy

In Figure 10.9 we examined Nessler's original method for matching the color of a sample to the color of a standard. Matching colors is a labor intensive process for the analyst and, not surprisingly, spectroscopic methods of analysis were slow to find favor. The 1930s and 1940s saw the introduction of photoelectric transducers for ultraviolet and visible radiation, and thermocouples for infrared radiation. As a result, modern instrumentation for absorption spectroscopy routinely became available in the 1940s—further progress has been rapid ever since.

10C.1   Instrumentation

Frequently an analyst must select from among several instruments of different design, the one instrument best suited for a particular analysis. In this section we examine several different instruments for molecular absorption spectroscopy, with an emphasis on their advantages and limitations. Methods of sample introduction also are covered in this section.
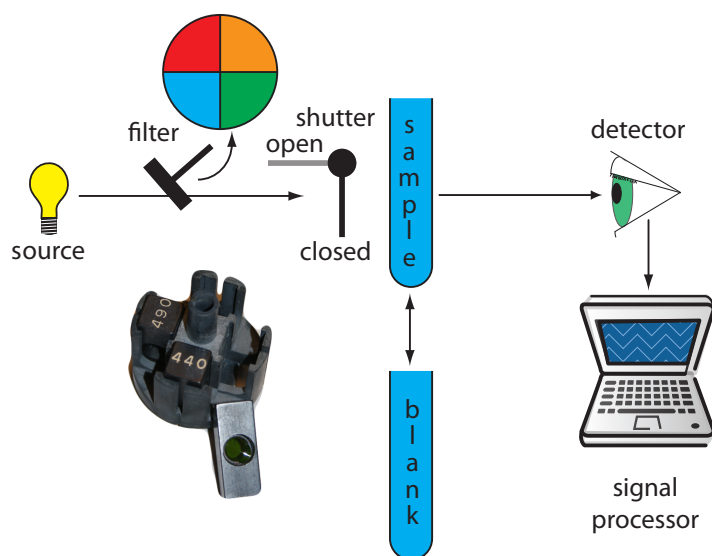
Figure 10.25 Schematic diagram of a filter photometer. The analyst either inserts a removable filter or the filters are placed in a carousel, an example of which is shown in the photographic inset. The analyst selects a filter by rotating it into place.

INSTRUMENT DESIGNS FOR MOLECULAR UV/VIS ABSORPTION

Filter Photometer. The simplest instrument for molecular UV/Vis absorption is a FILTER PHOTOMETER (Figure 10.25), which uses an absorption or interference filter to isolate a band of radiation. The filter is placed between the source and the sample to prevent the sample from decomposing when exposed to higher energy radiation. A filter photometer has a single optical path between the source and detector, and is called a SINGLE-BEAM instrument. The instrument is calibrated to 0% T while using a shutter to block the source radiation from the detector. After opening the shutter, the instrument is calibrated to 100% T using an appropriate blank. The blank is then replaced with the sample and its transmittance measured. Because the source's incident power and the sensitivity of the detector vary with wavelength, the photometer is recalibrated whenever the filter is changed. Photometers have the advantage of being relatively inexpensive, rugged, and easy to maintain. Another advantage of a photometer is its portability, making it easy to take into the field. Disadvantages of a photometer include the inability to record an absorption spectrum and the source's relatively large effective bandwidth, which limits the calibration curve's linearity.

Single-Beam Spectrophotometer. An instrument that uses a monochromator for wavelength selection is called a SPECTROPHOTOMETER. The simplest spectrophotometer is a single-beam instrument equipped with a fixed-wavelength monochromator (Figure 10.26). Single-beam spectrophotometers are calibrated and used in the same manner as a photometer. One example of a single-beam spectrophotometer is Thermo Scientific's Spectronic 20D+, which is shown in the photographic insert to Figure 10.26. The Spectronic 20D+ has a wavelength range of 340–625 nm (950 nm when using a red-sensitive detector), and a fixed effective bandwidth of 20 nm. Battery-operated, hand-held single-beam spectrophotometers are available, which are easy to transport into the field. Other single-beam

The percent transmittance varies between 0% and 100%. As we learned from Figure 10.21, we use a blank to determine P_0, which corresponds to 100% T. Even in the absence of light the detector records a signal. Closing the shutter allows us to assign 0% T to this signal. Together, setting 0% T and 100% T calibrates the instrument. The amount of light that passes through a sample produces a signal that is greater than or equal to 0% T and smaller than or equal to 100%T.
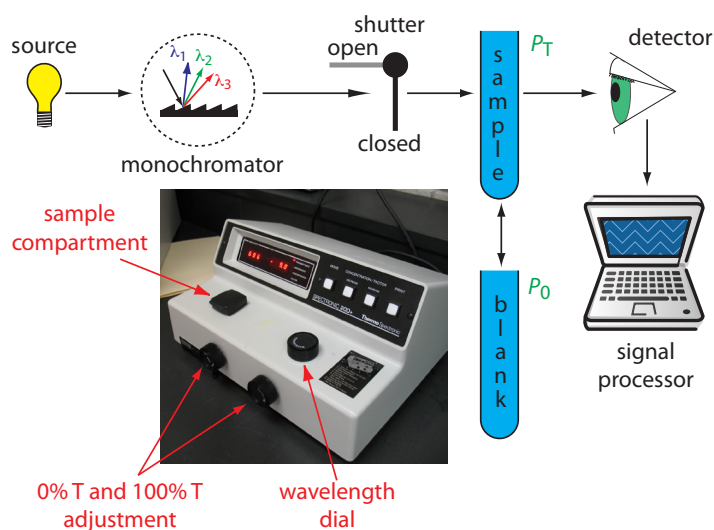
Figure 10.26 Schematic diagram of a fixed-wavelength, single-beam spectrophotometer. The photographic inset shows a typical instrument. The shutter remains closed until the sample or blank is placed in the sample compartment. The analyst manually selects the wavelength by adjusting the wavelength dial. Inset photo modified from: Adi (www.commons.wikipedia.org).

spectrophotometers also are available with effective bandwidths of 2–8 nm. Fixed wavelength single-beam spectrophotometers are not practical for recording spectra because manually adjusting the wavelength and recalibrating the spectrophotometer is awkward and time-consuming. The accuracy of a single-beam spectrophotometer is limited by the stability of its source and detector over time.

Double-Beam Spectrophotometer. The limitations of a fixed-wavelength, single-beam spectrophotometer is minimized by using a DOUBLE-BEAM spectrophotometer (Figure 10.27). A chopper controls the radiation's path, alternating it between the sample, the blank, and a shutter. The signal processor uses the chopper's speed of rotation to resolve the signal that reaches the detector into the transmission of the blank, P_0, and the sample, P_T. By including an opaque surface as a shutter, it also is possible to continuously adjust 0% T. The effective bandwidth of a double-beam spectrophotometer is controlled by adjusting the monochromator's entrance and exit slits. Effective bandwidths of 0.2–3.0 nm are common. A scanning monochromator allows for the automated recording of spectra. Double-beam instruments are more versatile than single-beam instruments, being useful for both quantitative and qualitative analyses, but also are more expensive and not particularly portable.

Diode Array Spectrometer. An instrument with a single detector can monitor only one wavelength at a time. If we replace a single photomultiplier with an array of photodiodes, we can use the resulting detector to record a full spectrum in as little as 0.1 s. In a diode array spectrometer the source
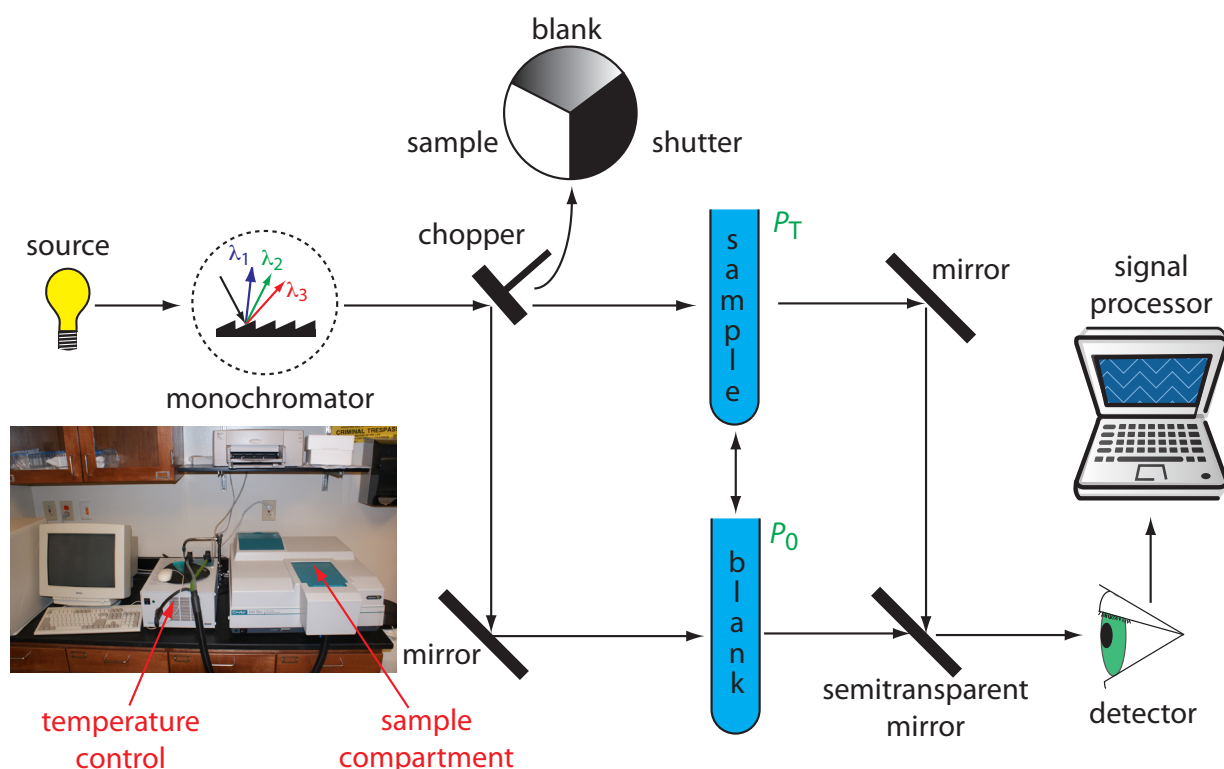
Figure 10.27 Schematic diagram of a scanning, double-beam spectrophotometer. A chopper directs the source's radiation, using a transparent window to pass radiation to the sample and a mirror to reflect radiation to the blank. The chopper's opaque surface serves as a shutter, which allows for a constant adjustment of the spectrophotometer's 0% T. The photographic insert shows a typical instrument. The module in the middle of the photo is a temperature control unit that makes it possible to heat or cool the sample to a constant temperature.

radiation passes through the sample and is dispersed by a grating (Figure 10.28). The photodiode array detector is situated at the grating's focal plane, with each diode recording the radiant power over a narrow range of wavelengths. Because we replace a full monochromator with just a grating, a diode array spectrometer is small and compact.

One advantage of a diode array spectrometer is the speed of data acquisition, which allows us to collect multiple spectra for a single sample. Individual spectra are added and averaged to obtain the final spectrum. This SIGNAL AVERAGING improves a spectrum's signal-to-noise ratio. If we add together n spectra, the sum of the signal at any point, x, increases as nS_x, where S_x is the signal. The noise at any point, N_x, is a random event, which increases as $\sqrt{n}\,N_x$ when we add together n spectra. The SIGNAL-TO-NOISE RATIO after n scans, $(S/N)_n$ is

$$\left(\frac{S}{N}\right)_n = \frac{nS_x}{\sqrt{n}\,N_x} = \sqrt{n}\,\frac{S_x}{N_x}$$

where S_x/N_x is the signal-to-noise ratio for a single scan. The impact of signal averaging is shown in Figure 10.29. The first spectrum shows the signal after one scan, which consists of a single, noisy peak. Signal averaging using 4 scans and 16 scans decreases the noise and improves the signal-to-
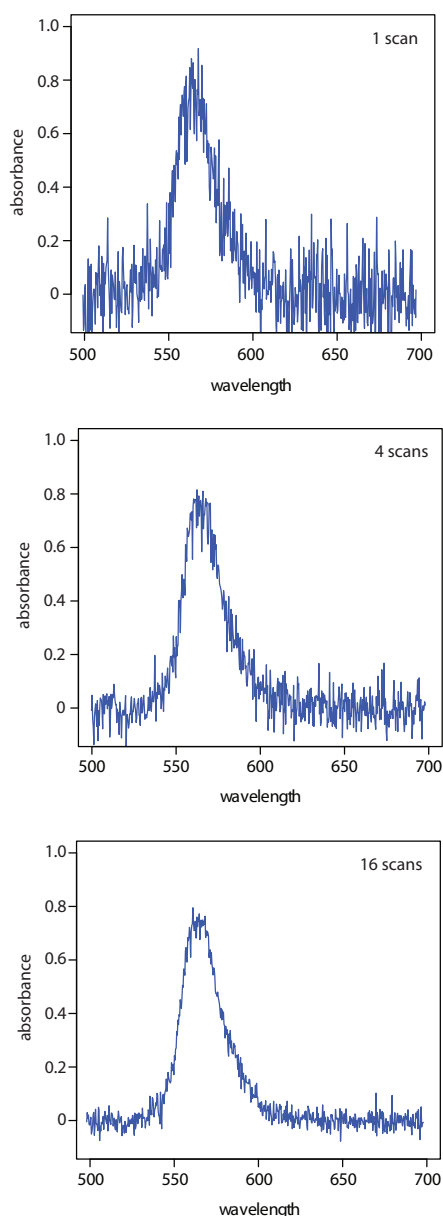
Figure 10.29 Effect of signal averaging on a spectrum's signal-to-noise ratio. From top to bottom: spectrum for a single scan; average spectrum after four scans; and average spectrum after adding 16 scans.

For more details on signals and noise, see Introduction to Signals and Noise by Steven Petrovic, an on-line resource that is part of the Analytical Sciences Digital Library.
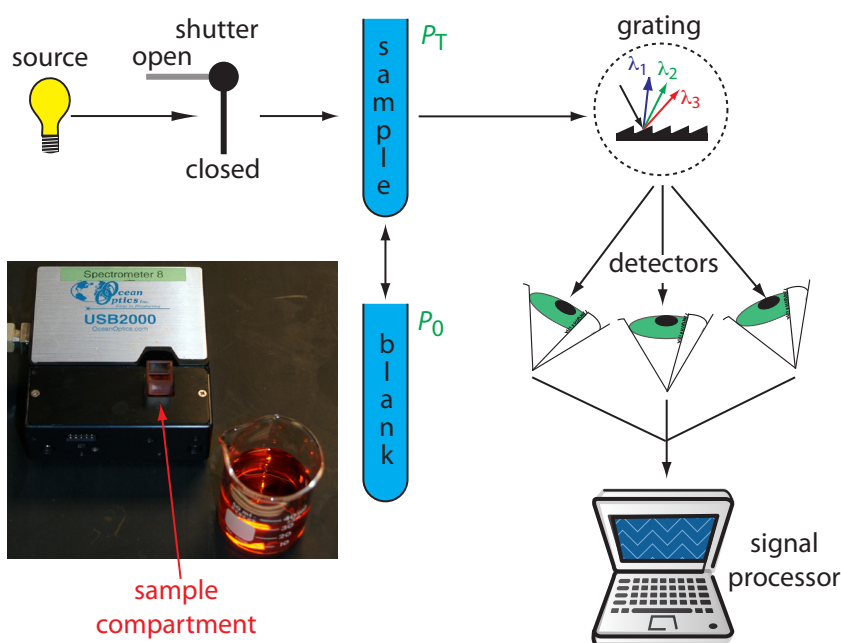
Figure 10.28 Schematic diagram of a diode array spectrophotometer. The photographic insert shows a typical instrument. Note that the 50-mL beaker provides a sense of scale. Because the spectrometer is small and compact, it is easy to transport into the field.

noise ratio. One disadvantage of a photodiode array is that the effective bandwidth per diode is roughly an order of magnitude larger than that for a high quality monochromator.

Sample Cells. The sample compartment provides a light-tight environment that limits stray radiation. Samples normally are in a liquid or solution state, and are placed in cells constructed with UV/Vis transparent materials, such as quartz, glass, and plastic (Figure 10.30). A quartz or fused-silica cell is required when working at a wavelength <300 nm where other materials show a significant absorption. The most common pathlength is 1 cm (10 mm), although cells with shorter (as little as 0.1 cm) and longer pathlengths (up to 10 cm) are available. Longer pathlength cells are useful when analyzing a very dilute solution or for gas samples. The highest quality cells allow the radiation to strike a flat surface at a 90° angle, minimizing the loss of radiation to reflection. A test tube often is used as a sample cell with simple, single-beam instruments, although differences in the cell's pathlength and optical properties add an additional source of error to the analysis.

If we need to monitor an analyte's concentration over time, it may not be possible to remove samples for analysis. This often is the case, for example, when monitoring an industrial production line or waste line, when monitoring a patient's blood, or when monitoring an environmental system, such as stream. With a FIBER-OPTIC PROBE we can analyze samples *in situ*. An example of a remote sensing fiber-optic probe is shown in Figure 10.31. The probe consists of two bundles of fiber-optic cable. One bundle transmits radiation from the source to the probe's tip, which is designed to

Figure 10.30 Examples of sample cells for UV/Vis spectroscopy. From left to right (with path lengths in parentheses): rectangular plastic cuvette (10.0 mm), rectangular quartz cuvette (5.000 mm), rectangular quartz cuvette (1.000 mm), cylindrical quartz cuvette (10.00 mm), cylindrical glass cuvette with quartz windows (100.0 mm). Cells often are available as a matched pair, which is important when using a double-beam instrument.

allow the sample to flow through the sample cell. Radiation from the source passes through the solution and is reflected back by a mirror. The second bundle of fiber-optic cable transmits the nonabsorbed radiation to the wavelength selector. Another design replaces the flow cell shown in Figure 10.31 with a membrane that contains a reagent that reacts with the analyte. When the analyte diffuses into the membrane it reacts with the reagent, producing a product that absorbs UV or visible radiation. The nonabsorbed radiation from the source is reflected or scattered back to the detector. Fiber optic probes that show chemical selectivity are called optrodes.[6]

6   (a) Seitz, W. R. *Anal. Chem.* **1984**, *56*, 16A–34A; (b) Angel, S. M. *Spectroscopy* **1987**, *2(2)*, 38–48.
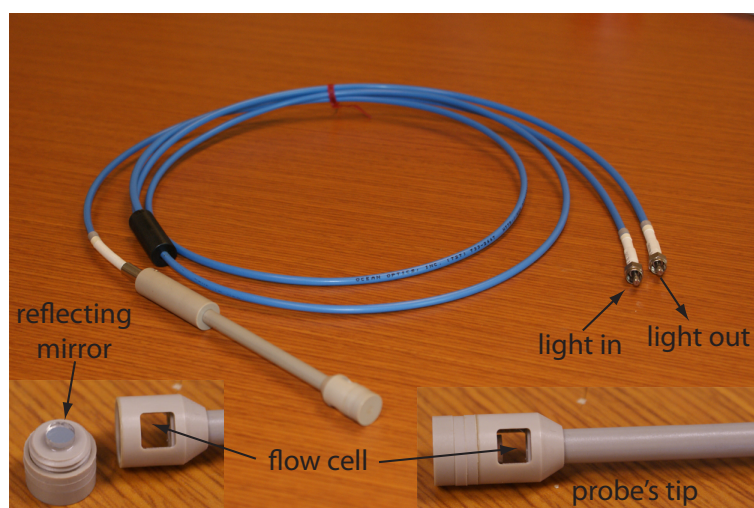
Figure 10.31 Example of a fiber-optic probe. The inset photographs at the bottom of the figure provide close-up views of the probe's flow cell and the reflecting mirror.

INSTRUMENT DESIGNS FOR INFRARED ABSORPTION

Filter Photometer. The simplest instrument for IR absorption spectroscopy is a filter photometer similar to that shown in Figure 10.25 for UV/Vis absorption. These instruments have the advantage of portability and typically are used as dedicated analyzers for gases such as HCN and CO.

Double-beam spectrophotometer. Infrared instruments using a monochromator for wavelength selection use double-beam optics similar to that shown in Figure 10.27. Double-beam optics are preferred over single-beam optics because the sources and detectors for infrared radiation are less stable than those for UV/Vis radiation. In addition, it is easier to correct for the absorption of infrared radiation by atmospheric CO_2 and H_2O vapor when using double-beam optics. Resolutions of 1–3 cm^{-1} are typical for most instruments.

Fourier transform spectrometer. In a Fourier transform infrared spectrometer, or FT–IR, the monochromator is replaced with an interferometer (Figure 10.13). Because an FT-IR includes only a single optical path, it is necessary to collect a separate spectrum to compensate for the absorbance of atmospheric CO_2 and H_2O vapor. This is done by collecting a background spectrum without the sample and storing the result in the instrument's computer memory. The background spectrum is removed from the sample's spectrum by taking the ratio the two signals. In comparison to other instrument designs, an FT–IR provides for rapid data acquisition, which allows for an enhancement in signal-to-noise ratio through signal-averaging.

Sample Cells. Infrared spectroscopy routinely is used to analyze gas, liquid, and solid samples. Sample cells are made from materials, such as NaCl and KBr, that are transparent to infrared radiation. Gases are analyzed using a cell with a pathlength of approximately 10 cm. Longer pathlengths are obtained by using mirrors to pass the beam of radiation through the sample several times.

A liquid samples may be analyzed using a variety of different sample cells (Figure 10.32). For non-volatile liquids a suitable sample is prepared by placing a drop of the liquid between two NaCl plates, forming a thin film that typically is less than 0.01 mm thick. Volatile liquids are placed in a sealed cell to prevent their evaporation.

The analysis of solution samples is limited by the solvent's IR absorbing properties, with CCl_4, CS_2, and $CHCl_3$ being the most common solvents. Solutions are placed in cells that contain two NaCl windows separated by a Teflon spacer. By changing the Teflon spacer, pathlengths from 0.015–1.0 mm are obtained.

Transparent solid samples are analyzed by placing them directly in the IR beam. Most solid samples, however, are opaque, and are first dispersed in a more transparent medium before recording the IR spectrum. If a suitable solvent is available, then the solid is analyzed by preparing a solution and
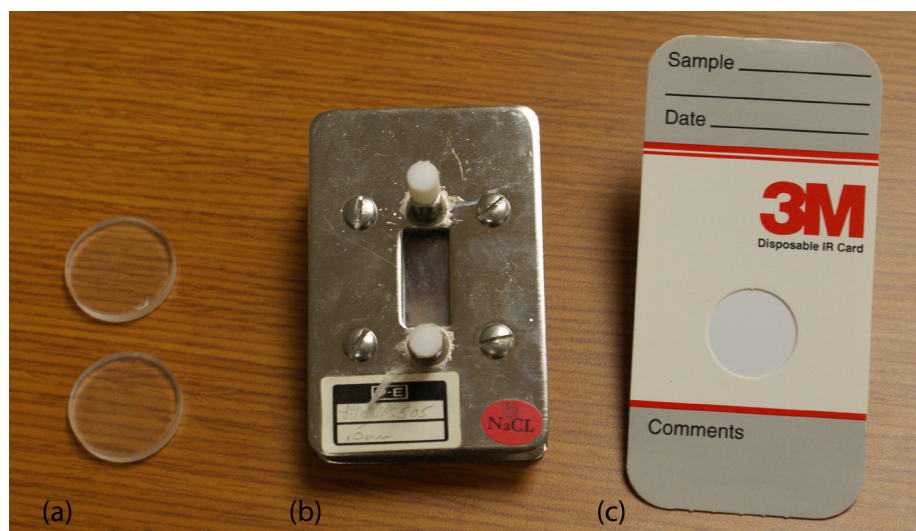
Figure 10.32 Three examples of IR sample cells: (a) NaCl salts plates; (b) fixed pathlength (0.5 mm) sample cell with NaCl windows; (c) disposable card with a polyethylene window that is IR transparent with the exception of strong absorption bands at 2918 cm^{-1} and 2849 cm^{-1}.

analyzing as described above. When a suitable solvent is not available, solid samples are analyzed by preparing a mull of the finely powdered sample with a suitable oil. Alternatively, the powdered sample is mixed with KBr and pressed into an optically transparent pellet.

The analysis of an aqueous sample is complicated by the solubility of the NaCl cell window in water. One approach to obtaining an infrared spectrum of an aqueous solution is to use ATTENUATED TOTAL REFLECTANCE instead of transmission. Figure 10.33 shows a diagram of a typical attenuated total reflectance (ATR) FT–IR instrument. The ATR cell consists of a high refractive index material, such as ZnSe or diamond, sandwiched between a low refractive index substrate and a lower refractive index sample. Radiation from the source enters the ATR crystal where it undergoes a series of internal reflections before exiting the crystal. During each reflection the radiation penetrates into the sample to a depth of a few microns, which results in a selective attenuation of the radiation at those wavelengths where the sample absorbs. ATR spectra are similar, but not identical, to those obtained by measuring the transmission of radiation.

Solid samples also can be analyzed using an ATR sample cell. After placing the solid in the sample slot, a compression tip ensures that it is in contact with the ATR crystal. Examples of solids analyzed by ATR include polymers, fibers, fabrics, powders, and biological tissue samples. Another reflectance method is diffuse reflectance, in which radiation is reflected from a rough surface, such as a powder. Powdered samples are mixed with a non-absorbing material, such as powdered KBr, and the reflected light is collected and analyzed. As with ATR, the resulting spectrum is similar to that obtained by conventional transmission methods.

Further details about these, and other methods for preparing solids for infrared analysis can be found in this chapter's additional resources.
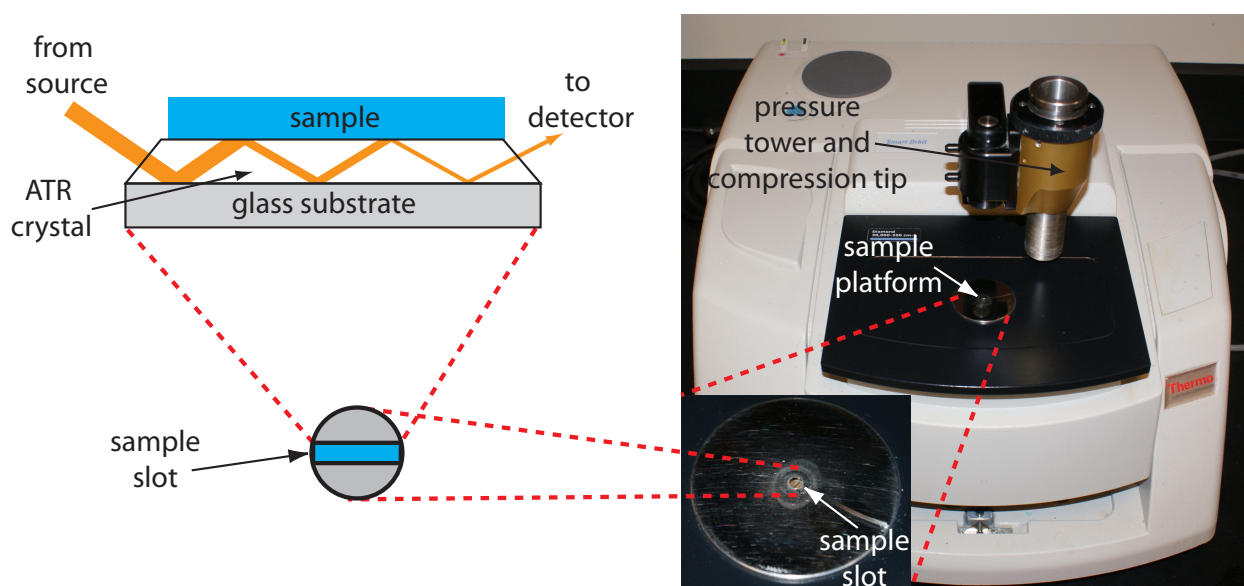
Figure 10.33 FT-IR spectrometer equipped with a diamond ATR sample cell. The inserts show a close-up photo of the sample platform, a sketch of the ATR's sample slot, and a schematic showing how the source's radiation interacts with the sample. The pressure tower is used to ensure proper contact of a solid sample with the ATR crystal.

10C.2   Quantitative Applications

The determination of an analyte's concentration based on its absorption of ultraviolet or visible radiation is one of the most frequently encountered quantitative analytical methods. One reason for its popularity is that many organic and inorganic compounds have strong absorption bands in the UV/Vis region of the electromagnetic spectrum. In addition, if an analyte does not absorb UV/Vis radiation—or if its absorbance is too weak—we often can react it with another species that is strongly absorbing. For example, a dilute solution of Fe^{2+} does not absorb visible light. Reacting Fe^{2+} with *o*-phenanthroline, however, forms an orange–red complex of $Fe(phen)_3^{2+}$ that has a strong, broad absorbance band near 500 nm. An additional advantage to UV/Vis absorption is that in most cases it is relatively easy to adjust experimental and instrumental conditions so that Beer's law is obeyed.

> Figure 10.18 shows the visible spectrum for $Fe(phen)_3^{2+}$.

A quantitative analysis based on the absorption of infrared radiation, although important, is encountered less frequently than with UV/Vis absorption. One reason is the greater tendency for instrumental deviations from Beer's law when using infrared radiation. Because an infrared absorption band is relatively narrow, any deviation due to the lack of monochromatic radiation is more pronounced. In addition, infrared sources are less intense than UV/Vis sources, which makes stray radiation more of a problem. Differences between the pathlengths for samples and for standards when using thin liquid films or KBr pellets are a problem, although an internal standard can correct for any difference in pathlength. Finally, establishing a 100% T ($A = 0$) baseline often is difficult because the optical properties of NaCl sample cells may change significantly with wavelength due to con-

> Another approach is to use a cell with a fixed pathlength, such as that shown in Figure 10.32b.
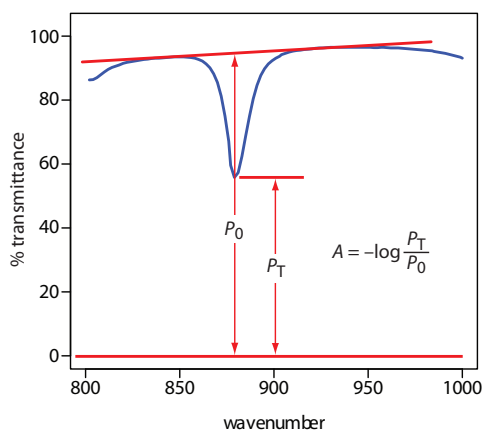
Figure 10.34 Method for determining absorbance from an IR spectrum.

tamination and degradation. We can minimize this problem by measuring absorbance relative to a baseline established for the absorption band. Figure 10.34 shows how this is accomplished.

ENVIRONMENTAL APPLICATIONS

The analysis of waters and wastewaters often relies on the absorption of ultraviolet and visible radiation. Many of these methods are outlined in Table 10.6. Several of these methods are described here in more detail.

Although the quantitative analysis of metals in waters and wastewaters is accomplished primarily by atomic absorption or atomic emission spectroscopy, many metals also can be analyzed following the formation of a colored metal–ligand complex. One advantage to these spectroscopic methods is that they easily are adapted to the analysis of samples in the field using a filter photometer. One ligand used for the analysis of several metals is diphenylthiocarbazone, also known as dithizone. Dithizone is not soluble in water, but when a solution of dithizone in $CHCl_3$ is shaken with an aqueous solution that contains an appropriate metal ion, a colored metal–dithizonate complex forms that is soluble in $CHCl_3$. The selectivity of dithizone is controlled by adjusting the sample's pH. For example, Cd^{2+} is extracted from solutions made strongly basic with NaOH, Pb^{2+} from solutions made basic with an NH_3/NH_4^+ buffer, and Hg^{2+} from solutions that are slightly acidic.

When chlorine is added to water the portion available for disinfection is called the chlorine residual. There are two forms of chlorine residual. The free chlorine residual includes Cl_2, HOCl, and OCl^-. The combined chlorine residual, which forms from the reaction of NH_3 with HOCl, consists of monochloramine, NH_2Cl, dichloramine, $NHCl_2$, and trichloramine, NCl_3. Because the free chlorine residual is more efficient as a disinfectant, there is an interest in methods that can distinguish between the total chlorine residual's different forms. One such method is the leuco crystal violet method. The free residual chlorine is determined by adding leuco crystal violet to the sample, which instantaneously oxidizes to give a blue-colored compound that is monitored at 592 nm. Completing the analysis in less

Atomic absorption is the subject of Section 10D and atomic emission is the subject of Section 10G.
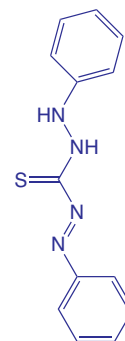
The structure of dithizone is shown above. See Chapter 7 for a discussion of extracting metal ions using dithizone.

Table 10.6 Examples of the Molecular UV/Vis Analysis of Waters and Wastewaters

Analyte	Method	λ (nm)
Trace Metals		
aluminum	react with Eriochrome cyanide R dye at pH 6; forms red to pink complex	535
arsenic	reduce to AsH_3 using Zn and react with silver diethyldithiocarbamate; forms red complex	535
cadmium	extract into $CHCl_3$ containing dithizone from a sample made basic with NaOH; forms pink to red complex	518
chromium	oxidize to Cr(VI) and react with diphenylcarbazide; forms red-violet product	540
copper	react with neocuprine in neutral to slightly acid solution and extract into $CHCl_3/CH_3OH$; forms yellow complex	457
iron	reduce to Fe^{2+} and react with *o*-phenanthroline; forms orange-red complex	510
lead	extract into $CHCl_3$ containing dithizone from sample made basic with NH_3/NH_4^+ buffer; forms cherry red complex	510
manganese	oxidize to MnO_4^- with persulfate; forms purple solution	525
mercury	extract into $CHCl_3$ containing dithizone from acidic sample; forms orange complex	492
zinc	react with zincon at pH 9; forms blue complex	620
Inorganic Nonmetals		
ammonia	reaction with hypochlorite and phenol using a manganous salt catalyst; forms blue indophenol as product	630
cyanide	react with chloroamine-T to form CNCl and then with a pyridine-barbituric acid; forms a red-blue dye	578
fluoride	react with red Zr-SPADNS lake; formation of ZrF_6^{2-} decreases color of the red lake	570
chlorine (residual)	react with leuco crystal violet; forms blue product	592
nitrate	react with Cd to form NO_2^- and then react with sulfanilamide and *N*-(1-napthyl)-ethylenediamine; forms red azo dye	543
phosphate	react with ammonium molybdate and then reduce with $SnCl_2$; forms molybdenum blue	690
Organics		
phenol	react with 4-aminoantipyrine and $K_3Fe(CN)_6$; forms yellow antipyrine dye	460
anionic surfactant	react with cationic methylene blue dye and extract into $CHCl_3$; forms blue ion pair	652

than five minutes prevents a possible interference from the combined chlorine residual. The total chlorine residual (free + combined) is determined by reacting a separate sample with iodide, which reacts with both chlorine residuals to form HOI. When the reaction is complete, leuco crystal violet is added and oxidized by HOI, giving the same blue-colored product. The combined chlorine residual is determined by difference.

The concentration of fluoride in drinking water is determined indirectly by its ability to form a complex with zirconium. In the presence of the dye SPADNS, a solution of zirconium forms a red colored compound, called a lake, that absorbs at 570 nm. When fluoride is added, the formation of the stable ZrF_6^{2-} complex causes a portion of the lake to dissociate, decreasing the absorbance. A plot of absorbance versus the concentration of fluoride, therefore, has a negative slope.

Spectroscopic methods also are used to determine organic constituents in water. For example, the combined concentrations of phenol and ortho- and meta- substituted phenols are determined by using steam distillation to separate the phenols from nonvolatile impurities. The distillate reacts with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of $K_3Fe(CN)_6$ to a yellow colored antipyrine dye. After extracting the dye into $CHCl_3$, its absorbance is monitored at 460 nm. A calibration curve is prepared using only the unsubstituted phenol, C_6H_5OH. Because the molar absorptivity of substituted phenols generally are less than that for phenol, the reported concentration represents the minimum concentration of phenolic compounds.

Molecular absorption also is used for the analysis of environmentally significant airborne pollutants. In many cases the analysis is carried out by collecting the sample in water, converting the analyte to an aqueous form that can be analyzed by methods such as those described in Table 10.6. For example, the concentration of NO_2 is determined by oxidizing NO_2 to NO_3^-. The concentration of NO_3^- is then determined by first reducing it to NO_2^- with Cd, and then reacting NO_2^- with sulfanilamide and N-(1-naphthyl)-ethylenediamine to form a red azo dye. Another important application is the analysis for SO_2, which is determined by collecting the sample in an aqueous solution of $HgCl_4^{2-}$ where it reacts to form $Hg(SO_3)_2^{2-}$. Addition of p-rosaniline and formaldehyde produces a purple complex that is monitored at 569 nm. Infrared absorption is useful for the analysis of organic vapors, including HCN, SO_2, nitrobenzene, methyl mercaptan, and vinyl chloride. Frequently, these analyses are accomplished using portable, dedicated infrared photometers.

CLINICAL APPLICATIONS

The analysis of clinical samples often is complicated by the complexity of the sample's matrix, which may contribute a significant background absorption at the desired wavelength. The determination of serum barbiturates provides one example of how this problem is overcome. The barbiturates are

In Chapter 9 we explored how the total chlorine residual can be determined by a redox titration; see Representative Method 9.3 for further details. The method described here allows us to divide the total chlorine residual into its component parts.

SPADNS, the structure of which is shown below, is an abbreviation for the sodium salt of 2-(4-sulfophenylazo)-1,8-dihydroxy-3,6-napthalenedisulfonic acid, which is a mouthful to say.
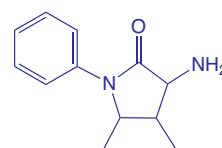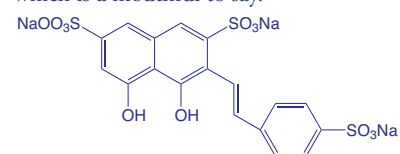
4-aminoantipyrene
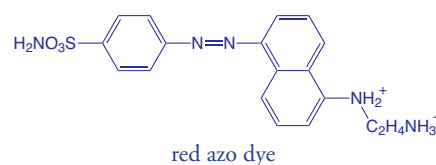
red azo dye

Table 10.7   Examples of the Molecular UV/Vis Analysis of Clinical Samples

Analyte	Method	λ(nm)
total serum protein	react with NaOH and Cu^{2+}; forms blue-violet complex	540
serum cholesterol	react with Fe^{3+} in presence of isopropanol, acetic acid, and H_2SO_4; forms blue-violet complex	540
uric acid	react with phosphotungstic acid; forms tungsten blue	710
serum barbiturates	extract into $CHCl_3$ to isolate from interferents and then extract into 0.45 M NaOH	260
glucose	react with o-toludine at $100\,^{\circ}C$; forms blue-green complex	630
protein-bound iodine	decompose protein to release iodide, which catalyzes redox reaction between Ce^{3+} and As^{3+}; forms yellow colored Ce^{4+}	420

first extracted from a sample of serum with $CHCl_3$ and then extracted from the $CHCl_3$ into 0.45 M NaOH (pH $\approx$ 13). The absorbance of the aqueous extract is measured at 260 nm, and includes contributions from the barbiturates as well as other components extracted from the serum sample. The pH of the sample is then lowered to approximately 10 by adding NH_4Cl and the absorbance remeasured. Because the barbiturates do not absorb at this pH, we can use the absorbance at pH 10, $A_{pH\,10}$, to correct the absorbance at pH 13, $A_{pH\,13}$

$$A_{barb} = A_{pH\,13} - \frac{V_{samp} + V_{NH_4Cl}}{V_{samp}} \times A_{pH\,10}$$

where A_{barb} is the absorbance due to the serum barbiturates and V_{samp} and V_{NH_4Cl} are the volumes of sample and NH_4Cl, respectively. Table 10.7 provides a summary of several other methods for analyzing clinical samples.

INDUSTRIAL ANALYSIS

UV/Vis molecular absorption is used for the analysis of a diverse array of industrial samples including pharmaceuticals, food, paint, glass, and metals. In many cases the methods are similar to those described in Table 10.6 and in Table 10.7. For example, the amount of iron in food is determined by bringing the iron into solution and analyzing using the o-phenanthroline method listed in Table 10.6.

Many pharmaceutical compounds contain chromophores that make them suitable for analysis by UV/Vis absorption. Products analyzed in this fashion include antibiotics, hormones, vitamins, and analgesics. One example of the use of UV absorption is in determining the purity of aspirin tablets, for which the active ingredient is acetylsalicylic acid. Salicylic acid, which is produced by the hydrolysis of acetylsalicylic acid, is an undesirable impurity in aspirin tablets, and should not be present at more than 0.01% w/w. Samples are screened for unacceptable levels of salicylic acid by monitoring the absorbance at a wavelength of 312 nm. Acetylsalicylic acid absorbs at 280 nm, but absorbs poorly at 312 nm. Conditions for preparing

the sample are chosen such that an absorbance of greater than 0.02 signifies an unacceptable level of salicylic acid.

FORENSIC APPLICATIONS

UV/Vis molecular absorption routinely is used for the analysis of narcotics and for drug testing. One interesting forensic application is the determination of blood alcohol using the Breathalyzer test. In this test a 52.5-mL breath sample is bubbled through an acidified solution of $K_2Cr_2O_7$, which oxidizes ethanol to acetic acid. The concentration of ethanol in the breath sample is determined by a decrease in the absorbance at 440 nm where the dichromate ion absorbs. A blood alcohol content of 0.10%, which is above the legal limit, corresponds to 0.025 mg of ethanol in the breath sample.

DEVELOPING A QUANTITATIVE METHOD FOR A SINGLE COMPONENT

To develop a quantitative analytical method, the conditions under which Beer's law is obeyed must be established. First, the most appropriate wavelength for the analysis is determined from an absorption spectrum. In most cases the best wavelength corresponds to an absorption maximum because it provides greater sensitivity and is less susceptible to instrumental limitations. Second, if the instrument has adjustable slits, then an appropriate slit width is chosen. The absorption spectrum also aids in selecting a slit width by choosing a width that is narrow enough to avoid instrumental limitations to Beer's law, but wide enough to increase the throughput of source radiation. Finally, a calibration curve is constructed to determine the range of concentrations for which Beer's law is valid. Additional considerations that are important in any quantitative method are the effect of potential interferents and establishing an appropriate blank.

Representative Method 10.1

Determination of Iron in Water and Wastewater

DESCRIPTION OF METHOD

Iron in the +2 oxidation state reacts with *o*-phenanthroline to form the orange-red $Fe(phen)_3^{2+}$ complex. The intensity of the complex's color is independent of the solution's acidity between a pH of 3 and 9. Because the complex forms more rapidly at lower pH levels, the reaction usually is carried out within a pH range of 3.0–3.5. Any iron present in the +3 oxidation state is reduced with hydroxylamine before adding *o*-phenanthroline. The most important interferents are strong oxidizing agents, polyphosphates, and metal ions such as Cu^{2+}, Zn^{2+}, Ni^{2+}, and Cd^{2+}. An interference from oxidizing agents is minimized by adding an excess of hydroxylamine, and an interference from polyphosphate is minimized by boiling the sample in the presence of acid. The absorbance of samples and standards are measured at a wavelength of 510 nm using a 1-cm cell

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of iron in water and wastewater provides an instructive example of a typical procedure. The description here is based on Method 3500- Fe B as published in *Standard Methods for the Examination of Water and Wastewater*, 20th Ed., American Public Health Association: Washington, D. C., 1998.

Figure 10.18 shows the visible spectrum for $Fe(phen)_3^{2+}$.
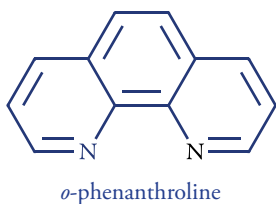
(longer pathlength cells also may be used). Beer's law is obeyed for concentrations of within the range of 0.2–4.0 mg Fe/L

PROCEDURE

For a sample that contains less than 2 mg Fe/L, directly transfer a 50-mL portion to a 125-mL Erlenmeyer flask. Samples that contain more than 2 mg Fe/L are diluted before acquiring the 50-mL portion. Add 2 mL of concentrated HCl and 1 mL of hydroxylamine to the sample. Bring the solution to a boil and continue boiling until the solution's volume is reduced to between 15 and 20 mL. After cooling to room temperature, transfer the solution to a 50-mL volumetric flask, add 10 mL of an ammonium acetate buffer, 2 mL of a 1000 ppm solution of *o*-phenanthroline, and dilute to volume. Allow 10–15 minutes for color development before measuring the absorbance, using distilled water to set 100% T. Calibration standards, including a blank, are prepared by the same procedure using a stock solution that contains a known concentration of Fe^{2+}.

QUESTIONS

1. Explain why strong oxidizing agents are interferents and why an excess of hydroxylamine prevents the interference.

   A strong oxidizing agent will oxidize some Fe^{2+} to Fe^{3+}. Because $Fe(phen)_3^{3+}$ does not absorb as strongly as $Fe(phen)_3^{2+}$, the absorbance is smaller than expected, which produces a negative determinate error. The excess hydroxylamine reacts with the oxidizing agents, removing them from the solution.

2. The color of the complex is stable between pH levels of 3 and 9. What are some possible complications at more acidic or at more basic pH's?

   Because *o*-phenanthroline is a weak base, its conditional formation constant for $Fe(phen)_3^{2+}$ becomes smaller at more acidic pH levels, where *o*-phenanthroline is present in its protonated form. The result is a decrease in absorbance and a less sensitive analytical method. When the pH is greater than 9, competition between OH^- and *o*-phenanthroline for Fe^{2+} also decreases the absorbance. In addition, if the pH is sufficiently basic there is a risk that the iron will precipitate as $Fe(OH)_2$.

3. Cadmium is an interferent because it forms a precipitate with *o*-phenanthroline. What effect does the formation of precipitate have on the determination of iron?

   Because *o*-phenanthroline is present in large excess (2000 µg of *o*-phenanthroline for 100 µg of Fe^{2+}), it is not likely that the interference is due to an insufficient amount of *o*-phenanthroline being available to react with the Fe^{2+}. The presence of a precipitate in the sample cell results in the scattering of radiation, which causes an

o-phenanthroline

In Chapter 9 we saw the same effect of pH on the complexation reactions between EDTA and metal ions.

Although scattering is a problem here, it can serve as the basis of a useful analytical method. See Section 10H for further details.

apparent increase in absorbance. Because the measured absorbance increases, the reported concentration is too high.

4. Even high quality ammonium acetate contains a significant amount of iron. Why is this source of iron not a problem?

Because all samples and standards are prepared using the same volume of ammonium acetate buffer, the contribution of this source of iron is accounted for by the calibration curve's reagent blank.

QUANTITATIVE ANALYSIS FOR A SINGLE ANALYTE

To determine the concentration of an analyte we measure its absorbance and apply Beer's law using any of the standardization methods described in Chapter 5. The most common methods are a normal calibration curve using external standards and the method of standard additions. A single point standardization also is possible, although we must first verify that Beer's law holds for the concentration of analyte in the samples and the standard.

Example 10.5

The determination of iron in an industrial waste stream is carried out by the *o*-phenanthroline described in Representative Method 10.1. Using the data in the following table, determine the mg Fe/L in the waste stream.

mg Fe/L	absorbance
0.00	0.000
1.00	0.183
2.00	0.364
3.00	0.546
4.00	0.727
sample	0.269

SOLUTION

Linear regression of absorbance versus the concentration of Fe in the standards gives the calibration curve shown to the right with the following equation.

$$A = 0.0006 + (0.1817 \text{ mg}^{-1}\text{L}) \times (\text{mg Fe/L})$$

Substituting the sample's absorbance into the calibration equation gives the concentration of Fe in the waste stream as 1.48 mg Fe/L
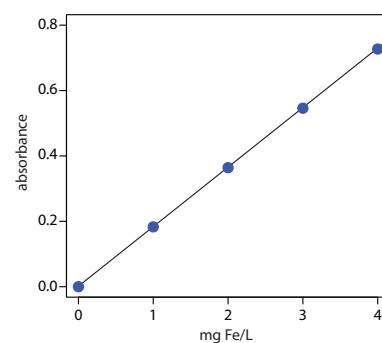
QUANTITATIVE ANALYSIS OF MIXTURES

Suppose we need to determine the concentration of two analytes, X and Y, in a sample. If each analyte has a wavelength where the other analyte does not absorb, then we can proceed using the approach in Example 10.5.

Practice Exercise 10.5

The concentration of Cu^{2+} in a sample is determined by reacting it with the ligand cuprizone and measuring its absorbance at 606 nm in a 1.00-cm cell. When a 5.00-mL sample is treated with cuprizone and diluted to 10.00 mL, the resulting solution has an absorbance of 0.118. A second 5.00-mL sample is mixed with 1.00 mL of a 20.00 mg/L standard of Cu^{2+}, treated with cuprizone and diluted to 10.00 mL, giving an absorbance of 0.162. Report the mg Cu^{2+}/L in the sample.

Click here to review your answer to this exercise.

Unfortunately, UV/Vis absorption bands are so broad that frequently it is not possible to find suitable wavelengths. Because Beer's law is additive the mixture's absorbance, A_{mix}, is

$$(A_{mix})_{\lambda_1} = (\varepsilon_X)_{\lambda_1} b C_X + (\varepsilon_Y)_{\lambda_1} b C_Y \qquad 10.11$$

where λ_1 is the wavelength at which we measure the absorbance. Because equation 10.11 includes terms for the concentration of both X and Y, the absorbance at one wavelength does not provide enough information to determine either C_X or C_Y. If we measure the absorbance at a second wavelength

$$(A_{mix})_{\lambda_2} = (\varepsilon_X)_{\lambda_2} b C_X + (\varepsilon_Y)_{\lambda_2} b C_Y \qquad 10.12$$

then we can determine C_X and C_Y by solving simultaneously equation 10.11 and equation 10.12. Of course, we also must determine the value for ε_X and ε_Y at each wavelength. For a mixture of n components, we must measure the absorbance at n different wavelengths.

Example 10.6

The concentrations of Fe^{3+} and Cu^{2+} in a mixture are determined following their reaction with hexacyanoruthenate (II), $Ru(CN)_6^{4-}$, which forms a purple-blue complex with Fe^{3+} ($\lambda_{max}=550$ nm) and a pale-green complex with Cu^{2+} ($\lambda_{max}=396$ nm).[7] The molar absorptivities (M^{-1} cm^{-1}) for the metal complexes at the two wavelengths are summarized in the following table.

	ε_{550}	ε_{396}
Fe^{3+}	9970	84
Cu^{2+}	34	856

When a sample that contains Fe^{3+} and Cu^{2+} is analyzed in a cell with a pathlength of 1.00 cm, the absorbance at 550 nm is 0.183 and the absorbance at 396 nm is 0.109. What are the molar concentrations of Fe^{3+} and Cu^{2+} in the sample?

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7   DiTusa, M. R.; Schlit, A. A. *J. Chem. Educ.* **1985**, *62*, 541–542.

SOLUTION

Substituting known values into equation 10.11 and equation 10.12 gives

$$A_{550} = 0.183 = 9970C_{Fe} + 34C_{Cu}$$

$$A_{396} = 0.109 = 84C_{Fe} + 856C_{Cu}$$

To determine C_{Fe} and C_{Cu} we solve the first equation for C_{Cu}

$$C_{Cu} = \frac{0.183 - 9970C_{Fe}}{34}$$

and substitute the result into the second equation.

$$0.109 = 84C_{Fe} + 856 \times \frac{0.183 - 9970C_{Fe}}{34}$$

$$= 4.607 - (2.51 \times 10^{5})\,C_{Fe}$$

Solving for C_{Fe} gives the concentration of Fe^{3+} as 1.8×10^{-5} M. Substituting this concentration back into the equation for the mixture's absorbance at 396 nm gives the concentration of Cu^{2+} as 1.3×10^{-4} M.

To obtain results with good accuracy and precision the two wavelengths should be selected so that $\varepsilon_X > \varepsilon_Y$ at one wavelength and $\varepsilon_X < \varepsilon_Y$ at the other wavelength. It is easy to appreciate why this is true. Because the absorbance at each wavelength is dominated by one analyte, any uncertainty in the concentration of the other analyte has less of an impact. Figure 10.35 shows that the choice of wavelengths for Practice Exercise 10.6 are reasonable. When the choice of wavelengths is not obvious, one method for locating the optimum wavelengths is to plot $\varepsilon_X/\varepsilon_Y$ as function of wavelength, and determine the wavelengths where $\varepsilon_X/\varepsilon_Y$ reaches maximum and minimum values.[8]

When the analyte's spectra overlap severely, such that $\varepsilon_X \approx \varepsilon_Y$ at all wavelengths, other computational methods may provide better accuracy and precision. In a multiwavelength linear regression analysis, for example, a mixture's absorbance is compared to that for a set of standard solutions at several wavelengths.[9] If A_{SX} and A_{SY} are the absorbance values for standard solutions of components X and Y at any wavelength, then

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Another approach is to multiply the first equation by 856/34 giving

$$4.607 = 251009C_{Fe} + 856C_{Cu}$$

Subtracting the second equation from this equation

$$4.607 = 251009C_{Fe} + 856C_{Cu}$$

$$-\,0.109 = 84C_{Fe} + 856C_{Cu}$$

$$\overline{4.498 = 250925C_{Fe}}$$

we find that C_{Fe} is 1.8×10^{-5}. Having determined C_{Fe} we can substitute back into one of the other equations to solve for C_{Cu}, which is 1.3×10^{-5}.

For example, in Example 10.6 the molar absorptivity for Fe^{3+} at 550 nm is 119× that for Cu^{2+}, and the molar absorptivity for Cu^{2+} at 396 nm is 10.2× that for Fe^{3+}.

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8 Mehra, M. C.; Rioux, J. *J. Chem. Educ.* **1982**, *59*, 688–689.
9 Blanco, M.; Iturriaga, H.; Maspoch, S.; Tarin, P. *J. Chem. Educ.* **1989**, *66*, 178–180.

Practice Exercise 10.6

The absorbance spectra for Cr^{3+} and Co^{2+} overlap significantly. To determine the concentration of these analytes in a mixture, its absorbance is measured at 400 nm and at 505 nm, yielding values of 0.336 and 0.187, respectively. The individual molar absorptivities (M^{-1} cm^{-1}) for Cr^{3+} are 15.2 at 400 nm and 0.533 at 505 nm; the values for Co^{2+} are 5.60 at 400 nm and 5.07 at 505 nm.

Click here to review your answer to this exercise.

Figure 10.35 Visible absorption spectra for **0.0250 M Cr³⁺**, **0.0750 M Co²⁺**, and for a **mixture of Cr³⁺ and Co²⁺**. The two wavelengths used to analyze the mixture of Cr^{3+} and Co^{2+} are shown by the dashed lines. The data for the two standard solutions are from reference 7.
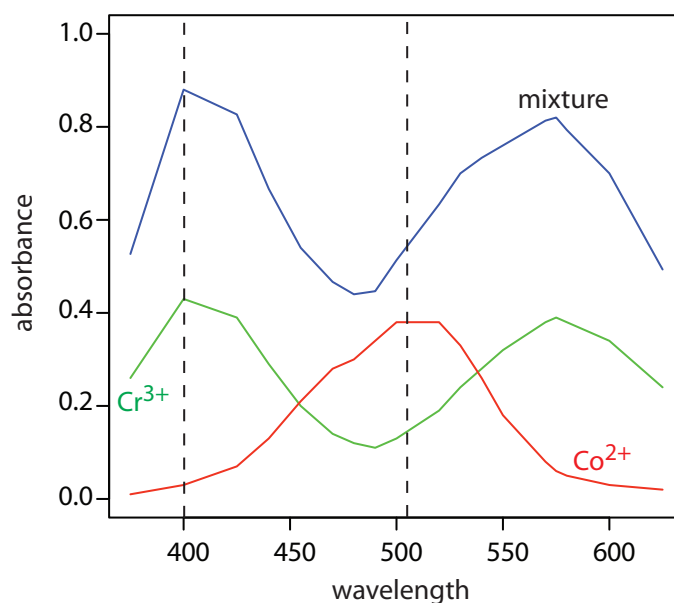
$$A_{SX} = \varepsilon_X b C_{SX} \qquad\qquad 10.13$$

$$A_{SY} = \varepsilon_Y b C_{SY} \qquad\qquad 10.14$$

where C_{SX} and C_{SY} are the known concentrations of X and Y in the standard solutions. Solving equation 10.13 and equation 10.14 for ε_X and for ε_Y, substituting into equation 10.11, and rearranging, gives

$$\frac{A_{mix}}{A_{SX}} = \frac{C_X}{C_{SX}} + \frac{C_Y}{C_{SY}} \times \frac{A_{SY}}{A_{SX}}$$

To determine C_X and C_Y the mixture's absorbance and the absorbances of the standard solutions are measured at several wavelengths. Graphing A_{mix}/A_{SX} versus A_{SY}/A_{SX} gives a straight line with a slope of C_Y/C_{SY} and a y-intercept of C_X/C_{SX}. This approach is particularly helpful when it is not possible to find wavelengths where $\varepsilon_X > \varepsilon_Y$ and $\varepsilon_X < \varepsilon_Y$.

The approach outlined here for a multi-wavelength linear regression uses a single standard solution for each analyte. A more rigorous approach uses multiple standards for each analyte. The math behind the analysis of such data—which we call a multiple linear regression—is beyond the level of this text. For more details about multiple linear regression see Brereton, R. G. *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*, Wiley: Chichester, England, 2003.

Example 10.7

Figure 10.35 shows visible absorbance spectra for a standard solution of 0.0250 M Cr³⁺, a standard solution of 0.0750 M Co²⁺, and a mixture that contains unknown concentrations of each ion. The data for these spectra are shown here.[10]

λ (nm)	A_{Cr}	A_{Co}	A_{mix}	λ (nm)	A_{Cr}	A_{Co}	A_{mix}
375	0.26	0.01	0.53	520	0.19	0.38	0.63
400	0.43	0.03	0.88	530	0.24	0.33	0.70
425	0.39	0.07	0.83	540	0.28	0.26	0.73
440	0.29	0.13	0.67	550	0.32	0.18	0.76
455	0.20	0.21	0.54	570	0.38	0.08	0.81

10 The data for the two standards are from Brewer, S. *Solving Problems in Analytical Chemistry*, John Wiley & Sons: New York, 1980.

λ (nm)	A_{Cr}	A_{Co}	A_{mix}	λ (nm)	A_{Cr}	A_{Co}	A_{mix}
470	0.14	0.28	0.47	575	0.39	0.06	0.82
480	0.12	0.30	0.44	580	0.38	0.05	0.79
490	0.11	0.34	0.45	600	0.34	0.03	0.70
500	0.13	0.38	0.51	625	0.24	0.02	0.49

Use a multiwavelength regression analysis to determine the composition of the unknown.

SOLUTION

First we need to calculate values for A_{mix}/A_{SX} and for A_{SY}/A_{SX}. Let's define X as Co^{2+} and Y as Cr^{3+}. For example, at a wavelength of 375 nm A_{mix}/A_{SX} is 0.53/0.01, or 53 and A_{SY}/A_{SX} is 0.26/0.01, or 26. Completing the calculation for all wavelengths and graphing A_{mix}/A_{SX} versus A_{SY}/A_{SX} gives the calibration curve shown in Figure 10.36. Fitting a straight-line to the data gives a regression model of

$$\frac{A_{mix}}{A_{SX}} = 0.636 + 2.01 \times \frac{A_{SY}}{A_{SX}}$$

Using the y-intercept, the concentration of Co^{2+} is

$$\frac{C_X}{C_{SX}} = \frac{[Co^{2+}]}{0.0750 \text{ M}} = 0.636$$

or $[Co^{2+}] = 0.048$ M; using the slope the concentration of Cr^{3+} is

$$\frac{C_Y}{C_{SY}} = \frac{[Cr^{3+}]}{0.0250 \text{ M}} = 2.01$$

or $[Cr^{3+}] = 0.050$ M.
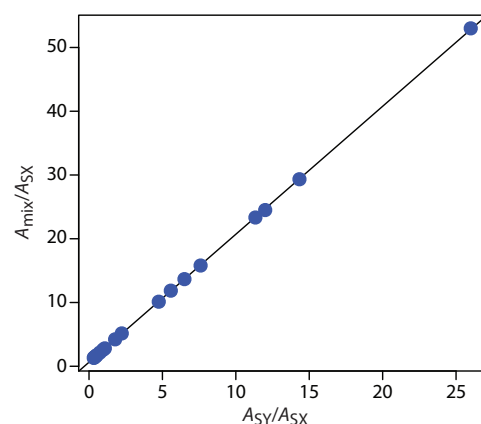
Figure 10.36 Multiwavelength linear regression analysis for the data in Example 10.7.

Practice Exercise 10.7

A mixture of MnO_4^- and $Cr_2O_7^{2-}$, and standards of 0.10 mM $KMnO_4$ and of 0.10 mM $K_2Cr_2O_7$ give the results shown in the following table. Determine the composition of the mixture. The data for this problem is from Blanco, M. C.; Iturriaga, H.; Maspoch, S.; Tarin, P. *J. Chem. Educ.* **1989**, *66*, 178–180.

λ (nm)	A_{Mn}	A_{Cr}	A_{mix}
266	0.042	0.410	0.766
288	0.082	0.283	0.571
320	0.168	0.158	0.422
350	0.125	0.318	0.672
360	0.056	0.181	0.366

Click here to review your answer to this exercise.

There are many additional ways to analyze mixtures spectrophotometrically, including generalized standard additions, H-point standard additions, and principal component regression to name three. Consult the chapter's additional resources for further information.

10C.3   Qualitative Applications

As discussed in Section 10B.1, ultraviolet, visible, and infrared absorption bands result from the absorption of electromagnetic radiation by specific valence electrons or bonds. The energy at which the absorption occurs, and the intensity of that absorption, is determined by the chemical environment of the absorbing moiety. For example, benzene has several ultraviolet absorption bands due to $\pi \rightarrow \pi^*$ transitions. The position and intensity of two of these bands, 203.5 nm ($\varepsilon = 7400$ M^{-1} cm^{-1}) and 254 nm ($\varepsilon = 204$ M^{-1} cm^{-1}), are sensitive to substitution. For benzoic acid, in which a carboxylic acid group replaces one of the aromatic hydrogens, the two bands shift to 230 nm ($\varepsilon = 11\,600$ M^{-1} cm^{-1}) and 273 nm ($\varepsilon = 970$ M^{-1} cm^{-1}). A variety of rules have been developed to aid in correlating UV/Vis absorption bands to chemical structure. Similar correlations are available for infrared absorption bands. For example a carbonyl's C=O stretch is sensitive to adjacent functional groups, appearing at 1650 cm^{-1} for acids, 1700 cm^{-1} for ketones, and 1800 cm^{-1} for acid chlorides. The interpretation of UV/Vis and IR spectra receives adequate coverage elsewhere in the chemistry curriculum, notably in organic chemistry, and is not considered further in this text.

With the availability of computerized data acquisition and storage it is possible to build digital libraries of standard reference spectra. The identity of an a unknown compound often can be determined by comparing its spectrum against a library of reference spectra, a process known as SPECTRAL SEARCHING. Comparisons are made using an algorithm that calculates the cumulative difference between the sample's spectrum and a reference spectrum. For example, one simple algorithm uses the following equation

$$D = \sum_{i=i}^{n} \left| (A_{sample})_i - (A_{reference})_i \right|$$

where D is the cumulative difference, A_{sample} is the sample's absorbance at wavelength or wavenumber i, $A_{reference}$ is the absorbance of the reference compound at the same wavelength or wavenumber, and n is the number of digitized points in the spectra. The cumulative difference is calculated for each reference spectrum. The reference compound with the smallest value of D is the closest match to the unknown compound. The accuracy of spectral searching is limited by the number and type of compounds included in the library, and by the effect of the sample's matrix on the spectrum.

Another advantage of computerized data acquisition is the ability to subtract one spectrum from another. When coupled with spectral searching it is possible to determine the identity of several components in a sample without the need of a prior separation step by repeatedly searching and subtracting reference spectra. An example is shown in Figure 10.37 in which the composition of a two-component mixture is determined by successive searching and subtraction. Figure 10.37a shows the spectrum of the mixture. A search of the spectral library selects cocaine·HCl (Figure 10.37b) as

Figure 10.37 Identifying the components of a mixture by spectral searching and subtracting. (a) IR spectrum of the mixture; (b) Reference IR spectrum of cocaine·HCl; (c) Result of subtracting the spectrum of cocaine·HCl from the mixture's spectrum; (d) Reference IR spectrum of mannitol; and (e) The residual spectrum after removing mannitol's contribution to the mixture's spectrum.

IR spectra traditionally are displayed using percent transmittance, %T, along the *y*-axis (for example, see Figure 10.16). Because absorbance—not percent transmittance—is a linear function of concentration, spectral searching and spectral subtraction, is easier to do when displaying absorbance on the *y*-axis.

a likely component of the mixture. Subtracting the reference spectrum for cocaine·HCl from the mixture's spectrum leaves a result (Figure 10.37c) that closely matches mannitol's reference spectrum (Figure 10.37d). Subtracting the reference spectrum for mannitol leaves a small residual signal (Figure 10.37e).

10C.4 Characterization Applications

Molecular absorption, particularly in the UV/Vis range, has been used for a variety of different characterization studies, including determining the stoichiometry of metal–ligand complexes and determining equilibrium constants. Both of these examples are examined in this section.

STOICHIOMETRY OF A METAL-LIGAND COMPLEX

We can determine the stoichiometry of the metal–ligand complexation reaction

$$M + yL \rightleftharpoons ML_y$$
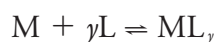
using one of three methods: the method of continuous variations, the mole-ratio method, and the slope-ratio method. Of these approaches, the METHOD OF CONTINUOUS VARIATIONS, also called Job's method, is the most popular. In this method a series of solutions is prepared such that the total moles of metal and of ligand, n_{total}, in each solution is the same. If $(n_M)_i$ and $(n_L)_i$ are, respectively, the moles of metal and ligand in solution i, then

$$n_{total} = (n_M)_i + (n_L)_i$$

The relative amount of ligand and metal in each solution is expressed as the mole fraction of ligand, $(X_L)_i$, and the mole fraction of metal, $(X_M)_i$,

$$(X_L)_i = \frac{(n_L)_i}{n_{total}}$$

$$(X_M)_i = 1 - \frac{(n_L)_i}{n_{total}} = \frac{(n_M)_i}{n_{total}}$$

The concentration of the metal–ligand complex in any solution is determined by the limiting reagent, with the greatest concentration occurring when the metal and the ligand are mixed stoichiometrically. If we monitor the complexation reaction at a wavelength where only the metal–ligand complex absorbs, a graph of absorbance versus the mole fraction of ligand has two linear branches—one when the ligand is the limiting reagent and a second when the metal is the limiting reagent. The intersection of the two branches represents a stoichiometric mixing of the metal and the ligand. We use the mole fraction of ligand at the intersection to determine the value of y for the metal–ligand complex ML_y.

You also can plot the data as absorbance versus the mole fraction of metal. In this case, y is equal to $(1-X_M)/X_M$.

$$y = \frac{n_L}{n_M} = \frac{X_L}{X_M} = \frac{X_L}{1 - X_L}$$

Example 10.8

To determine the formula for the complex between Fe^{2+} and *o*-phenanthroline, a series of solutions is prepared in which the total concentration of metal and ligand is held constant at 3.15×10^{-4} M. The absorbance of each solution is measured at a wavelength of 510 nm. Using the following data, determine the formula for the complex.

X_L	absorbance	X_L	absorbance
0.000	0.000	0.600	0.693
0.100	0.116	0.700	0.809
0.200	0.231	0.800	0.693
0.300	0.347	0.900	0.347
0.400	0.462	1.000	0.000
0.500	0.578		

To prepare these solutions I first prepared a solution of 3.15×10^{-4} M Fe^{2+} and a solution of 3.15×10^{-4} M o-phenanthroline. Because the two stock solutions have the same concentration, diluting a portion of one solution with the other solution gives a mixture in which the combined concentration of o-phenanthroline and Fe^{2+} is 3.15×10^{-4} M. Because each solution has the same volume, each solution also contains the same total moles of metal and ligand.

SOLUTION

A plot of absorbance versus the mole fraction of ligand is shown in Figure 10.38. To find the maximum absorbance, we extrapolate the two linear portions of the plot. The two lines intersect at a mole fraction of ligand of 0.75. Solving for y gives

$$y = \frac{X_L}{1 - X_L} = \frac{0.75}{1 - 0.75} = 3$$

The formula for the metal–ligand complex is $Fe(phen)_3^{2+}$.
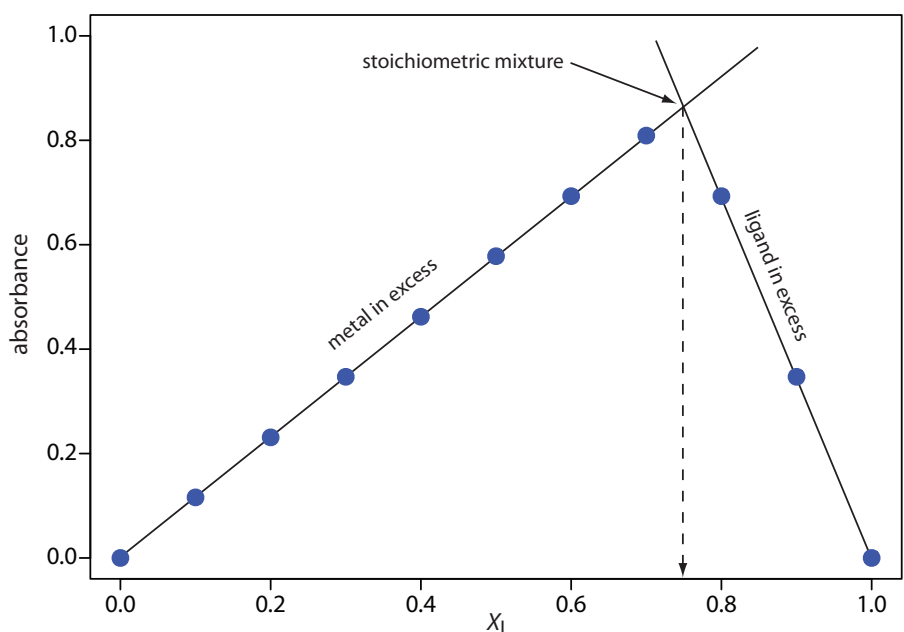
Figure 10.38 Continuous variations plot for Example 10.8. The photo shows the solutions used to gather the data. Each solution is displayed directly below its corresponding point on the continuous variations plot.

Practice Exercise 10.8

Use the continuous variations data in the following table to determine the formula for the complex between Fe^{2+} and SCN^-. The data for this problem is adapted from Meloun, M.; Havel, J.; Högfeldt, E. *Computation of Solution Equilibria*, Ellis Horwood: Chichester, England, 1988, p. 236.

X_L	absorbance	X_L	absorbance	X_L	absorbance	X_L	absorbance
0.0200	0.068	0.2951	0.670	0.5811	0.790	0.8923	0.325
0.0870	0.262	0.3887	0.767	0.6860	0.701	0.9787	0.071
0.1792	0.471	0.4964	0.807	0.7885	0.540		

Click here to review your answer to this exercise.

Several precautions are necessary when using the method of continuous variations. First, the metal and the ligand must form only one metal–ligand complex. To determine if this condition is true, plots of absorbance versus X_L are constructed at several different wavelengths and for several different values of n_{total}. If the maximum absorbance does not occur at the same value of X_L for each set of conditions, then more than one metal–ligand complex is present. A second precaution is that the metal–ligand complex's absorbance must obey Beer's law. Third, if the metal–ligand complex's formation constant is relatively small, a plot of absorbance versus X_L may show significant curvature. In this case it often is difficult to determine the stoichiometry by extrapolation. Finally, because the stability of a metal–ligand complex may be influenced by solution conditions, it is necessary to control carefully the composition of the solutions. When the ligand is a weak base, for example, each solutions must be buffered to the same pH.

In the MOLE-RATIO METHOD the moles of one reactant, usually the metal, is held constant, while the moles of the other reactant is varied. The absorbance is monitored at a wavelength where the metal–ligand complex absorbs. A plot of absorbance as a function of the ligand-to-metal mole ratio, n_L/n_M, has two linear branches that intersect at a mole–ratio corresponding to the complex's formula. Figure 10.39a shows a mole-ratio plot for the formation of a 1:1 complex in which the absorbance is monitored at a wavelength where only the complex absorbs. Figure 10.39b shows a mole-ratio plot for a 1:2 complex in which all three species—the metal, the ligand, and the complex—absorb at the selected wavelength. Unlike the method of continuous variations, the mole-ratio method can be used for complexation reactions that occur in a stepwise fashion if there is a difference in the molar absorptivities of the metal–ligand complexes, and if the formation constants are sufficiently different. A typical mole-ratio plot for the step-wise formation of ML and ML_2 is shown in Figure 10.39c.

For both the method of continuous variations and the mole-ratio method, we determine the complex's stoichiometry by extrapolating absorbance data from conditions in which there is a linear relationship between absorbance and the relative amounts of metal and ligand. If a metal–ligand
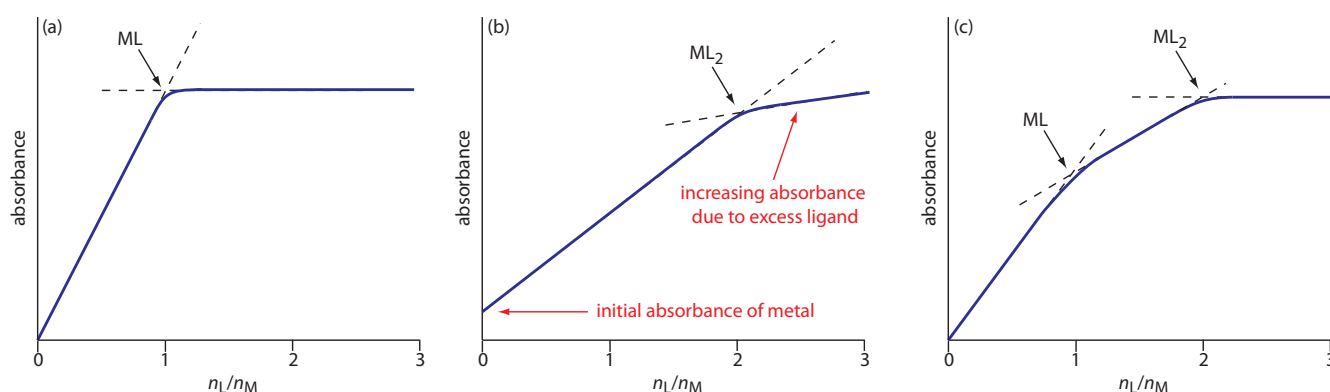
Figure 10.39 Mole-ratio plots for: (a) a 1:1 metal–ligand complex in which only the complex absorbs; (b) a 1:2 metal–ligand complex in which the metal, the ligand, and the complex absorb; and (c) the stepwise formation of a 1:1 and a 1:2 metal–ligand complex.

complex is very weak, a plot of absorbance versus X_L or n_L/n_M becomes so curved that it is impossible to determine the stoichiometry by extrapolation. In this case the slope-ratio is used.

In the SLOPE-RATIO METHOD two sets of solutions are prepared. The first set of solutions contains a constant amount of metal and a variable amount of ligand, chosen such that the total concentration of metal, C_M, is much larger than the total concentration of ligand, C_L. Under these conditions we may assume that essentially all the ligand reacts to form the metal–ligand complex. The concentration of the complex, which has the general form M_xL_y, is

$$[M_xL_y] = \frac{C_L}{y}$$

If we monitor the absorbance at a wavelength where only M_xL_y absorbs, then

$$A = \varepsilon b[M_xL_y] = \frac{\varepsilon b C_L}{y}$$

and a plot of absorbance versus C_L is linear with a slope, s_L, of

$$s_L = \frac{\varepsilon b}{y}$$

A second set of solutions is prepared with a fixed concentration of ligand that is much greater than a variable concentration of metal; thus

$$[M_xL_y] = \frac{C_M}{x}$$

$$A = \varepsilon b[M_xL_y] = \frac{\varepsilon b C_M}{x}$$

$$s_M = \frac{\varepsilon b}{x}$$

A ratio of the slopes provides the relative values of x and y.

$$\frac{s_M}{s_L} = \frac{\varepsilon b/x}{\varepsilon b/y} = \frac{y}{x}$$

An important assumption in the slope-ratio method is that the complexation reaction continues to completion in the presence of a sufficiently large excess of metal or ligand. The slope-ratio method also is limited to systems in which only a single complex forms and for which Beer's law is obeyed.

DETERMINATION OF EQUILIBRIUM CONSTANTS

Another important application of molecular absorption spectroscopy is the determination of equilibrium constants. Let's consider, as a simple example, an acid–base reaction of the general form

$$HIn\,(aq) + H_2O\,(l) \rightleftharpoons H_3O^+\,(aq) + In^-\,(aq)$$
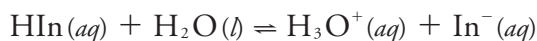
where HIn and In^- are the conjugate weak acid and weak base forms of an acid–base indicator. The equilibrium constant for this reaction is

$$K_a = \frac{[H_3O^+]\,[A^-]}{[HA]}$$

To determine the equilibrium constant's value, we prepare a solution in which the reaction is in a state of equilibrium and determine the equilibrium concentration for H_3O^+, HIn, and In^-. The concentration of H_3O^+ is easy to determine by measuring the solution's pH. To determine the concentration of HIn and In^- we can measure the solution's absorbance.

If both HIn and In^- absorb at the selected wavelength, then, from equation 10.6, we know that

$$A = \varepsilon_{HIn}b[HIn] + \varepsilon_{In^-}b[In^-] \qquad 10.15$$

where ε_{HIn} and $\varepsilon_{In}-$ are the molar absorptivities for HIn and In^-. The indicator's total concentration, C, is given by a mass balance equation

$$C = [HIn] + [In^-] \qquad 10.16$$

Solving equation 10.16 for [HIn] and substituting into equation 10.15 gives

$$A = \varepsilon_{HIn}b(C - [In^-]) + \varepsilon_{In^-}b[In^-]$$

which we simplify to

$$A = \varepsilon_{HIn}bC - \varepsilon_{HIn}b[In^-] + \varepsilon_{In^-}b[In^-]$$

$$A = A_{HIn} + b[In^-](\varepsilon_{In^-} - \varepsilon_{HIn}) \qquad 10.17$$

where A_{HIn}, which is equal to $\varepsilon_{HIn}bC$, is the absorbance when the pH is acidic enough that essentially all the indicator is present as HIn. Solving equation 10.17 for the concentration of In^- gives

$$[In^-] = \frac{A - A_{HIn}}{b(\varepsilon_{In^-} - \varepsilon_{HIn})} \qquad 10.18$$

Proceeding in the same fashion, we derive a similar equation for the concentration of HIn

$$[\text{HIn}] = \frac{A_{\text{In}^-} - A}{b(\varepsilon_{\text{In}^-} - \varepsilon_{\text{HIn}})} \qquad 10.19$$

where A_{In}, which is equal to $\varepsilon_{\text{In}^-} bC$, is the absorbance when the pH is basic enough that only In^- contributes to the absorbance. Substituting equation 10.18 and equation 10.19 into the equilibrium constant expression for HIn gives

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{In}^-]}{[\text{HIn}]} = [\text{H}_3\text{O}^+] \times \frac{A - A_{\text{HIn}}}{A_{\text{In}^-} - A} \qquad 10.20$$

We can use equation 10.20 to determine K_a in one of two ways. The simplest approach is to prepare three solutions, each of which contains the same amount, C, of indicator. The pH of one solution is made sufficiently acidic such that $[\text{HIn}] \gg [\text{In}^-]$. The absorbance of this solution gives A_{HIn}. The value of A_{In^-} is determined by adjusting the pH of the second solution such that $[\text{In}^-] \gg [\text{HIn}]$. Finally, the pH of the third solution is adjusted to an intermediate value, and the pH and absorbance, A, recorded. The value of K_a is calculated using equation 10.20.

Example 10.9

The acidity constant for an acid–base indicator is determined by preparing three solutions, each of which has a total concentration of indicator equal to 5.00×10^{-5} M. The first solution is made strongly acidic with HCl and has an absorbance of 0.250. The second solution is made strongly basic and has an absorbance of 1.40. The pH of the third solution is 2.91 and has an absorbance of 0.662. What is the value of K_a for the indicator?

SOLUTION

The value of K_a is determined by making appropriate substitutions into 10.20 where $[\text{H}_3\text{O}^+]$ is 1.23×10^{-3}; thus

$$K_a = (1.23 \times 10^{-3}) \times \frac{0.662 - 0.250}{1.40 - 0.662} = 6.87 \times 10^{-4}$$

Practice Exercise 10.9

To determine the K_a of a merocyanine dye, the absorbance of a solution of 3.5×10^{-4} M dye was measured at a pH of 2.00, a pH of 6.00, and a pH of 12.00, yielding absorbances of 0.000, 0.225, and 0.680, respectively. What is the value of K_a for this dye? The data for this problem is adapted from Lu, H.; Rutan, S. C. *Anal. Chem.*, **1996**, *68*, 1381–1386.

Click here to review your answer to this exercise.

A second approach for determining K_a is to prepare a series of solutions, each of which contains the same amount of indicator. Two solutions are used to determine values for A_{HIn} and A_{In}. Taking the log of both sides of equation 10.20 and rearranging leave us with the following equation.

$$\log \frac{A - A_{HIn}}{A_{In^-} - A} = \text{pH} - \text{p}K_a \qquad 10.21$$

A plot of $\log[(A-A_{HIn})/(A_{In}-A)]$ versus pH is a straight-line with a slope of $+1$ and a y-intercept of $-\text{p}K_a$.

Practice Exercise 10.10

To determine the K_a for the indicator bromothymol blue, the absorbance of each a series of solutions that contain the same concentration of bromothymol blue is measured at pH levels of 3.35, 3.65, 3.94, 4.30, and 4.64, yielding absorbance values of 0.170, 0.287, 0.411, 0.562, and 0.670, respectively. Acidifying the first solution to a pH of 2 changes its absorbance to 0.006, and adjusting the pH of the last solution to 12 changes its absorbance to 0.818. What is the value of K_a for bromothymol blue? The data for this problem is from Patterson, G. S. *J. Chem. Educ.*, **1999**, *76*, 395–398.

Click here to review your answer to this exercise.

In developing these approaches for determining K_a we considered a relatively simple system in which the absorbance of HIn and In⁻ are easy to measure and for which it is easy to determine the concentration of H_3O^+. In addition to acid–base reactions, we can adapt these approaches to any reaction of the general form

$$X(aq) + Y(aq) \rightleftharpoons Z(aq)$$
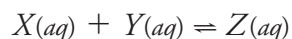
including metal–ligand complexation reactions and redox reactions, provided we can determine spectrophotometrically the concentration of the product, Z, and one of the reactants, either X or Y, and that we can determine the concentration of the other reactant by some other method. With appropriate modifications, a more complicated system in which we cannot determine the concentration of one or more of the reactants or products also is possible.[11]

10C.5 Evaluation of UV/Vis and IR Spectroscopy

SCALE OF OPERATION

Molecular UV/Vis absorption routinely is used for the analysis of trace analytes in macro and meso samples. Major and minor analytes are determined by diluting the sample before analysis, and concentrating a sample

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11 Ramette, R. W. *Chemical Equilibrium and Analysis*, Addison-Wesley: Reading, MA, 1981, Chapter 13.

may allow for the analysis of ultratrace analytes. The scale of operations for infrared absorption is generally poorer than that for UV/Vis absorption.

ACCURACY

Under normal conditions a relative error of 1–5% is easy to obtained with UV/Vis absorption. Accuracy usually is limited by the quality of the blank. Examples of the type of problems that are encountered include the presence of particulates in the sample that scatter radiation, and the presence of interferents that react with analytical reagents. In the latter case the interferent may react to form an absorbing species, which leads to a positive determinate error. Interferents also may prevent the analyte from reacting, which leads to a negative determinate error. With care, it is possible to improve the accuracy of an analysis by as much as an order of magnitude.

PRECISION

In absorption spectroscopy, precision is limited by indeterminate errors—primarily instrumental noise—which are introduced when we measure absorbance. Precision generally is worse for low absorbances where $P_0 \approx P_T$, and for high absorbances where P_T approaches 0. We might expect, therefore, that precision will vary with transmittance.

We can derive an expression between precision and transmittance by applying the propagation of uncertainty as described in Chapter 4. To do so we rewrite Beer's law as

$$C = -\frac{1}{\varepsilon b} \log T \qquad\qquad 10.22$$

Table 4.10 in Chapter 4 helps us complete the propagation of uncertainty for equation 10.22; thus, the absolute uncertainty in the concentration, s_C, is

$$s_C = -\frac{0.4343}{\varepsilon b} \times \frac{s_T}{T} \qquad\qquad 10.23$$

where s_T is the absolute uncertainty in the transmittance. Dividing equation 10.23 by equation 10.22 gives the relative uncertainty in concentration, s_C/C, as

$$\frac{s_C}{C} = \frac{0.4343 s_T}{T \log T}$$

If we know the transmittance's absolute uncertainty, then we can determine the relative uncertainty in concentration for any measured transmittance.

Determining the relative uncertainty in concentration is complicated because s_T is a function of the transmittance. As shown in Table 10.8, three categories of indeterminate instrumental error are observed.[12] A constant s_T is observed for the uncertainty associated with reading %T on a meter's analog or digital scale. Typical values are ±0.2–0.3% (a k_1 of ±0.002–0.003) for an analog scale and ±0.001% a (k_1 of ±0.00001) for a digital scale.

12 Rothman, L. D.; Crouch, S. R.; Ingle, J. D. Jr. *Anal. Chem.* **1975**, *47*, 1226–1233.

Table 10.8 Effect of Indeterminate Errors on Relative Uncertainty in Concentration		
Category	Sources of Indeterminate Error	Relative Uncertainty in Concentration
$s_T = k_1$	%T readout resolution noise in thermal detectors	$\dfrac{s_C}{C} = \dfrac{0.4343k_1}{T\log T}$
$s_T = k_2\sqrt{T^2 + T}$	noise in photon detectors	$\dfrac{s_C}{C} = \dfrac{0.4343k_2}{\log T}\sqrt{1 + \dfrac{1}{T}}$
$s_T = k_3 T$	positioning of sample cell fluctuations in source intensity	$\dfrac{s_C}{C} = \dfrac{0.4343k_3}{\log T}$

A constant s_T also is observed for the thermal transducers used in infrared spectrophotometers. The effect of a constant s_T on the relative uncertainty in concentration is shown by curve A in Figure 10.40. Note that the relative uncertainty is very large for both high absorbances and low absorbances, reaching a minimum when the absorbance is 0.4343. This source of indeterminate error is important for infrared spectrophotometers and for inexpensive UV/Vis spectrophotometers. To obtain a relative uncertainty in concentration of ±1–2%, the absorbance is kept within the range 0.1–1.

Values of s_T are a complex function of transmittance when indeterminate errors are dominated by the noise associated with photon detectors. Curve B in Figure 10.40 shows that the relative uncertainty in concentration is very large for low absorbances, but is smaller at higher absorbances. Although the relative uncertainty reaches a minimum when the absorbance is 0.963, there is little change in the relative uncertainty for absorbances between 0.5 and 2. This source of indeterminate error generally limits the precision of high quality UV/Vis spectrophotometers for mid-to-high absorbances.
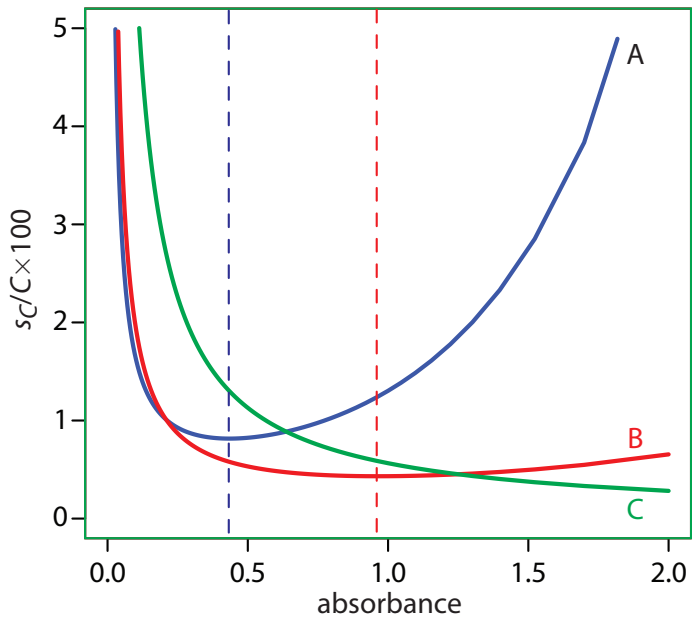
Figure 10.40 Percent relative uncertainty in concentration as a function of absorbance for the categories of indeterminate errors in Table 10.8. **A**: $k_1 = \pm 0.0030$; **B**: $k_2 = \pm 0.0030$; and **C**: $k_3 = \pm 0.0130$. The dashed lines correspond to the minimum uncertainty for **curve A** (absorbance of 0.4343) and for **curve B** (absorbance of 0.963).

Finally, the value of s_T is directly proportional to transmittance for indeterminate errors that result from fluctuations in the source's intensity and from uncertainty in positioning the sample within the spectrometer. The latter is particularly important because the optical properties of a sample cell are not uniform. As a result, repositioning the sample cell may lead to a change in the intensity of transmitted radiation. As shown by curve C in Figure 10.40, the effect is important only at low absorbances. This source of indeterminate errors usually is the limiting factor for high quality UV/Vis spectrophotometers when the absorbance is relatively small.

When the relative uncertainty in concentration is limited by the %T readout resolution, it is possible to improve the precision of the analysis by redefining 100% T and 0% T. Normally 100% T is established using a blank and 0% T is established while preventing the source's radiation from reaching the detector. If the absorbance is too high, precision is improved by resetting 100% T using a standard solution of analyte whose concentration is less than that of the sample (Figure 10.41a). For a sample whose absorbance is too low, precision is improved by redefining 0% T using a standard solution of the analyte whose concentration is greater than that of the analyte (Figure 10.41b). In this case a calibration curve is required because a linear relationship between absorbance and concentration no longer exists. Precision is further increased by combining these two methods (Figure 10.41c). Again, a calibration curve is necessary since the relationship between absorbance and concentration is no longer linear.

Figure 10.41 Methods for improving the precision of absorption methods: (a) high-absorbance method; (b) low-absorbance method; (c) maximum precision method.

See Figure 10.24 for an example of how the choice of wavelength affects a calibration curve's sensitivity.

SENSITIVITY

The sensitivity of a molecular absorption method, which is the slope of a Beer's law calibration curve, is the product of the analyte's absorptivity and the pathlength of the sample cell (εb). You can improve a method's sensitivity by selecting a wavelength where absorbance is at a maximum or by increasing pathlength.

SELECTIVITY

Selectivity rarely is a problem in molecular absorption spectrophotometry. In many cases it is possible to find a wavelength where only the analyte absorbs. When two or more species do contribute to the measured absorbance, a multicomponent analysis is still possible, as shown in Example 10.6 and Example 10.7.

TIME, COST, AND EQUIPMENT

The analysis of a sample by molecular absorption spectroscopy is relatively rapid, although additional time is required if we need to convert a nonabsorbing analyte into an absorbing form. The cost of UV/Vis instrumentation ranges from several hundred dollars for a simple filter photometer, to more than $50,000 for a computer-controlled, high-resolution double-

beam instrument equipped with variable slit widths, and operating over an extended range of wavelengths. Fourier transform infrared spectrometers can be obtained for as little as $15,000–$20,000, although more expensive models are available.

10D   Atomic Absorption Spectroscopy

Guystav Kirchoff and Robert Bunsen first used atomic absorption—along with atomic emission—in 1859 and 1860 as a means for identify atoms in flames and hot gases. Although atomic emission continued to develop as an analytical technique, progress in atomic absorption languished for almost a century. Modern atomic absorption spectroscopy has its beginnings in 1955 as a result of the independent work of A. C. Walsh and C. T. J. Alkemade.[13] Commercial instruments were in place by the early 1960s, and the importance of atomic absorption as an analytical technique soon was evident.

10D.1   Instrumentation

What reagent we choose to use to bring an analyte into solution depends on our research goals. If we need to know the total amount of metal in the sediment, then we might try a microwave digestion using a mixture of concentrated acids, such as HNO_3, HCl, and HF. This destroys the sediment's matrix and brings everything into solution. On the other hand, if our interest is biologically available metals, we might extract the sample under milder conditions using, for example, a dilute solution of HCl or CH_3COOH at room temperature.

Atomic absorption spectrophotometers use the same single-beam or double-beam optics described earlier for molecular absorption spectrophotometers (see Figure 10.26 and Figure 10.27). There is, however, an important additional need in atomic absorption spectroscopy: we first must covert the analyte into free atoms. In most cases the analyte is in solution form. If the sample is a solid, then we must bring the analyte into solution before the analysis. When analyzing a lake sediment for Cu, Zn, and Fe, for example, we bring the analytes into solution as Cu^{2+}, Zn^{2+}, and Fe^{3+} by extracting them with a suitable reagent. For this reason, only the introduction of solution samples is considered in this chapter.

Atomization

The process of converting an analyte to a free gaseous atom is called ATOMIZATION. Converting an aqueous analyte into a free atom requires that we strip away the solvent, volatilize the analyte, and, if necessary, dissociate the analyte into free atoms. Desolvating an aqueous solution of $CuCl_2$, for example, leaves us with solid particulates of $CuCl_2$. Converting the particulate $CuCl_2$ to gas phases atoms of Cu and Cl requires thermal energy.

$$CuCl_2(aq) \longrightarrow CuCl_2(s) \longrightarrow Cu(g) + 2Cl(g)$$

There are two common atomization methods: flame atomization and electrothermal atomization, although a few elements are atomized using other methods.

13   (a) Walsh, A. *Anal. Chem.* **1991**, *63*, 933A–941A; (b) Koirtyohann, S. R. *Anal. Chem.* **1991**, *63*, 1024A–1031A; (c) Slavin, W. *Anal. Chem.* **1991**, *63*, 1033A–1038A.
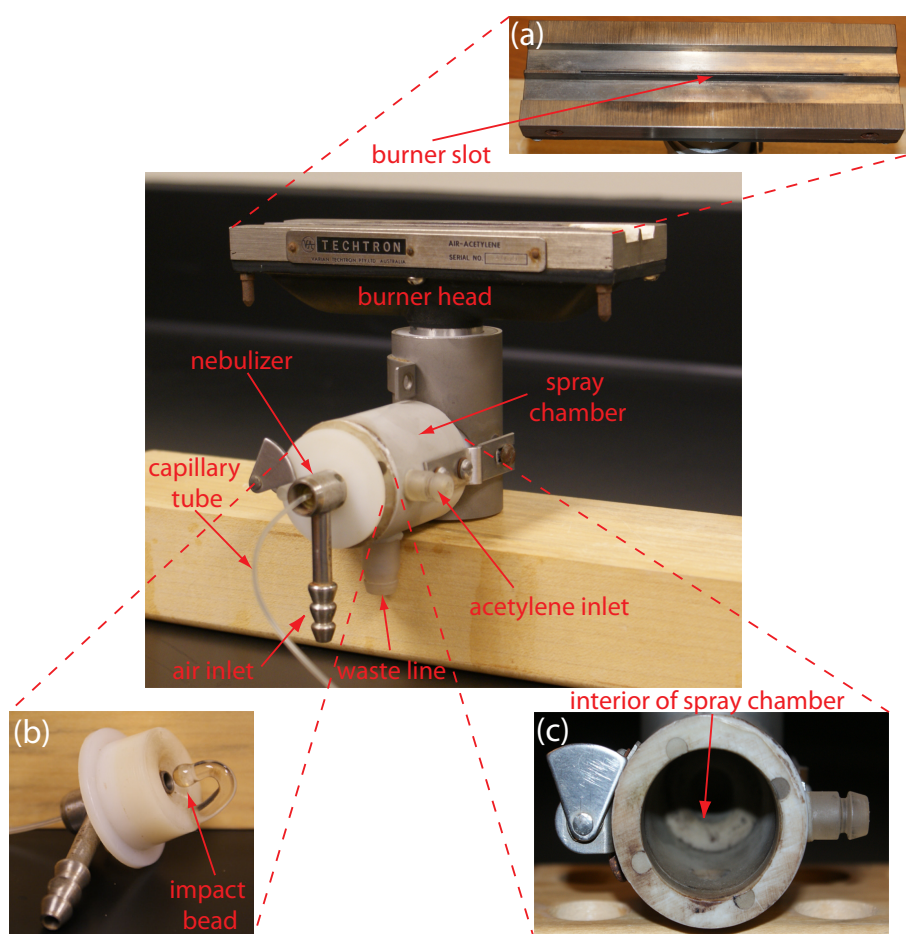
Figure 10.42 Flame atomization assembly with expanded views of (a) the burner head showing the burner slot where the flame is located; (b) the nebulizer's impact bead; and (c) the interior of the spray chamber. Although the unit shown here is from an instrument dating to the 1970s, the basic components of a modern flame AA spectrometer are the same.

FLAME ATOMIZER

Figure 10.42 shows a typical flame atomization assembly with close-up views of several key components. In the unit shown here, the aqueous sample is drawn into the assembly by passing a high-pressure stream of compressed air past the end of a capillary tube immersed in the sample. When the sample exits the nebulizer it strikes a glass impact bead, which converts it into a fine aerosol mist within the spray chamber. The aerosol mist is swept through the spray chamber by the combustion gases—compressed air and acetylene in this case—to the burner head where the flame's thermal energy desolvates the aerosol mist to a dry aerosol of small, solid particulates. The flame's thermal energy then volatilizes the particles, producing a vapor that consists of molecular species, ionic species, and free atoms.

Burner. The slot burner in Figure 10.42a provides a long optical path-length and a stable flame. Because absorbance is directly proportional to pathlength, a long pathlength provides greater sensitivity. A stable flame minimizes uncertainty due to fluctuations in the flame.

The burner is mounted on an adjustable stage that allows the entire assembly to move horizontally and vertically. Horizontal adjustments ensure the flame is aligned with the instrument's optical path. Vertical adjustments

Compressed air is one of the two gases whose combustion produces the flame.
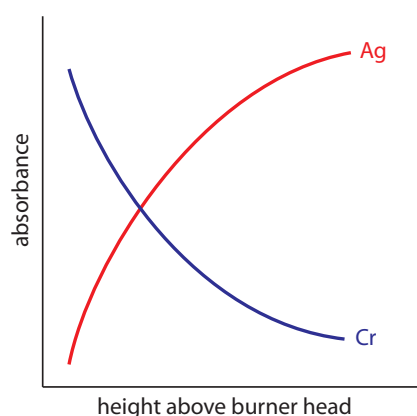
Figure 10.43 Absorbance versus height profiles for Ag and Cr in flame atomic absorption spectroscopy.
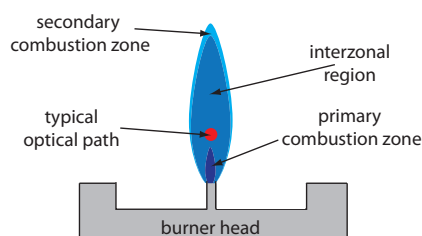
Figure 10.44 Profile of typical flame using a slot burner. The relative size of each zone depends on many factors, including the choice of fuel and oxidant, and their relative proportions.

change the height within the flame from which absorbance is monitored. This is important because two competing processes affect the concentration of free atoms in the flame. The more time an analyte spends in the flame the greater the atomization efficiency; thus, the production of free atoms increases with height. On the other hand, a longer residence time allows more opportunity for the free atoms to combine with oxygen to form a molecular oxide. As seen in Figure 10.43, for a metal this is easy to oxidize, such as Cr, the concentration of free atoms is greatest just above the burner head. For a metal, such as Ag, which is difficult to oxidize, the concentration of free atoms increases steadily with height.

Flame. The flame's temperature, which affects the efficiency of atomization, depends on the fuel–oxidant mixture, several examples of which are listed in Table 10.9. Of these, the air–acetylene and the nitrous oxide–acetylene flames are the most popular. Normally the fuel and oxidant are mixed in an approximately stoichiometric ratio; however, a fuel-rich mixture may be necessary for easily oxidized analytes.

Figure 10.44 shows a cross-section through the flame, looking down the source radiation's optical path. The primary combustion zone usually is rich in gas combustion products that emit radiation, limiting is usefulness for atomic absorption. The interzonal region generally is rich in free atoms and provides the best location for measuring atomic absorption. The hottest part of the flame typically is 2–3 cm above the primary combustion zone. As atoms approach the flame's secondary combustion zone, the decrease in temperature allows for formation of stable molecular species.

Sample Introduction. The most common means for introducing a sample into a flame atomizer is a continuous aspiration in which the sample flows through the burner while we monitor absorbance. Continuous aspiration is sample intensive, typically requiring from 2–5 mL of sample.

Flame microsampling allows us to introduce a discrete sample of fixed volume, and is useful if we have a limited amount of sample or when the sample's matrix is incompatible with the flame atomizer. For example, continuously aspirating a sample that has a high concentration of dissolved solids—sea water, for example, comes to mind—may build-up a solid deposit on the burner head that obstructs the flame and that lowers the absorbance. Flame microsampling is accomplished using a micropipet to place 50–250 μL of sample in a Teflon funnel connected to the nebulizer, or by

Table 10.9	Fuels and Oxidants Used for Flame Combustion	
fuel	oxidant	temperature range (°C)
natural gas	air	1700–1900
hydrogen	air	2000–2100
acetylene	air	2100–2400
acetylene	nitrous oxide	2600–2800
acetylene	oxygen	3050–3150

dipping the nebulizer tubing into the sample for a short time. Dip sampling usually is accomplished with an automatic sampler. The signal for flame microsampling is a transitory peak whose height or area is proportional to the amount of analyte that is injected.

Advantages and Disadvantages of Flame Atomization. The principal advantage of flame atomization is the reproducibility with which the sample is introduced into the spectrophotometer; a significant disadvantage is that the efficiency of atomization is quite poor. There are two reasons for poor atomization efficiency. First, the majority of the aerosol droplets produced during nebulization are too large to be carried to the flame by the combustion gases. Consequently, as much as 95% of the sample never reaches the flame. A second reason for poor atomization efficiency is that the large volume of combustion gases significantly dilutes the sample. Together, these contributions to the efficiency of atomization reduce sensitivity because the analyte's concentration in the flame may be a factor of 2.5×10^{-6} less than that in solution.[14]

This is the reason for the waste line shown at the bottom of the spray chamber in Figure 10.42.

ELECTROTHERMAL ATOMIZERS

A significant improvement in sensitivity is achieved by using the resistive heating of a graphite tube in place of a flame. A typical electrothermal atomizer, also known as a GRAPHITE FURNACE, consists of a cylindrical graphite tube approximately 1–3 cm in length and 3–8 mm in diameter. As shown in Figure 10.45, the graphite tube is housed in an sealed assembly that has an optically transparent window at each end. A continuous stream of inert gas is passed through the furnace, which protects the graphite tube from oxidation and removes the gaseous products produced during atomization. A power supply is used to pass a current through the graphite tube, resulting in resistive heating.

Samples of between 5–50 μL are injected into the graphite tube through a small hole at the top of the tube. Atomization is achieved in three stages. In the first stage the sample is dried to a solid residue using a current that raises the temperature of the graphite tube to about 110 °C. In the second stage, which is called ashing, the temperature is increased to between 350–

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14 Ingle, J. D.; Crouch, S. R. *Spectrochemical Analysis*, Prentice-Hall: Englewood Cliffs, NJ, 1988; p. 275.
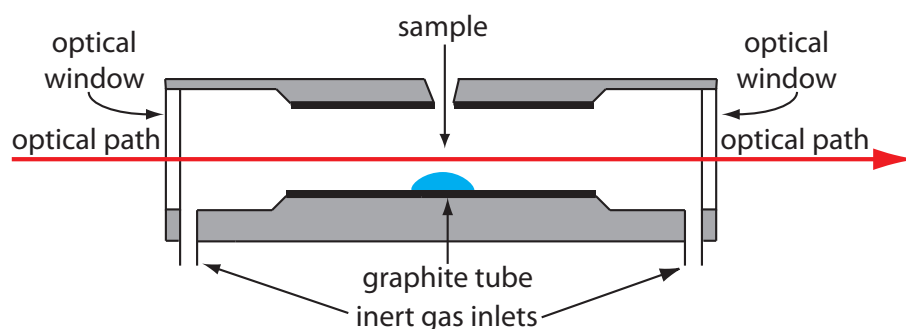
Figure 10.45 Diagram showing a cross-section of an electrothermal analyzer.

1200 °C. At these temperatures organic material in the sample is converted to CO_2 and H_2O, and volatile inorganic materials are vaporized. These gases are removed by the inert gas flow. In the final stage the sample is atomized by rapidly increasing the temperature to between 2000–3000 °C. The result is a transient absorbance peak whose height or area is proportional to the absolute amount of analyte injected into the graphite tube. Together, the three stages take approximately 45–90 s, with most of this time used for drying and ashing the sample.

Electrothermal atomization provides a significant improvement in sensitivity by trapping the gaseous analyte in the small volume within the graphite tube. The analyte's concentration in the resulting vapor phase is as much as $1000\times$ greater than in a flame atomization.[15] This improvement in sensitivity—and the resulting improvement in detection limits—is offset by a significant decrease in precision. Atomization efficiency is influenced strongly by the sample's contact with the graphite tube, which is difficult to control reproducibly.

MISCELLANEOUS ATOMIZATION METHODS

A few elements are atomized by using a chemical reaction to produce a volatile product. Elements such as As, Se, Sb, Bi, Ge, Sn, Te, and Pb, for example, form volatile hydrides when they react with $NaBH_4$ in the presence of acid. An inert gas carries the volatile hydride to either a flame or to a heated quartz observation tube situated in the optical path. Mercury is determined by the cold-vapor method in which it is reduced to elemental mercury with $SnCl_2$. The volatile Hg is carried by an inert gas to an unheated observation tube situated in the instrument's optical path.

10D.2   Quantitative Applications

Atomic absorption is used widely for the analysis of trace metals in a variety of sample matrices. Using Zn as an example, there are standard atomic absorption methods for its determination in samples as diverse as water and wastewater, air, blood, urine, muscle tissue, hair, milk, breakfast cereals, shampoos, alloys, industrial plating baths, gasoline, oil, sediments, and rocks.

Developing a quantitative atomic absorption method requires several considerations, including choosing a method of atomization, selecting the wavelength and slit width, preparing the sample for analysis, minimizing spectral and chemical interferences, and selecting a method of standardization. Each of these topics is considered in this section.

DEVELOPING A QUANTITATIVE METHOD

Flame or Electrothermal Atomization? The most important factor in choosing a method of atomization is the analyte's concentration. Because

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15   Parsons, M. L.; Major, S.; Forster, A. R. *Appl. Spectrosc.* **1983**, *37*, 411–418.

of its greater sensitivity, it takes less analyte to achieve a given absorbance when using electrothermal atomization. Table 10.10, which compares the amount of analyte needed to achieve an absorbance of 0.20 when using flame atomization and electrothermal atomization, is useful when selecting an atomization method. For example, flame atomization is the method of choice if our samples contain 1–10 mg Zn^{2+}/L, but electrothermal atomization is the best choice for samples that contain 1–10 µg Zn^{2+}/L.

Selecting the Wavelength and Slit Width. The source for atomic absorption is a hollow cathode lamp that consists of a cathode and anode enclosed within a glass tube filled with a low pressure of an inert gas, such as Ne or Ar (Figure 10.46). Applying a potential across the electrodes ionizes the filler gas. The positively charged gas ions collide with the negatively charged cathode, sputtering atoms from the cathode's surface. Some of the sputtered atoms are in the excited state and emit radiation characteristic of the metal(s) from which the cathode is manufactured. By fashioning the cathode from the metallic analyte, a hollow cathode lamp provides emission lines that correspond to the analyte's absorption spectrum.

Because atomic absorption lines are narrow, we need to use a line source instead of a continuum source (compare, for example, Figure 10.18 with Figure 10.20). The effective bandwidth when using a continuum source is roughly 1000× larger than an atomic absorption line; thus, $P_T \approx P_0$, $\%T \approx 100$, and $A \approx 0$. Because a hollow cathode lamp is a line source, P_T and P_0 have different values giving a $\%T < 100$ and $A > 0$.

Table 10.10 Concentration of Analyte That Yields an Absorbance of 0.20		
	Concentration (mg/L)[a]	
element	flame atomization	electrothermal atomization
Ag	1.5	0.0035
Al	40	0.015
As	40[b]	0.050
Ca	0.8	0.003
Cd	0.6	0.001
Co	2.5	0.021
Cr	2.5	0.0075
Cu	1.5	0.012
Fe	2.5	0.006
Hg	70[b]	0.52
Mg	0.15	0.00075
Mn	1	0.003
Na	0.3	0.00023
Ni	2	0.024
Pb	5	0.080
Pt	70	0.29
Sn	50[b]	0.023
Zn	0.3	0.00071

[a] Source: Varian Cookbook, SpectraAA Software Version 4.00 Pro.

[b] As: 10 mg/L by hydride vaporization; Hg: 11.5 mg/L by cold-vapor; and Sn:18 mg/L by hydride vaporization
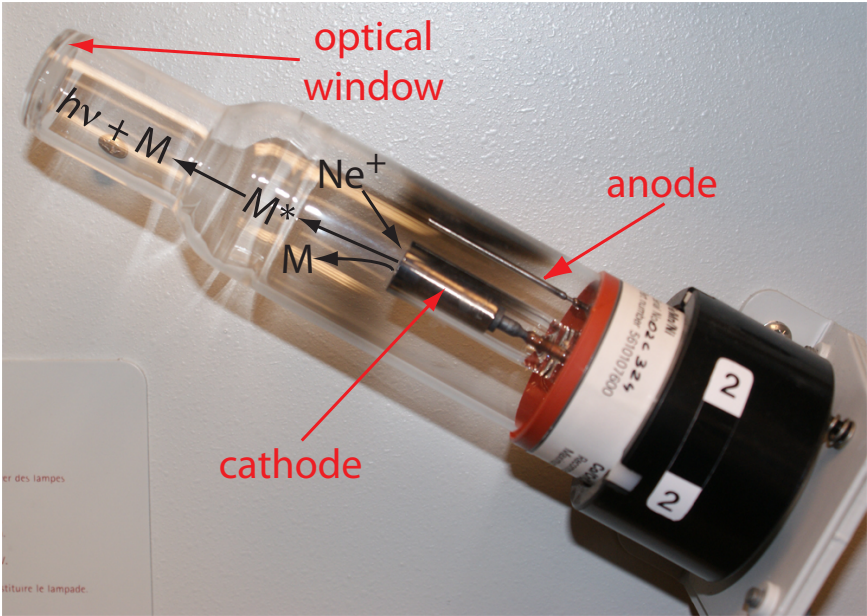
Figure 10.46 Photo of a typical multielemental hollow cathode lamp. The cathode in this lamp is fashioned from an alloy of Co, Cr, Cu, Fe, Mn, and Ni, and is surrounded by a glass shield that isolates it from the anode. The lamp is filled with Ne gas. Also shown is the process that leads to atomic emission. Note the black deposit of sputtered metal on the outer wall of the hollow cathode lamp. See the text for additional details.

Each element in a hollow cathode lamp provides several atomic emission lines that we can use for atomic absorption. Usually the wavelength that provides the best sensitivity is the one we choose to use, although a less sensitive wavelength may be more appropriate for a sample that has higher concentration of analyte. For the Cr hollow cathode lamp in Table 10.11, the best sensitivity is obtained using a wavelength of 357.9 nm.

Another consideration is the emission line's intensity. If several emission lines meet our requirements for sensitivity, we may wish to use the emission line with the largest relative P_0 because there is less uncertainty in measuring P_0 and P_T. When analyzing a sample that is ≈ 10 mg Cr/L, for example, the first three wavelengths in Table 10.11 provide an appropriate sensitivity; the wavelengths of 425.5 nm and 429.0 nm, however, have a greater P_0 and will provide less uncertainty in the measured absorbance.

Table 10.11 Atomic Emission Lines for a Cr Hollow Cathode Lamp			
wavelength (nm)	slit width (nm)	mg Cr/L giving $A = 0.20$	P_0 (relative)
357.9	0.2	2.5	40
425.4	0.2	12	85
429.0	0.5	20	100
520.5	0.2	1500	15
520.8	0.2	500	20

The emission spectrum for a hollow cathode lamp includes, in addition to the analyte's emission lines, additional emission lines from impurities present in the metallic cathode and from the filler gas. These additional lines are a potential source of stray radiation that could result in an instrumental deviation from Beer's law. The monochromator's slit width is set as wide as possible to improve the throughput of radiation and narrow enough to eliminate these sources of stray radiation.

Preparing the Sample. Flame and electrothermal atomization require that the analyte is in solution. Solid samples are brought into solution by dissolving in an appropriate solvent. If the sample is not soluble it is digested, either on a hot-plate or by microwave, using HNO_3, H_2SO_4, or $HClO_4$. Alternatively, we can extract the analyte using a Soxhlet extractor. Liquid samples are analyzed directly or the analytes extracted if the matrix is incompatible with the method of atomization. A serum sample, for instance, is difficult to aspirate when using flame atomization and may produce an unacceptably high background absorbance when using electrothermal atomization. A liquid–liquid extraction using an organic solvent and a chelating agent frequently is used to concentrate analytes. Dilute solutions of Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, and Zn^{2+}, for example, are concentrated by extracting with a solution of ammonium pyrrolidine dithiocarbamate in methyl isobutyl ketone.

Minimizing Spectral Interference. A spectral interference occurs when an analyte's absorption line overlaps with an interferent's absorption line or band. Because they are so narrow, the overlap of two atomic absorption lines seldom is a problem. On the other hand, a molecule's broad absorption band or the scattering of source radiation is a potentially serious spectral interference.

An important consideration when using a flame as an atomization source is its effect on the measured absorbance. Among the products of combustion are molecular species that exhibit broad absorption bands and particulates that scatter radiation from the source. If we fail to compensate for these spectral interferences, then the intensity of transmitted radiation is smaller than expected. The result is an apparent increase in the sample's absorbance. Fortunately, absorption and scattering of radiation by the flame are corrected by analyzing a blank.

Spectral interferences also occur when components of the sample's matrix other than the analyte react to form molecular species, such as oxides and hydroxides. The resulting absorption and scattering constitutes the sample's background and may present a significant problem, particularly at wavelengths below 300 nm where the scattering of radiation becomes more important. If we know the composition of the sample's matrix, then we can prepare our samples using an identical matrix. In this case the background absorption is the same for both the samples and the standards. Alternatively, if the background is due to a known matrix component, then we can add

that component in excess to all samples and standards so that the contribution of the naturally occurring interferent is insignificant. Finally, many interferences due to the sample's matrix are eliminated by increasing the atomization temperature. For example, switching to a higher temperature flame helps prevents the formation of interfering oxides and hydroxides.

If the identity of the matrix interference is unknown, or if it is not possible to adjust the flame or furnace conditions to eliminate the interference, then we must find another method to compensate for the background interference. Several methods have been developed to compensate for matrix interferences, and most atomic absorption spectrophotometers include one or more of these methods.

One of the most common methods for BACKGROUND CORRECTION is to use a continuum source, such as a D_2 lamp. Because a D_2 lamp is a continuum source, absorbance of its radiation by the analyte's narrow absorption line is negligible. Only the background, therefore, absorbs radiation from the D_2 lamp. Both the analyte and the background, on the other hand, absorb the hollow cathode's radiation. Subtracting the absorbance for the D_2 lamp from that for the hollow cathode lamp gives a corrected absorbance that compensates for the background interference. Although this method of background correction is effective, it does assume that the background absorbance is constant over the range of wavelengths passed by the monochromator. If this is not true, then subtracting the two absorbances underestimates or overestimates the background.

Minimizing Chemical Interferences. The quantitative analysis of some elements is complicated by chemical interferences that occur during atomization. The most common chemical interferences are the formation of nonvolatile compounds that contain the analyte and ionization of the analyte.

One example of the formation of a nonvolatile compound is the effect of PO_4^{3-} or Al^{3+} on the flame atomic absorption analysis of Ca^{2+}. In one study, for example, adding 100 ppm Al^{3+} to a solution of 5 ppm Ca^{2+} decreased calcium ion's absorbance from 0.50 to 0.14, while adding 500 ppm PO_4^{3-} to a similar solution of Ca^{2+} decreased the absorbance from 0.50 to 0.38. These interferences are attributed to the formation of nonvolatile particles of $Ca_3(PO_4)_2$ and an Al–Ca–O oxide.[16]

When using flame atomization, we can minimize the formation of nonvolatile compounds by increasing the flame's temperature by changing the fuel-to-oxidant ratio or by switching to a different combination of fuel and oxidant. Another approach is to add a releasing agent or a protecting agent to the sample. A RELEASING AGENT is a species that reacts preferentially with the interferent, releasing the analyte during atomization. For example, Sr^{2+} and La^{3+} serve as releasing agents for the analysis of Ca^{2+} in the presence of PO_4^{3-} or Al^{3+}. Adding 2000 ppm $SrCl_2$ to the Ca^{2+}/PO_4^{3-} and to the Ca^{2+}/Al^{3+} mixtures described in the previous paragraph increased the absorbance to 0.48. A PROTECTING AGENT reacts with the analyte to form

Other methods of background correction have been developed, including Zeeman effect background correction and Smith–Hieftje background correction, both of which are included in some commercially available atomic absorption spectrophotometers. Consult the chapter's additional resources for additional information.

16 Hosking, J. W.; Snell, N. B.; Sturman, B. T. *J. Chem. Educ.* **1977**, *54*, 128–130.

a stable volatile complex. Adding 1% w/w EDTA to the Ca^{2+}/PO_4^{3-} solution described in the previous paragraph increased the absorbance to 0.52.

An ionization interference occurs when thermal energy from the flame or the electrothermal atomizer is sufficient to ionize the analyte

$$M(s) \rightleftharpoons M^+(aq) + e^- \qquad 10.24$$
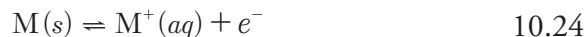
where M is the analyte. Because the absorption spectra for M and M^+ are different, the position of the equilibrium in reaction 10.24 affects the absorbance at wavelengths where M absorbs. To limit ionization we add a high concentration of an IONIZATION SUPPRESSOR, which is a species that ionizes more easily than the analyte. If the ionization suppressor's concentration is sufficient, then the increased concentration of electrons in the flame pushes reaction 10.24 to the left, preventing the analyte's ionization. Potassium and cesium frequently are used as an ionization suppressor because of their low ionization energy.

Standardizing the Method. Because Beer's law also applies to atomic absorption, we might expect atomic absorption calibration curves to be linear. In practice, however, most atomic absorption calibration curves are nonlinear or linear over a limited range of concentrations. Nonlinearity in atomic absorption is a consequence of instrumental limitations, including stray radiation from the hollow cathode lamp and the variation in molar absorptivity across the absorption line. Accurate quantitative work, therefore, requires a suitable means for computing the calibration curve from a set of standards.

When possible, a quantitative analysis is best conducted using external standards. Unfortunately, matrix interferences are a frequent problem, particularly when using electrothermal atomization. For this reason the method of standard additions often is used. One limitation to this method of standardization, however, is the requirement of a linear relationship between absorbance and concentration.

Most instruments include several different algorithms for computing the calibration curve. The instrument in my lab, for example, includes five algorithms. Three of the algorithms fit absorbance data using linear, quadratic, or cubic polynomial functions of the analyte's concentration. It also includes two algorithms that fit the concentrations of the standards to quadratic functions of the absorbance.

Representative Method 10.2

Determination of Cu and Zn in Tissue Samples

DESCRIPTION OF METHOD

Copper and zinc are isolated from tissue samples by digesting the sample with HNO_3 after first removing any fatty tissue. The concentration of copper and zinc in the supernatant are determined by atomic absorption using an air-acetylene flame.

PROCEDURE

Tissue samples are obtained by a muscle needle biopsy and dried for 24–30 h at 105 °C to remove all traces of moisture. The fatty tissue in a dried sample is removed by extracting overnight with anhydrous ether. After removing the ether, the sample is dried to obtain the fat-free dry

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of Cu and Zn in biological tissues provides an instructive example of a typical procedure. The description here is based on Bhattacharya, S. K.; Goodwin, T. G.; Crawford, A. J. *Anal. Lett.* **1984**, *17*, 1567–1593, and Crawford, A. J.; Bhattacharya, S. K. Varian Instruments at Work, Number AA–46, April 1985.

tissue weight (FFDT). The sample is digested at 68 °C for 20–24 h using 3 mL of 0.75 M HNO_3. After centrifuging at 2500 rpm for 10 minutes, the supernatant is transferred to a 5-mL volumetric flask. The digestion is repeated two more times, for 2–4 hours each, using 0.9-mL aliquots of 0.75 M HNO_3. These supernatants are added to the 5-mL volumetric flask, which is diluted to volume with 0.75 M HNO_3. The concentrations of Cu and Zn in the diluted supernatant are determined by flame atomic absorption spectroscopy using an air-acetylene flame and external standards. Copper is analyzed at a wavelength of 324.8 nm with a slit width of 0.5 nm, and zinc is analyzed at 213.9 nm with a slit width of 1.0 nm. Background correction using a D_2 lamp is necessary for zinc. Results are reported as µg of Cu or Zn per gram of FFDT.

QUESTIONS

1. Describe the appropriate matrix for the external standards and for the blank?

   The matrix for the standards and the blank should match the matrix of the samples; thus, an appropriate matrix is 0.75 M HNO_3. Any interferences from other components of the sample matrix are minimized by background correction.

2. Why is a background correction necessary for the analysis of Zn, but not for the analysis of Cu?

   Background correction compensates for background absorption and scattering due to interferents in the sample. Such interferences are most severe when using a wavelength less than 300 nm. This is the case for Zn, but not for Cu.

3. A Cu hollow cathode lamp has several emission lines, the properties of which are shown in the following table. Explain why this method uses the line at 324.8 nm.

wavelength (nm)	slit width (nm)	mg Cu/L for $A = 0.20$	P_0 (relative)
217.9	0.2	15	3
218.2	0.2	15	3
222.6	0.2	60	5
244.2	0.2	400	15
249.2	0.5	200	24
324.8	0.5	1.5	100
327.4	0.5	3	87

   With 1.5 mg Cu/L giving an absorbance of 0.20, the emission line at 324.8 nm has the best sensitivity. In addition, it is the most intense emission line, which decreases the uncertainty in the measured absorbance.

Example 10.10

To evaluate the method described in Representative Method 10.2, a series of external standard is prepared and analyzed, providing the results shown here.[17]

μg Cu/mL	absorbance	μg Cu/mL	absorbance
0.000	0.000	0.500	0.033
0.100	0.006	0.600	0.039
0.200	0.013	0.700	0.046
0.300	0.020	1.000	0.066
0.400	0.026		

A bovine liver standard reference material is used to evaluate the method's accuracy. After drying and extracting the sample, a 11.23-mg FFDT tissue sample gives an absorbance of 0.023. Report the amount of copper in the sample as μg Cu/g FFDT.

SOLUTION

Linear regression of absorbance versus the concentration of Cu in the standards gives the calibration curve shown to the right and the following calibration equation.

$$A = -0.0002 + 0.0661 \times \frac{\mu g\ Cu}{mL}$$

Substituting the sample's absorbance into the calibration equation gives the concentration of copper as 0.351 μg/mL. The concentration of copper in the tissue sample, therefore, is

$$\frac{\dfrac{0.351\,\mu g\ Cu}{mL} \times 5.000\ mL}{0.01123\ g\ sample} = 156\ \mu g\ Cu/g\ FFDT$$
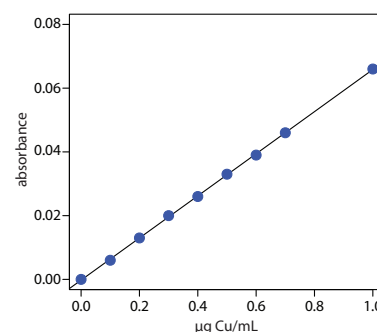
10D.3 - Evaluation of Atomic Absorption Spectroscopy

SCALE OF OPERATION

Atomic absorption spectroscopy is ideally suited for the analysis of trace and ultratrace analytes, particularly when using electrothermal atomization. For minor and major analytes, sample are diluted before the analysis. Most analyses use a macro or a meso sample. The small volume requirement for electrothermal atomization or for flame microsampling, however, makes practical the analysis of micro and ultramicro samples.

See Figure 3.5 to review the meaning of macro and meso for describing samples, and the meaning of major, minor, and ultratrace for describing analytes.

17 Crawford, A. J.; Bhattacharya, S. K. "Microanalysis of Copper and Zinc in Biopsy-Sized Tissue Specimens by Atomic Absorption Spectroscopy Using a Stoichiometric Air-Acetylene Flame," Varian Instruments at Work, Number AA–46, April 1985.

ACCURACY

If spectral and chemical interferences are minimized, an accuracy of 0.5–5% is routinely attainable. When the calibration curve is nonlinear, accuracy is improved by using a pair of standards whose absorbances closely bracket the sample's absorbance and assuming that the change in absorbance is linear over this limited concentration range. Determinate errors for electrothermal atomization often are greater than those obtained with flame atomization due to more serious matrix interferences.

PRECISION

For an absorbance greater than 0.1–0.2, the relative standard deviation for atomic absorption is 0.3–1% for flame atomization and 1–5% for electrothermal atomization. The principle limitation is the uncertainty in the concentration of free analyte atoms that result from variations in the rate of aspiration, nebulization, and atomization for a flame atomizer, and the consistency of injecting samples for electrothermal atomization.

SENSITIVITY

The sensitivity of a flame atomic absorption analysis is influenced by the flame's composition and by the position in the flame from which we monitor the absorbance. Normally the sensitivity of an analysis is optimized by aspirating a standard solution of analyte and adjusting the fuel-to-oxidant ratio, the nebulizer flow rate, and the height of the burner, to give the greatest absorbance. With electrothermal atomization, sensitivity is influenced by the drying and ashing stages that precede atomization. The temperature and time at each stage is optimized for each type of sample.

See Chapter 14 for several strategies for optimizing experiments.

Sensitivity also is influenced by the sample's matrix. We already noted, for example, that sensitivity is decreased by a chemical interference. An increase in sensitivity may be realized by adding a low molecular weight alcohol, ester, or ketone to the solution, or by using an organic solvent.

SELECTIVITY

Due to the narrow width of absorption lines, atomic absorption provides excellent selectivity. Atomic absorption is used for the analysis of over 60 elements at concentrations at or below the level of μg/L.

TIME, COST, AND EQUIPMENT

The analysis time when using flame atomization is short, with sample throughputs of 250–350 determinations per hour when using a fully automated system. Electrothermal atomization requires substantially more time per analysis, with maximum sample throughputs of 20–30 determinations per hour. The cost of a new instrument ranges from between $10,000–$50,000 for flame atomization, and from $18,000–$70,000 for electro-

thermal atomization. The more expensive instruments in each price range include double-beam optics, automatic samplers, and can be programmed for multielemental analysis by allowing the wavelength and hollow cathode lamp to be changed automatically.

10E   Emission Spectroscopy

An analyte in an excited state possesses an energy, E_2, that is greater than its energy when it is in a lower energy state, E_1. When the analyte returns to its lower energy state—a process we call RELAXATION—the excess energy, ΔE

$$\triangle E = E_2 - E_1$$

is released. Figure 10.4 shows a simplified picture of this process.

The amount of time an analyte, A, spends in its excited state—what we call the excited state's LIFETIME—is short, typically 10^{-5}–10^{-9} s for an electronic excited state and 10^{-15} s for a vibrational excited state. Relaxation of the analyte's excited state, A^*, occurs through several mechanisms, including collisions with other species in the sample, photochemical reactions, and the emission of photons. In the first process, which we call vibrational relaxation or nonradiative relaxation, the excess energy is released as heat.

$$A^* \longrightarrow A + \text{heat}$$

Relaxation by a photochemical reaction may involve simple decomposition

$$A^* \longrightarrow X + Y$$
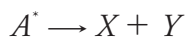
or a reaction between A^* and another species

$$A^* + Z \longrightarrow X + Y$$

In both cases the excess energy is used up in the chemical reaction or released as heat.

In the third mechanism, the excess energy is released as a photon of electromagnetic radiation.

$$A^* \longrightarrow A + h\nu$$

The release of a photon following thermal excitation is called emission and that following the absorption of a photon is called photoluminescence. In chemiluminescence and bioluminescence, excitation results from a chemical or a biochemical reaction, respectively. Spectroscopic methods based on photoluminescence are the subject of the next section and atomic emission is covered in Section 10G.

10F   Photoluminescence Spectroscopy

Photoluminescence is divided into two categories: fluorescence and phosphorescence. A pair of electrons that occupy the same electronic ground state have opposite spins and are in a singlet spin state (Figure 10.47a).
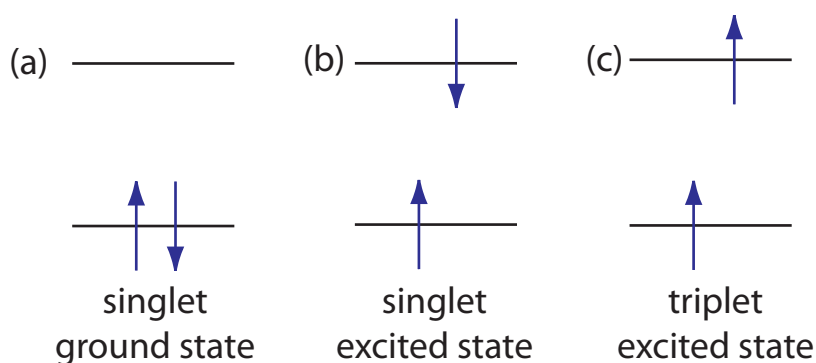
Figure 10.47 Electron configurations for (a) a singlet ground state; (b) a singlet excited state; and (c) a triplet excited state.

When an analyte absorbs an ultraviolet or a visible photon, one of its valence electrons moves from the ground state to an excited state with a conservation of the electron's spin (Figure 10.47b). Emission of a photon from a SINGLET EXCITED STATE to the singlet ground state—or between any two energy levels with the same spin—is called FLUORESCENCE. The probability of fluorescence is very high and the average lifetime of an electron in the excited state is only 10^{-5}–10^{-8} s. Fluorescence, therefore, rapidly decays once the source of excitation is removed.

In some cases an electron in a singlet excited state is transformed to a TRIPLET EXCITED STATE (Figure 10.47c) in which its spin no is longer paired with the ground state. Emission between a triplet excited state and a singlet ground state—or between any two energy levels that differ in their respective spin states–is called PHOSPHORESCENCE. Because the average lifetime for phosphorescence ranges from 10^{-4}–10^{4} s, phosphorescence may continue for some time after we remove the excitation source.

The use of molecular fluorescence for qualitative analysis and for semiquantitative analysis dates to the early to mid 1800s, with more accurate quantitative methods appearing in the 1920s. Instrumentation for fluorescence spectroscopy using a filter or a monochromator for wavelength selection appeared in, respectively, the 1930s and 1950s. Although the discovery of phosphorescence preceded that of fluorescence by almost 200 years, qualitative and quantitative applications of molecular phosphorescence did not receive much attention until after the development of fluorescence instrumentation.

As you might expect, the persistence of long-lived phosphorescence made it more noticeable.

10F.1   Fluorescence and Phosphorescence Spectra

To appreciate the origin of fluorescence and phosphorescence we must consider what happens to a molecule following the absorption of a photon. Let's assume the molecule initially occupies the lowest vibrational energy level of its electronic ground state, which is the singlet state labeled S_0 in Figure 10.48. Absorption of a photon excites the molecule to one of several vibrational energy levels in the first excited electronic state, S_1, or the second electronic excited state, S_2, both of which are singlet states. Relaxation to the ground state occurs by a number of mechanisms, some of which result in the emission of a photon and others that occur without the emis-

Figure 10.48 Energy level diagram for a molecule that shows pathways for the deactivation of an excited state: *vr* is vibrational relaxation; *ic* is internal conversion; *ec* is external conversion; and *isc* is an intersystem crossing. The lowest vibrational energy for each electronic state is indicated by the thicker line. The electronic ground state is shown in **black** and the three electronic excited states are shown in **green**. The **absorption**, **fluorescence**, and **phosphorescence** of photons also are shown.

sion of a photon. These relaxation mechanisms are shown in Figure 10.48. The most likely relaxation pathway from any excited state is the one with the shortest lifetime.

RADIATIONLESS DEACTIVATION

When a molecule relaxes without emitting a photon we call the process RADIATIONLESS DEACTIVATION. One example of radiationless deactivation is VIBRATIONAL RELAXATION, in which a molecule in an excited vibrational energy level loses energy by moving to a lower vibrational energy level in the same electronic state. Vibrational relaxation is very rapid, with an average lifetime of $<10^{-12}$ s. Because vibrational relaxation is so efficient, a molecule in one of its excited state's higher vibrational energy levels quickly returns to the excited state's lowest vibrational energy level.

Another form of radiationless deactivation is an INTERNAL CONVERSION in which a molecule in the ground vibrational level of an excited state passes directly into a higher vibrational energy level of a lower energy electronic state of the same spin state. By a combination of internal conversions and vibrational relaxations, a molecule in an excited electronic state may return to the ground electronic state without emitting a photon. A related form of radiationless deactivation is an EXTERNAL CONVERSION in which excess energy is transferred to the solvent or to another component of the sample's matrix.

Let's use Figure 10.48 to illustrate how a molecule can relax back to its ground state without emitting a photon. Suppose our molecule is in the highest vibrational energy level of the second electronic excited state. After a series of vibrational relaxations brings the molecule to the lowest vibrational energy level of S_2, it undergoes an internal conversion into a higher vibrational energy level of the first excited electronic state. Vibrational relaxations bring the molecule to the lowest vibrational energy level of S_1. Following an internal conversion into a higher vibrational energy level of the ground state, the molecule continues to undergo vibrational relaxation until it reaches the lowest vibrational energy level of S_0.

A final form of radiationless deactivation is an INTERSYSTEM CROSSING in which a molecule in the ground vibrational energy level of an excited electronic state passes into one of the higher vibrational energy levels of a lower energy electronic state with a different spin state. For example, an intersystem crossing is shown in [Figure 10.48](#) between the singlet excited state S_1 and the triplet excited state T_1.

RELAXATION BY FLUORESCENCE

Fluorescence occurs when a molecule in an excited state's lowest vibrational energy level returns to a lower energy electronic state by emitting a photon. Because molecules return to their ground state by the fastest mechanism, fluorescence is observed only if it is a more efficient means of relaxation than a combination of internal conversions and vibrational relaxations.

A quantitative expression of fluorescence efficiency is the FLUORESCENT QUANTUM YIELD, Φ_f, which is the fraction of excited state molecules that return to the ground state by fluorescence. The fluorescent quantum yields range from 1 when every molecule in an excited state undergoes fluorescence, to 0 when fluorescence does not occur.

The intensity of fluorescence, I_f, is proportional to the amount of radiation absorbed by the sample, $P_0 - P_T$, and the fluorescent quantum yield

$$I_f = k\Phi_f(P_0 - P_T) \qquad 10.25$$

where k is a constant that accounts for the efficiency of collecting and detecting the fluorescent emission. From Beer's law we know that

$$\frac{P_T}{P_0} = 10^{-\varepsilon bC} \qquad 10.26$$

where C is the concentration of the fluorescing species. Solving equation 10.26 for P_T and substituting into equation 10.25 gives, after simplifying

$$I_f = k\Phi_f P_0(1 - 10^{-\varepsilon bC}) \qquad 10.27$$

When $\varepsilon bC < 0.01$, which often is the case when the analyte's concentration is small, equation 10.27 simplifies to

$$I_f = 2.303k\Phi_f \varepsilon bC P_0 = k'P_0 \qquad 10.28$$

where k' is a collection of constants. The intensity of fluorescence, therefore, increases with an increase in the quantum efficiency, the source's incident power, and the molar absorptivity and the concentration of the fluorescing species.

Fluorescence generally is observed when the molecule's lowest energy absorption is a $\pi \rightarrow \pi^*$ transition, although some $n \rightarrow \pi^*$ transitions show weak fluorescence. Many unsubstituted, nonheterocyclic aromatic compounds have a favorable fluorescence quantum yield, although substitutions on the aromatic ring can effect Φ_f significantly. For example, the presence of an electron-withdrawing group, such as $-NO_2$, decreases Φ_f, while adding an electron-donating group, such as $-OH$, increases Φ_f. Fluorescence also increases for aromatic ring systems and for aromatic molecules

with rigid planar structures. Figure 10.49 shows the fluorescence of quinine under a UV lamp.

A molecule's fluorescent quantum yield also is influenced by external variables, such as temperature and solvent. Increasing the temperature generally decreases Φ_f because more frequent collisions between the molecule and the solvent increases external conversion. A decrease in the solvent's viscosity decreases Φ_f for similar reasons. For an analyte with acidic or basic functional groups, a change in pH may change the analyte's structure and its fluorescent properties.

As shown in Figure 10.48, fluorescence may return the molecule to any of several vibrational energy levels in the ground electronic state. Fluorescence, therefore, occurs over a range of wavelengths. Because the change in energy for fluorescent emission generally is less than that for absorption, a molecule's fluorescence spectrum is shifted to higher wavelengths than its absorption spectrum.

RELAXATION BY PHOSPHORESCENCE

A molecule in a triplet electronic excited state's lowest vibrational energy level normally relaxes to the ground state by an intersystem crossing to a singlet state or by an external conversion. Phosphorescence occurs when the molecule relaxes by emitting a photon. As shown in Figure 10.48, phosphorescence occurs over a range of wavelengths, all of which are at lower energies than the molecule's absorption band. The intensity of phosphorescence, I_p, is given by an equation similar to equation 10.28 for fluorescence

$$I_p = 2.303 k \Phi_p \varepsilon b C P_0 = k' P_0 \qquad 10.29$$

where Φ_p is the PHOSPHORESCENT QUANTUM YIELD.

Phosphorescence is most favorable for molecules with $n \rightarrow \pi^*$ transitions, which have a higher probability for an intersystem crossing than $\pi \rightarrow \pi^*$ transitions. For example, phosphorescence is observed with aromatic molecules that contain carbonyl groups or heteroatoms. Aromatic compounds that contain halide atoms also have a higher efficiency for phosphorescence. In general, an increase in phosphorescence corresponds to a decrease in fluorescence.

Because the average lifetime for phosphorescence can be quite long, ranging from 10^{-4}–10^4 s, the phosphorescent quantum yield usually is quite small. An improvement in Φ_p is realized by decreasing the efficiency of external conversion. This is accomplished in several ways, including lowering the temperature, using a more viscous solvent, depositing the sample on a solid substrate, or trapping the molecule in solution. Figure 10.50 shows an example of phosphorescence.

EXCITATION VERSUS EMISSION SPECTRA

Photoluminescence spectra are recorded by measuring the intensity of emitted radiation as a function of either the excitation wavelength or the
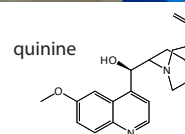
Figure 10.49 Tonic water, which contains quinine, is fluorescent when placed under a UV lamp. Source: Splarka (commons.wikipedia.org).
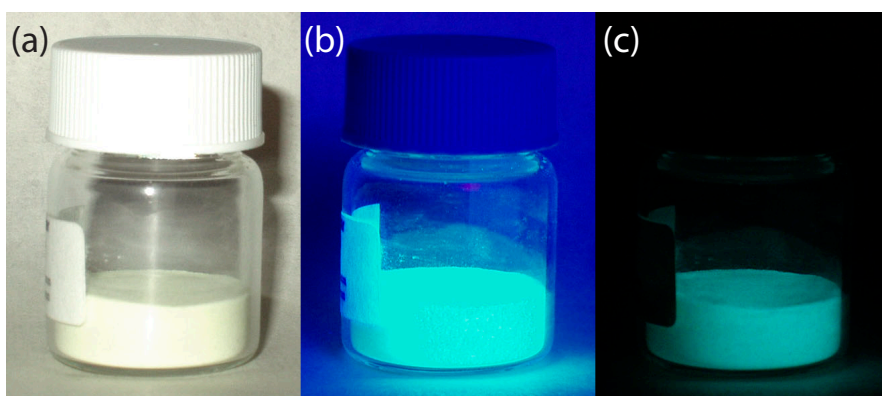
Figure 10.50 An europium doped strontium silicate-aluminum oxide powder under (a) natural light, (b) a long-wave UV lamp, and (c) in total darkness. The photo taken in total darkness shows the phosphorescent emission. Source: modified from Splarka (commons.wikipedia.org).

emission wavelength. An EXCITATION SPECTRUM is obtained by monitoring emission at a fixed wavelength while varying the excitation wavelength. When corrected for variations in the source's intensity and the detector's response, a sample's excitation spectrum is nearly identical to its absorbance spectrum. The excitation spectrum provides a convenient means for selecting the best excitation wavelength for a quantitative or qualitative analysis.

In an EMISSION SPECTRUM a fixed wavelength is used to excite the sample and the intensity of emitted radiation is monitored as function of wavelength. Although a molecule has a single excitation spectrum, it has two emission spectra, one for fluorescence and one for phosphorescence. Figure 10.51 shows the UV absorption spectrum and the UV fluorescence emission spectrum for quinine.

10F.2 Instrumentation

The basic instrumentation for monitoring fluorescence and phosphorescence—a source of radiation, a means of selecting a narrow band of radiation, and a detector—are the same as those for absorption spectroscopy. The unique demands of fluorescence and phosphorescence, however, re-
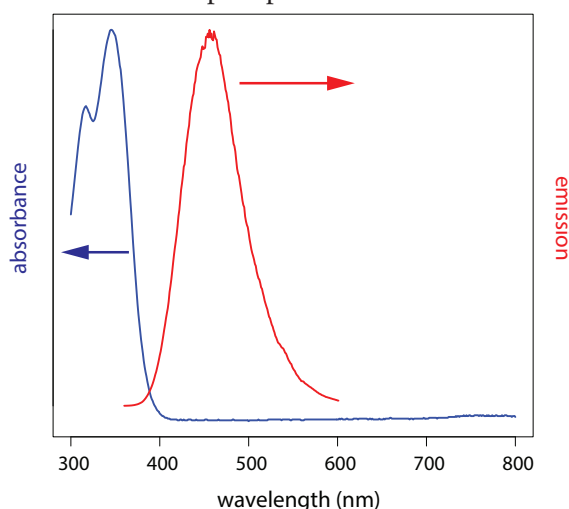
Figure 10.51 Absorbance spectrum and fluorescence emission spectrum for quinine in 0.05 M H_2SO_4. The emission spectrum uses an excitation wavelength of 350 nm with a bandwidth of 20 nm. Both spectra are normalized so that the maximum absorbance is 1.00 and the maximum emission is 1.00. The actual maximum absorbance is 0.444 and the actual maximum emission is 126747. Source: data from Daniel Scott, Department of Chemistry & Biochemistry, DePauw University.
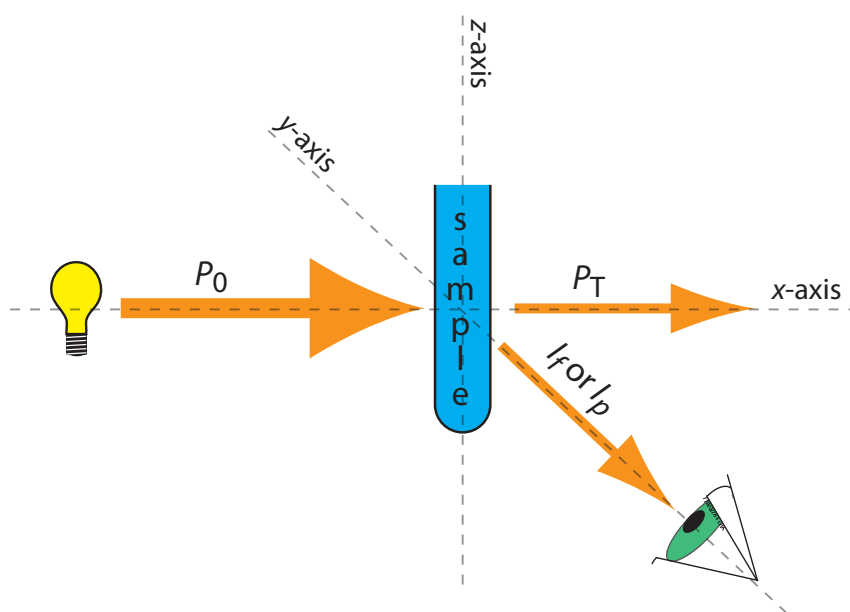
Figure 10.52 Schematic diagram showing the orientation of the source and the detector when measuring fluorescence and phosphorescence. Contrast this to Figure 10.21, which shows the orientation for absorption spectroscopy.

quire some modifications to the instrument designs seen earlier in Figure 10.25 (filter photometer), Figure 10.26 (single-beam spectrophotometer), Figure 10.27 (double-beam spectrophotometer), and Figure 10.28 (diode array spectrometer). The most important difference is that the detector cannot be placed directly across from the source. Figure 10.52 shows why this is the case. If we place the detector along the source's axis it receives both the transmitted source radiation, P_T, and the fluorescent, I_f, or phosphorescent, I_p, radiation. Instead, we rotate the director and place it at 90° to the source.

INSTRUMENTS FOR MEASURING FLUORESCENCE

Figure 10.53 shows the basic design of an instrument for measuring fluorescence, which includes two wavelength selectors, one for selecting the source's excitation wavelength and one for selecting the analyte's emission wavelength. In a FLUORIMETER the excitation and emission wavelengths are selected using absorption or interference filters. The excitation source for a fluorimeter usually is a low-pressure Hg vapor lamp that provides intense emission lines distributed throughout the ultraviolet and visible region. When a monochromator is used to select the excitation and the emission wavelengths, the instrument is called a SPECTROFLUOROMETER. With a monochromator the excitation source usually is a high-pressure Xe arc lamp, which has a continuous emission spectrum. Either instrumental design is appropriate for quantitative work, although only a spectrofluorometer can record an excitation or emission spectrum.

A Hg vapor lamp has emission lines at 254, 312, 365, 405, 436, 546, 577, 691, and 773 nm

The sample cells for molecular fluorescence are similar to those for molecular absorption (see Figure 10.30). Remote sensing using a fiber optic probe (see Figure 10.31) is possible using with either a fluorimeter or spectrofluorometer. An analyte that is fluorescent is monitored directly. For an analyte that is not fluorescent, a suitable fluorescent probe molecule is
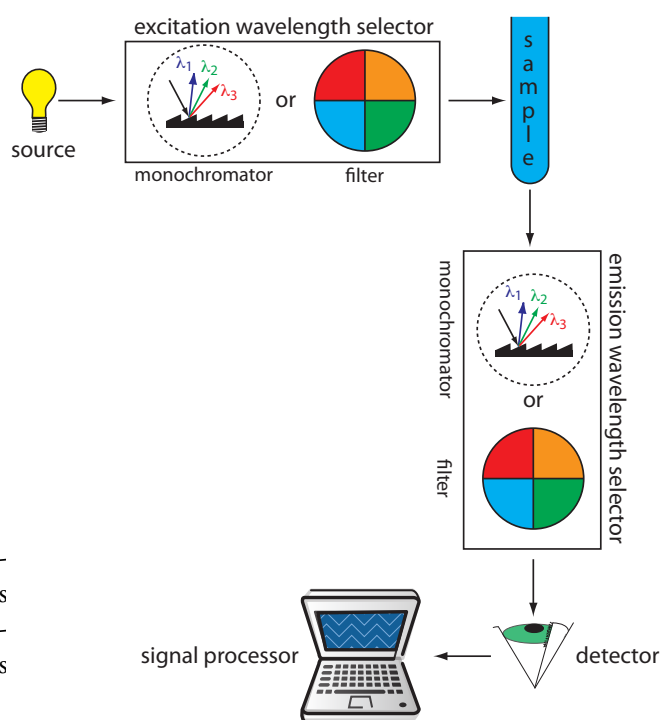
Figure 10.53 Schematic diagram for measuring fluorescence showing the placement of the wavelength selectors for excitation and emission. When a filter is used the instrument is called a fluorimeter and when a monochromator is used the instrument is called a spectrofluorimeter.

incorporated into the tip of the fiber optic probe. The analyte's reaction with the probe molecule leads to an increase or decrease in fluorescence.

INSTRUMENTS FOR MEASURING PHOSPHORESCENCE

An instrument for molecular phosphorescence must discriminate between phosphorescence and fluorescence. Because the lifetime for fluorescence is shorter than that for phosphorescence, discrimination is achieved by incorporating a delay between exciting the sample and measuring the phosphorescent emission. Figure 10.54 shows how two out-of-phase choppers allow us to block fluorescent emission from reaching the detector when the sample is being excited and to prevent the source radiation from causing fluorescence when we are measuring the phosphorescent emission.

Because phosphorescence is such a slow process, we must prevent the excited state from relaxing by external conversion. One way this is accomplished is by dissolving the sample in a suitable organic solvent, usually a mixture of ethanol, isopentane, and diethylether. The resulting solution is frozen at liquid-N_2 temperatures to form an optically clear solid. The solid matrix minimizes external conversion due to collisions between the analyte and the solvent. External conversion also is minimized by immobilizing the sample on a solid substrate, making possible room temperature measurements. One approach is to place a drop of a solution that contains the analyte on a small disc of filter paper. After drying the sample under a heat lamp, the sample is placed in the spectrofluorometer for analysis. Other solid substrates include silica gel, alumina, sodium acetate, and sucrose. This approach is particularly useful for the analysis of thin layer chromatography plates.

Figure 10.54 Schematic diagram showing how choppers are used to prevent fluorescent emission from interfering with the measurement of phosphorescent emission.

10F.3   Quantitative Applications

Molecular fluorescence and, to a lesser extent, phosphorescence are used for the direct or indirect quantitative analysis of analytes in a variety of matrices. A direct quantitative analysis is possible when the analyte's fluorescent or phosphorescent quantum yield is favorable. If the analyte is not fluorescent or phosphorescent, or if the quantum yield is unfavorable, then an indirect analysis may be feasible. One approach is to react the analyte with a reagent to form a product that is fluorescent or phosphorescent. Another approach is to measure a decrease in fluorescence or phosphorescence when the analyte is added to a solution that contains a fluorescent or phosphorescent probe molecule. A decrease in emission is observed when the reaction between the analyte and the probe molecule enhances radiationless deactivation or results in a nonemitting product. The application of fluorescence and phosphorescence to inorganic and organic analytes are considered in this section.

INORGANIC ANALYTES

Except for a few metal ions, most notably UO_2^+, most inorganic ions are not sufficiently fluorescent for a direct analysis. Many metal ions are determined indirectly by reacting with an organic ligand to form a fluorescent or, less commonly, a phosphorescent metal–ligand complex. One example is the reaction of Al^{3+} with the sodium salt of 2, 4, 3′-trihydroxyazobenzene-5′-sulfonic acid—also known as alizarin garnet R—which forms a fluorescent metal–ligand complex (Figure 10.55). The analysis is carried out using an excitation wavelength of 470 nm, with fluorescence monitored at 500 nm. Table 10.12 provides additional examples of chelating reagents that form fluorescent metal–ligand complexes with metal ions. A few inorganic nonmetals are determined by their ability to decrease, or quench, the fluorescence of another species. One example is the analysis for F^- based on its ability to quench the fluorescence of the Al^{3+}–alizarin garnet R complex.

ORGANIC ANALYTES

As noted earlier, organic compounds that contain aromatic rings generally are fluorescent and aromatic heterocycles often are phosphorescent. Table 10.13 provides examples of several important biochemical, pharmaceutical, and environmental compounds that are analyzed quantitatively by fluorimetry or phosphorimetry. If an organic analyte is not naturally fluorescent or phosphorescent, it may be possible to incorporate it into a chemical reaction that produces a fluorescent or phosphorescent product. For example, the enzyme creatine phosphokinase is determined by using it to catalyze the formation of creatine from phosphocreatine. Reacting the creatine with ninhydrin produces a fluorescent product of unknown structure.
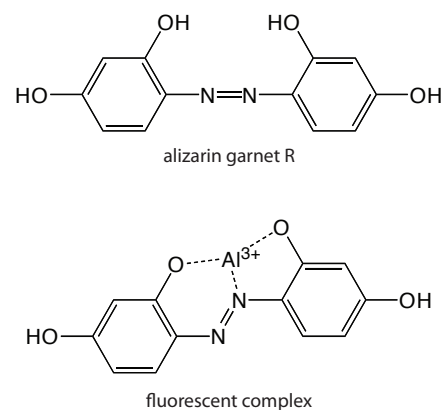
Figure 10.55 Structure of alizarin garnet R and its metal–ligand complex with Al^{3+}.

Table 10.12 Chelating Agents for the Fluorescence Analysis of Metal Ions	
chelating agent	metal ions
8-hydroxyquinoline	Al^{3+}, Be^{2+}, Zn^{2+}, Li^{+}, Mg^{2+} (and others)
flavonal	Zr^{2+}, Sn^{4+}
benzoin	$B_4O_7^{2-}$, Zn^{2+}
2′, 3, 4′, 5, 7-pentahydroxyflavone	Be^{2+}
2-(*o*-hydroxyphenyl) benzoxazole	Cd^{2+}

Table 10.13 Examples of Naturally Photoluminescent Organic Analytes	
class	compounds (F = fluorescence; P = phosphorescence)
aromatic amino acids	phenylalanine (F) tyrosine (F) tryptophan (F, P)
vitamins	vitamin A (F) vitamin B2 (F) vitamin B6 (F) vitamin B12 (F) vitamin E (F) folic acid (F)
catecholamines	dopamine (F) norepinephrine (F)
pharmaceuticals and drugs	quinine (F) salicylic acid (F, P) morphine (F) barbiturates (F) LSD (F) codeine (P) caffeine (P) sulfanilamide (P)
environmental pollutants	pyrene (F) benzo[a]pyrene (F) organothiophosphorous pesticides (F) carbamate insecticides (F) DDT (P)

STANDARDIZING THE METHOD

From equation 10.28 and equation 10.29 we know that the intensity of fluorescence or phosphorescence is a linear function of the analyte's concentration provided that the sample's absorbance of source radiation ($A = \varepsilon b C$) is less than approximately 0.01. Calibration curves often are linear over four to six orders of magnitude for fluorescence and over two to four orders of magnitude for phosphorescence. For higher concentrations of analyte the

calibration curve becomes nonlinear because the assumptions that led to equation 10.28 and equation 10.29 no longer apply. Nonlinearity may be observed for smaller concentrations of analyte fluorescent or phosphorescent contaminants are present. As discussed earlier, quantum efficiency is sensitive to temperature and sample matrix, both of which must be controlled when using external standards. In addition, emission intensity depends on the molar absorptivity of the photoluminescent species, which is sensitive to the sample matrix.

Representative Method 10.3

Determination of Quinine in Urine

DESCRIPTION OF METHOD

Quinine is an alkaloid used to treat malaria. It is a strongly fluorescent compound in dilute solutions of H_2SO_4 ($\Phi_f = 0.55$). Quinine's excitation spectrum has absorption bands at 250 nm and 350 nm and its emission spectrum has a single emission band at 450 nm. Quinine is excreted rapidly from the body in urine and is determined by measuring its fluorescence following its extraction from the urine sample.

PROCEDURE

Transfer a 2.00-mL sample of urine to a 15-mL test tube and use 3.7 M NaOH to adjust its pH to between 9 and 10. Add 4 mL of a 3:1 (v/v) mixture of chloroform and isopropanol and shake the contents of the test tube for one minute. Allow the organic and the aqueous (urine) layers to separate and transfer the organic phase to a clean test tube. Add 2.00 mL of 0.05 M H_2SO_4 to the organic phase and shake the contents for one minute. Allow the organic and the aqueous layers to separate and transfer the aqueous phase to the sample cell. Measure the fluorescent emission at 450 nm using an excitation wavelength of 350 nm. Determine the concentration of quinine in the urine sample using a set of external standards in 0.05 M H_2SO_4, prepared from a 100.0 ppm solution of quinine in 0.05 M H_2SO_4. Use distilled water as a blank.

QUESTIONS

1. Chloride ion quenches the intensity of quinine's fluorescent emission. For example, in the presence of 100 ppm NaCl (61 ppm Cl⁻) quinine's emission intensity is only 83% of its emission intensity in the absence of chloride. The presence of 1000 ppm NaCl (610 ppm Cl⁻) further reduces quinine's fluorescent emission to less than 30% of its emission intensity in the absence of chloride. The concentration of chloride in urine typically ranges from 4600–6700 ppm Cl⁻. Explain how this procedure prevents an interference from chloride.

    The procedure uses two extractions. In the first of these extractions, quinine is separated from urine by extracting it into a mixture of

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of quinine in urine provides an instructive example of a typical procedure. The description here is based on Mule, S. J.; Hushin, P. L. *Anal. Chem.* **1971**, *43*, 708–711, and O'Reilly, J. E.; *J. Chem. Educ.* **1975**, *52*, 610–612.

Figure 10.49 shows the fluorescence of the quinine in tonic water.

chloroform and isopropanol, leaving the chloride ion behind in the original sample.

2. Samples of urine may contain small amounts of other fluorescent compounds, which will interfere with the analysis if they are carried through the two extractions. Explain how you can modify the procedure to take this into account?

    One approach is to prepare a blank that uses a sample of urine known to be free of quinine. Subtracting the blank's fluorescent signal from the measured fluorescence from urine samples corrects for the interfering compounds.

3. The fluorescent emission for quinine at 450 nm can be induced using an excitation frequency of either 250 nm or 350 nm. The fluorescent quantum efficiency is the same for either excitation wavelength. Quinine's absorption spectrum shows that ε_{250} is greater than ε_{350}. Given that quinine has a stronger absorbance at 250 nm, explain why its fluorescent emission intensity is greater when using 350 nm as the excitation wavelength.

    From equation 10.28 we know that I_f is a function of the following terms: k, Φ_f, P_0, ε, b, and C. We know that Φ_f, b, and C are the same for both excitation wavelengths and that ε is larger for a wavelength of 250 nm; we can, therefore, ignore these terms. The greater emission intensity when using an excitation wavelength of 350 nm must be due to a larger value for P_0 or k. In fact, P_0 at 350 nm for a high-pressure Xe arc lamp is about 170% of that at 250 nm. In addition, the sensitivity of a typical photomultiplier detector (which contributes to the value of k) at 350 nm is about 140% of that at 250 nm.

Example 10.11

To evaluate the method described in Representative Method 10.3, a series of external standard are prepared and analyzed, providing the results shown in the following table. All fluorescent intensities are corrected using a blank prepared from a quinine-free sample of urine. The fluorescent intensities are normalized by setting I_f for the highest concentration standard to 100.

[quinine] (µg/mL)	I_f
1.00	10.11
3.00	30.20
5.00	49.84
7.00	69.89
10.00	100.0

After ingesting 10.0 mg of quinine, a volunteer provides a urine sample 24-h later. Analysis of the urine sample gives a relative emission intensity

of 28.16. Report the concentration of quinine in the sample in mg/L and the percent recovery for the ingested quinine.

SOLUTION

Linear regression of the relative emission intensity versus the concentration of quinine in the standards gives the calibration curve shown to the right and the following calibration equation.

$$I_f = 0.122 + 9.978 \times \frac{\text{g quinine}}{\text{mL}}$$

Substituting the sample's relative emission intensity into the calibration equation gives the concentration of quinine as 2.81 μg/mL. Because the volume of urine taken, 2.00 mL, is the same as the volume of 0.05 M H_2SO_4 used to extract the quinine, the concentration of quinine in the urine also is 2.81 μg/mL. The recovery of the ingested quinine is

$$\frac{\dfrac{2.81\,\mu\text{g quinine}}{\text{mL urine}} \times 2.00\ \text{mL urine} \times \dfrac{1\ \text{mg}}{1000\ \mu\text{g}}}{10.0\ \text{mg quinine ingested}} \times 100 = 0.0562\%$$
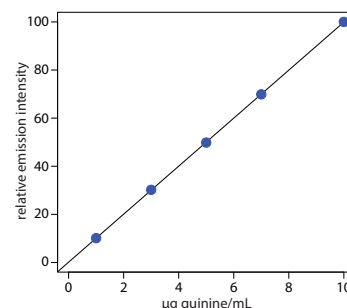
It can take 10–11 days for the body to completely excrete quinine so it is not surprising that such a small amount of quinine is recovered from this sample of urine.

10F.4   Evaluation of Photoluminescence Spectroscopy

SCALE OF OPERATION

Photoluminescence spectroscopy is used for the routine analysis of trace and ultratrace analytes in macro and meso samples. Detection limits for fluorescence spectroscopy are influenced by the analyte's quantum yield. For an analyte with $\Phi_f > 0.5$, a picomolar detection limit is possible when using a high quality spectrofluorometer. For example, the detection limit for quinine sulfate, for which Φ is 0.55, generally is between 1 part per billion and 1 part per trillion. Detection limits for phosphorescence are somewhat higher, with typical values in the nanomolar range for low-temperature phosphorimetry and in the micromolar range for room-temperature phosphorimetry using a solid substrate.

See Figure 3.5 to review the meaning of macro and meso for describing samples, and the meaning of major, minor, and ultratrace for describing analytes.

ACCURACY

The accuracy of a fluorescence method generally is between 1–5% when spectral and chemical interferences are insignificant. Accuracy is limited by the same types of problems that affect other optical spectroscopic methods. In addition, accuracy is affected by interferences that affect the fluorescent quantum yield. The accuracy of phosphorescence is somewhat greater than that for fluorescence.
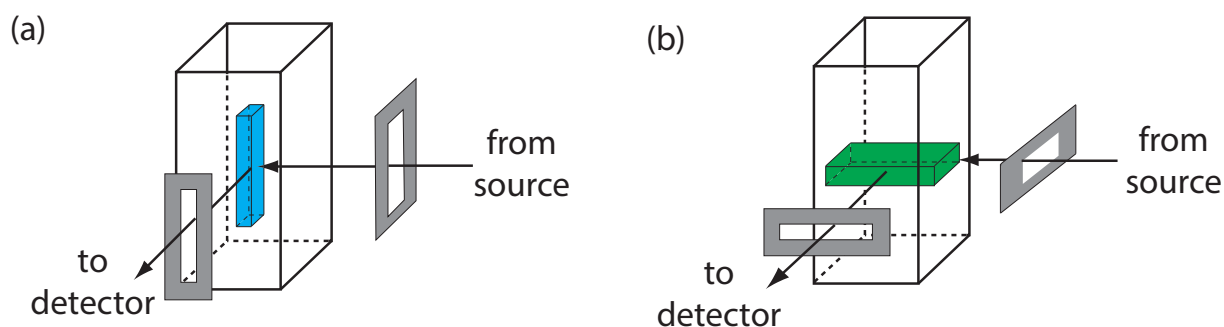
Figure 10.56 Use of slit orientation to change the volume from which fluorescence is measured: (a) vertical slit orientation; (b) horizontal slit orientation. Suppose the slit's dimensions are 0.1 mm × 3 mm. In (a) the dimensions of the **sampling volume** are 0.1 mm × 0.1 mm × 3 mm, or 0.03 mm^3. For (b) the dimensions of the **sampling volume** are 0.1 mm × 3 mm × 3 mm, or 0.9 mm^3, a 30-fold increase in the sampling volume.

PRECISION

The relative standard deviation for fluorescence usually is between 0.5–2% when the analyte's concentration is well above its detection limit. Precision usually is limited by the stability of the excitation source. The precision for phosphorescence often is limited by reproducibility in preparing samples for analysis, with relative standard deviations of 5–10% being common.

SENSITIVITY

From equation 10.28 and equation 10.29 we know that the sensitivity of a fluorescent or a phosphorescent method is affected by a number of parameters. We already have considered the importance of quantum yield and the effect of temperature and solution composition on Φ_f and Φ_p. Besides quantum yield, sensitivity is improved by using an excitation source that has a greater emission intensity, P_0, at the desired wavelength, and by selecting an excitation wavelength for which the analyte has a greater molar absorptivity, ε. Another approach for improving sensitivity is to increase the volume from which emission is monitored. Figure 10.56 shows how rotating a monochromator's slits from their usual vertical orientation to a horizontal orientation increases the sampling volume. The result can increase the emission from the sample by 5–30×.

SELECTIVITY

The selectivity of fluorescence and phosphorescence is superior to that of absorption spectrophotometry for two reasons: first, not every compound that absorbs radiation is fluorescent or phosphorescent; and, second, selectivity between an analyte and an interferent is possible if there is a difference in either their excitation or their emission spectra. The total emission intensity is a linear sum of that from each fluorescent or phosphorescent species. The analysis of a sample that contains n analytes, therefore, is accomplished by measuring the total emission intensity at n wavelengths.

As with other optical spectroscopic methods, fluorescent and phosphorescent methods provide a rapid means for analyzing samples and are capable of automation. Fluorimeters are relatively inexpensive, ranging from several hundred to several thousand dollars, and often are satisfactory for quantitative work. Spectrofluorometers are more expensive, with models often exceeding $50,000.

10G   Atomic Emission Spectroscopy

The focus of this section is on the emission of ultraviolet and visible radiation following the thermal excitation of atoms. Atomic emission spectroscopy has a long history. Qualitative applications based on the color of flames were used in the smelting of ores as early as 1550 and were more fully developed around 1830 with the observation of atomic spectra generated by flame emission and spark emission.[18] Quantitative applications based on the atomic emission from electric sparks were developed by Lockyer in the early 1870 and quantitative applications based on flame emission were pioneered by Lundegardh in 1930. Atomic emission based on emission from a plasma was introduced in 1964.

For an on-line introduction to much of the material in this section, see Atomic Emission Spectroscopy (AES) by Tomas Spudich and Alexander Scheeline, a resource that is part of the Analytical Sciences Digital Library.

10G.1   Atomic Emission Spectra

Atomic emission occurs when a valence electron in a higher energy atomic orbital returns to a lower energy atomic orbital. Figure 10.57 shows a portion of the energy level diagram for sodium, which consists of a series of discrete lines at wavelengths that correspond to the difference in energy between two atomic orbitals.

The intensity of an atomic emission line, I_e, is proportional to the number of atoms, N^*, that populate the excited state,

$$I_e = kN^* \qquad 10.30$$

where k is a constant that accounts for the efficiency of the transition. If a system of atoms is in thermal equilibrium, the population of excited state i is related to the total concentration of atoms, N, by the Boltzmann distribution. For many elements at temperatures of less than 5000 K the Boltzmann distribution is approximated as

$$N^* = N\left(\frac{g_i}{g_o}\right)e^{-E_i/kT} \qquad 10.31$$

where g_i and g_0 are statistical factors that account for the number of equivalent energy levels for the excited state and the ground state, E_i is the energy of the excited state relative to a ground state energy, E_0, k is Boltzmann's constant (1.3807×10^{-23} J/K), and T is the temperature in kelvin. From equation 10.31 we expect that excited states with lower energies have larger

Figure 10.57 Valence shell energy level diagram for sodium. The wavelengths corresponding to several transitions are shown. Note that this is the same energy level diagram as Figure 10.19.

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18 Dawson, J. B. *J. Anal. At. Spectrosc.* **1991**, *6*, 93–98.

populations and more intense emission lines. We also expect emission intensity to increase with temperature.

10G.2 Equipment

An atomic emission spectrometer is similar in design to the instrumentation for atomic absorption. In fact, it is easy to adapt most flame atomic absorption spectrometers for atomic emission by turning off the hollow cathode lamp and monitoring the difference between the emission intensity when aspirating the sample and when aspirating a blank. Many atomic emission spectrometers, however, are dedicated instruments designed to take advantage of features unique to atomic emission, including the use of plasmas, arcs, sparks, and lasers as atomization and excitation sources, and an enhanced capability for multielemental analysis.

ATOMIZATION AND EXCITATION

Atomic emission requires a means for converting into a free gaseous atom an analyte that is present in a solid, liquid, or solution sample. The same source of thermal energy used for atomization usually serves as the excitation source. The most common methods are flames and plasmas, both of which are useful for liquid or solution samples. Solid samples are analyzed by dissolving in a solvent and using a flame or plasma atomizer.

FLAME SOURCES

A Meker burner is similar to the more common Bunsen burner found in most laboratories; it is designed to allow for higher temperatures and for a larger diameter flame.

Atomization and excitation in flame atomic emission is accomplished with the same nebulization and spray chamber assembly used in atomic absorption (Figure 10.42). The burner head consists of a single or multiple slots, or a Meker-style burner. Older atomic emission instruments often used a total consumption burner in which the sample is drawn through a capillary tube and injected directly into the flame.

PLASMA SOURCES

A PLASMA is a hot, partially ionized gas that contains an abundant concentration of cations and electrons. The plasma used in atomic emission is formed by ionizing a flowing stream of argon gas, producing argon ions and electrons. A plasma's high temperature results from resistive heating as the electrons and argon ions move through the gas. Because a plasma operates at a much higher temperature than a flame, it provides for a better atomization efficiency and a higher population of excited states.

A schematic diagram of the inductively coupled plasma source (ICP) is shown in Figure 10.58. The ICP torch consists of three concentric quartz tubes, surrounded at the top by a radio-frequency induction coil. The sample is mixed with a stream of Ar using a nebulizer, and is carried to the plasma through the torch's central capillary tube. Plasma formation is initiated by a spark from a Tesla coil. An alternating radio-frequency cur-

rent in the induction coil creates a fluctuating magnetic field that induces the argon ions and the electrons to move in a circular path. The resulting collisions with the abundant unionized gas give rise to resistive heating, providing temperatures as high as 10 000 K at the base of the plasma, and between 6000 and 8000 K at a height of 15–20 mm above the coil, where emission usually is measured. At these high temperatures the outer quartz tube must be thermally isolated from the plasma. This is accomplished by the tangential flow of argon shown in the schematic diagram.

MULTIELEMENTAL ANALYSIS

Atomic emission spectroscopy is ideally suited for a multielemental analysis because all analytes in a sample are excited simultaneously. If the instrument includes a scanning monochromator, we can program it to move rapidly to an analyte's desired wavelength, pause to record its emission intensity, and then move to the next analyte's wavelength. This sequential analysis allows for a sampling rate of 3–4 analytes per minute.

Another approach to a multielemental analysis is to use a multichannel instrument that allows us to monitor simultaneously many analytes. A simple design for a multichannel spectrometer, shown in Figure 10.59, couples a monochromator with multiple detectors that are positioned in a semicircular array around the monochromator at positions that correspond to the wavelengths for the analytes.

Figure 10.58 Schematic diagram of an inductively coupled plasma torch.

10G.3 Quantitative Applications

Atomic emission is used widely for the analysis of trace metals in a variety of sample matrices. The development of a quantitative atomic emission method requires several considerations, including choosing a source for
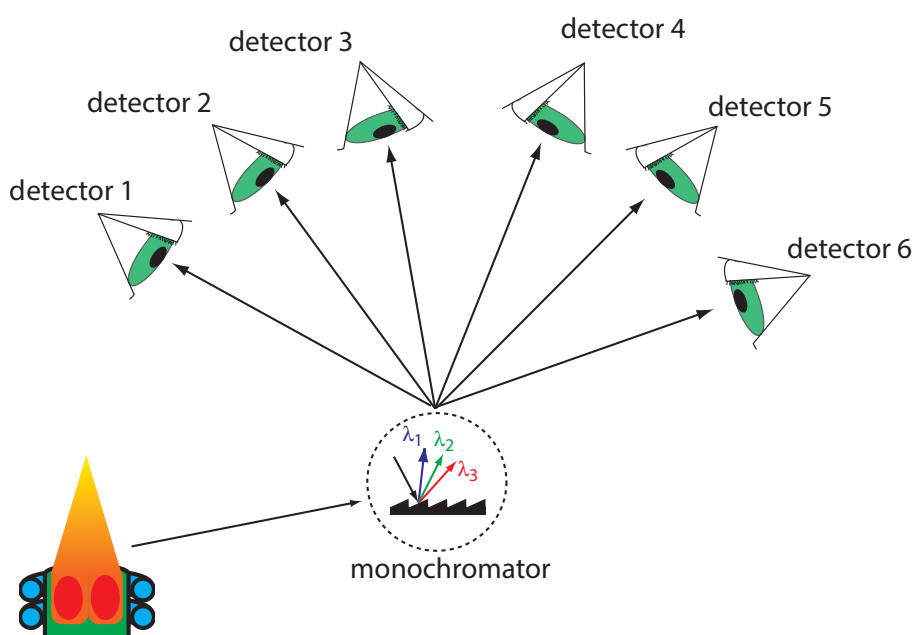
Figure 10.59 Schematic diagram of a multichannel atomic emission spectrometer for the simultaneous analysis of several elements. Instruments may contain as many as 48–60 detectors.

atomization and excitation, selecting a wavelength and slit width, preparing the sample for analysis, minimizing spectral and chemical interferences, and selecting a method of standardization.

CHOICE OF ATOMIZATION AND EXCITATION SOURCE

Except for the alkali metals, detection limits when using an ICP are significantly better than those obtained with flame emission (Table 10.14). Plasmas also are subject to fewer spectral and chemical interferences. For these reasons a plasma emission source is usually the better choice.

SELECTING THE WAVELENGTH AND SLIT WIDTH

The choice of wavelength is dictated by the need for sensitivity and the need to avoid interferences from the emission lines of other constituents in the sample. Because an analyte's atomic emission spectrum has an abundance of emission lines—particularly when using a high temperature plasma source—it is inevitable that there will be some overlap between emission lines. For example, an analysis for Ni using the atomic emission line at 349.30 nm is complicated by the atomic emission line for Fe at 349.06 nm.

Table 10.14 Detection Limits for Atomic Emission[a]		
	detection limit in µg/mL	
element	flame emission	ICP
Ag	2	0.2
Al	3	0.2
As	2000	2
Ca	0.1	0.0001
Cd	300	0.07
Co	5	0.1
Cr	1	0.08
Fe	10	0.09
Hg	150	1
K	0.01	30
Li	0.001	0.02
Mg	1	0.003
Mn	1	0.01
Na	0.01	0.1
Ni	10	0.2
Pb	0.2	1
Pt	2000	0.9
Sn	100	3
Zn	1000	0.1

[a] Source: Parsons, M. L.; Major, S.; Forster, A. R.; App. Spectrosc. 1983, 37, 411–418.

A narrower slit width provides better resolution, but at the cost of less radia-tion reaching the detector. The easiest approach to selecting a wavelength is to record the sample's emission spectrum and look for an emission line that provides an intense signal and is resolved from other emission lines.

PREPARING THE SAMPLE

Flame and plasma sources are best suited for samples in solution and in liquid form. Although a solid sample can be analyzed by directly inserting it into the flame or plasma, they usually are first brought into solution by digestion or extraction.
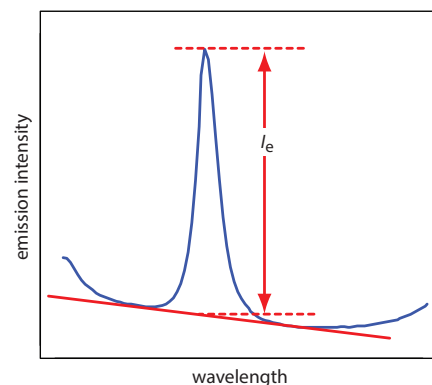
MINIMIZING SPECTRAL INTERFERENCES

The most important spectral interference is broad, background emission from the flame or plasma and emission bands from molecular species. This background emission is particularly severe for flames because the tempera-ture is insufficient to break down refractory compounds, such as oxides and hydroxides. Background corrections for flame emission are made by scanning over the emission line and drawing a baseline (Figure 10.60). Because a plasma's temperature is much higher, a background interference due to molecular emission is less of a problem. Although emission from the plasma's core is strong, it is insignificant at a height of 10–30 mm above the core where measurements normally are made.

Figure 10.60 Method for correcting an analyte's emission for the flame's background emission.

MINIMIZING CHEMICAL INTERFERENCES

Flame emission is subject to the same types of chemical interferences as atomic absorption; they are minimized using the same methods: by ad-justing the flame's composition and by adding protecting agents, releasing agents, or ionization suppressors. An additional chemical interference re-sults from SELF-ABSORPTION. Because the flame's temperature is greatest at its center, the concentration of analyte atoms in an excited state is greater at the flame's center than at its outer edges. If an excited state atom in the flame's center emits a photon, then a ground state atom in the cooler, outer regions of the flame may absorb the photon, which decreases the emission intensity. For higher concentrations of analyte self-absorption may invert the center of the emission band (Figure 10.61).

Chemical interferences when using a plasma source generally are not significant because the plasma's higher temperature limits the formation of nonvolatile species. For example, PO_4^{3-} is a significant interferent when analyzing samples for Ca^{2+} by flame emission, but has a negligible effect when using a plasma source. In addition, the high concentration of elec-trons from the ionization of argon minimizes ionization interferences.

Figure 10.61 Atomic emission lines for (a) a low concentration of analyte, and (b) a high concentration of analyte showing the effect of self-absorption.

STANDARDIZING THE METHOD

From [equation 10.30](#) we know that emission intensity is proportional to the population of the analyte's excited state, N^*. If the flame or plasma is in thermal equilibrium, then the excited state population is proportional to the analyte's total population, N, through the Boltzmann distribution ([equation 10.31](#)).

A calibration curve for flame emission usually is linear over two to three orders of magnitude, with ionization limiting linearity when the analyte's concentrations is small and self-absorption limiting linearity at higher concentrations of analyte. When using a plasma, which suffers from fewer chemical interferences, the calibration curve often is linear over four to five orders of magnitude and is not affected significantly by changes in the matrix of the standards.

Emission intensity is affected significantly by many parameters, including the temperature of the excitation source and the efficiency of atomization. An increase in temperature of 10 K, for example, produces a 4% increase in the fraction of Na atoms in the $3p$ excited state, an uncertainty in the signal that may limit the use of external standards. The method of internal standards is used when the variations in source parameters are difficult to control. To compensate for changes in the temperature of the excitation source, the internal standard is selected so that its emission line is close to the analyte's emission line. In addition, the internal standard should be subject to the same chemical interferences to compensate for changes in atomization efficiency. To accurately correct for these errors the analyte and internal standard emission lines are monitored simultaneously.

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of sodium in salt substitutes provides an instructive example of a typical procedure. The description here is based on Goodney, D. E. *J. Chem. Educ.* **1982**, *59*, 875–876.

Representative Method 10.4

Determination of Sodium in a Salt Substitute

DESCRIPTION OF METHOD

Salt substitutes, which are used in place of table salt for individuals on low-sodium diets, replaces NaCl with KCl. Depending on the brand, fumaric acid, calcium hydrogen phosphate, or potassium tartrate also are present. Although intended to be sodium-free, salt substitutes contain small amounts of NaCl as an impurity. Typically, the concentration of sodium in a salt substitute is about 100 μg/g The exact concentration of sodium is determined by flame atomic emission. Because it is difficult to match the matrix of the standards to that of the sample, the analysis is accomplished by the method of standard additions.

PROCEDURE

A sample is prepared by placing an approximately 10-g portion of the salt substitute in 10 mL of 3 M HCl and 100 mL of distilled water. After the sample has dissolved, it is transferred to a 250-mL volumetric flask and diluted to volume with distilled water. A series of standard additions is

prepared by placing 25-mL portions of the diluted sample into separate 50-mL volumetric flasks, spiking each with a known amount of an approximately 10 mg/L standard solution of Na^+, and diluting to volume. After zeroing the instrument with an appropriate blank, the instrument is optimized at a wavelength of 589.0 nm while aspirating a standard solution of Na^+. The emission intensity is measured for each of the standard addition samples and the concentration of sodium in the salt substitute is reported in µg/g.

QUESTIONS

1. Potassium ionizes more easily than sodium. What problem might this present if you use external standards prepared from a stock solution of 10 mg Na/L instead of using a set of standard additions?

    Because potassium is present at a much higher concentration than is sodium, its ionization suppresses the ionization of sodium. Normally suppressing ionization is a good thing because it increases emission intensity. In this case, however, the difference between the standard's matrix and the sample's matrix means that the sodium in a standard experiences more ionization than an equivalent amount of sodium in a sample. The result is a determinate error.

2. One way to avoid a determinate error when using external standards is to match the matrix of the standards to that of the sample. We could, for example, prepare external standards using reagent grade KCl to match the matrix to that of the sample. Why is this not a good idea for this analysis?

    Sodium is a common contaminant in many chemicals. Reagent grade KCl, for example, may contain 40–50 µg Na/g. This is a significant source of sodium, given that the salt substitute contains approximately 100 µg Na/g.

3. Suppose you decide to use an external standardization. Given the previous questions, is the result of your analysis likely to underestimate or to overestimate the amount of sodium in the salt substitute?

    The solid black line in Figure 10.62 shows the ideal calibration curve, assuming we match the standard's matrix to the sample's matrix, and that we do so without adding any additional sodium. If we prepare the external standards without adding KCl, the emission for each standard decreases due to increased ionization. This is shown by the lower of the two dashed red lines. Preparing the standards by adding reagent grade KCl increases the concentration of sodium due to its contamination. Because we underestimate the actual concentration of sodium in the standards, the resulting calibration curve is shown by the other dashed red line. In both cases, the sample's emission results in our overestimating the concentration of sodium in the sample.
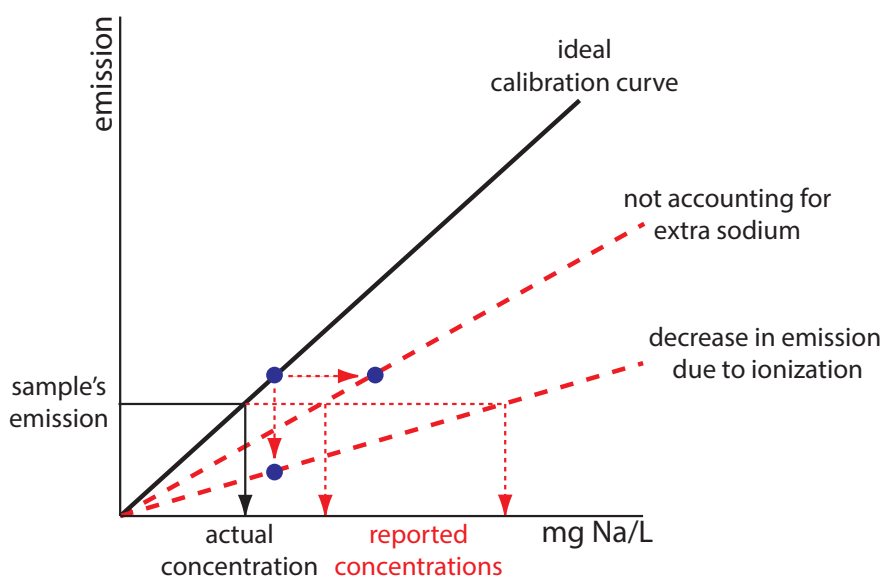
Figure 10.62 External standards calibration curves for the flame atomic emission analysis of Na in a salt substitute. The solid **black** line shows the ideal calibration curve assuming we match the matrix of the samples and the standards using pure KCl. The lower of the two dashed **red** lines shows the effect of failing to add KCl to the external standards, which decreases emission. The other dashed **red** line shows the effect of using KCl that is contaminated with NaCl, which causes us to underestimate the concentration of Na in the standards. In both cases, the result is a positive determinate error in the analysis of samples.

4. One problem with analyzing salt samples is their tendency to clog the aspirator and burner assembly. What effect does this have on the analysis?

    Clogging the aspirator and burner assembly decreases the rate of aspiration, which decreases the analyte's concentration in the flame. The result is a decrease in the emission intensity and a negative determinate error.

Example 10.12

See Section 5C.3 in Chapter 5 to review the method of standard additions.

To evaluate the method described in Representative Method 10.4, a series of standard additions is prepared using a 10.0077-g sample of a salt substitute. The results of a flame atomic emission analysis of the standards is shown here.[19]

added Na (µg/mL)	I_e (arb. units)
0.000	1.79
0.420	2.63
1.051	3.54
2.102	4.94
3.153	6.18

What is the concentration of sodium, in µg/g, in the salt substitute.

19 Goodney, D. E. *J. Chem. Educ.* **1982**, *59*, 875–876

SOLUTION

Linear regression of emission intensity versus the concentration of added Na gives the standard additions calibration curve shown to the right, which has the following calibration equation.

$$I_e = 1.97 + 1.37 \times \frac{\mu g\ Na}{mL}$$

The concentration of sodium in the sample is the absolute value of the calibration curve's x-intercept. Substituting zero for the emission intensity and solving for sodium's concentration gives a result of 1.44 μg Na/mL. The concentration of sodium in the salt substitute is

$$\frac{\dfrac{1.44\ \mu g\ Na}{mL} \times \dfrac{50.00\ mL}{25.00\ mL} \times 250.0\ mL}{10.0077\ g\ sample} = 71.9\ \mu g\ Na/g$$
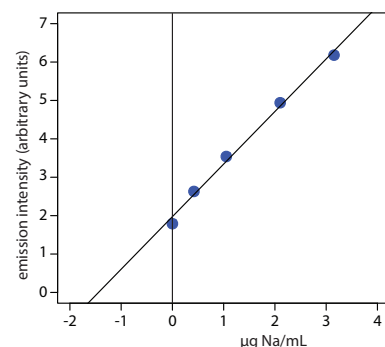
10G.4 Evaluation of Atomic Emission Spectroscopy

SCALE OF OPERATION

The scale of operations for atomic emission is ideal for the direct analysis of trace and ultratrace analytes in macro and meso samples. With appropriate dilutions, atomic emission can be applied to major and minor analytes.

ACCURACY

When spectral and chemical interferences are insignificant, atomic emission can achieve quantitative results with accuracies of 1–5%. For flame emission, accuracy frequently is limited by chemical interferences. Because the higher temperature of a plasma source gives rise to more emission lines, accuracy when using plasma emission often is limited by stray radiation from overlapping emission lines.

PRECISION

For samples and standards in which the analyte's concentration exceeds the detection limit by at least a factor of 50, the relative standard deviation for both flame and plasma emission is about 1–5%. Perhaps the most important factor that affect precision is the stability of the flame's or the plasma's temperature. For example, in a 2500 K flame a temperature fluctuation of ±2.5 K gives a relative standard deviation of 1% in emission intensity. Significant improvements in precision are realized when using internal standards.

SENSITIVITY

Sensitivity is influenced by the temperature of the excitation source and the composition of the sample matrix. Sensitivity is optimized by aspirating a

standard solution of analyte and maximizing the emission by adjusting the flame's composition and the height from which we monitor the emission. Chemical interferences, when present, decrease the sensitivity of the analysis. Because the sensitivity of plasma emission is less affected by the sample matrix, a calibration curve prepared using standards in a matrix of distilled water is possible even for samples that have more complex matrices.

SELECTIVITY

The selectivity of atomic emission is similar to that of atomic absorption. Atomic emission has the further advantage of rapid sequential or simultaneous analysis of multiple analytes.

TIME, COST, AND EQUIPMENT

Sample throughput with atomic emission is rapid when using an automated system that can analyze multiple analytes. For example, sampling rates of 3000 determinations per hour are possible using a multichannel ICP, and sampling rates of 300 determinations per hour when using a sequential ICP. Flame emission often is accomplished using an atomic absorption spectrometer, which typically costs between $10,000–$50,000. Sequential ICP's range in price from $55,000–$150,000, while an ICP capable of simultaneous multielemental analysis costs between $80,000–$200,000. Combination ICP's that are capable of both sequential and simultaneous analysis range in price from $150,000–$300,000. The cost of Ar, which is consumed in significant quantities, can not be overlooked when considering the expense of operating an ICP.

10H   Spectroscopy Based on Scattering

The blue color of the sky during the day and the red color of the sun at sunset are the result of light scattered by small particles of dust, molecules of water, and other gases in the atmosphere. The efficiency of a photon's scattering depends on its wavelength. We see the sky as blue during the day because violet and blue light scatter to a greater extent than other, longer wavelengths of light. For the same reason, the sun appears red at sunset because red light is less efficiently scattered and is more likely to pass through the atmosphere than other wavelengths of light. The scattering of radiation has been studied since the late 1800s, with applications beginning soon thereafter. The earliest quantitative applications of scattering, which date from the early 1900s, used the elastic scattering of light by colloidal suspensions to determine the concentration of colloidal particles.

10H.1   Origin of Scattering

If we send a focused, monochromatic beam of radiation with a wavelength λ through a medium of particles with dimensions $<1.5\lambda$, the radiation

scatters in all directions. For example, visible radiation of 500 nm is scattered by particles as large as 750 nm in the longest dimension. Two general categories of scattering are recognized. In elastic scattering, radiation is first absorbed by the particles and then emitted without undergoing a change in the radiation's energy. When the radiation emerges with a change in energy, the scattering is inelastic. Only elastic scattering is considered in this text.

Elastic scattering is divided into two types: Rayleigh, or small-particle scattering, and large-particle scattering. Rayleigh scattering occurs when the scattering particle's largest dimension is less than 5% of the radiation's wavelength. The intensity of the scattered radiation is proportional to its frequency to the fourth power, ν^4—which accounts for the greater scattering of blue light than red light—and is distributed symmetrically (Figure 10.63a). For larger particles, scattering increases in the forward direction and decreases in the backward direction as the result of constructive and destructive interferences (Figure 10.63b).
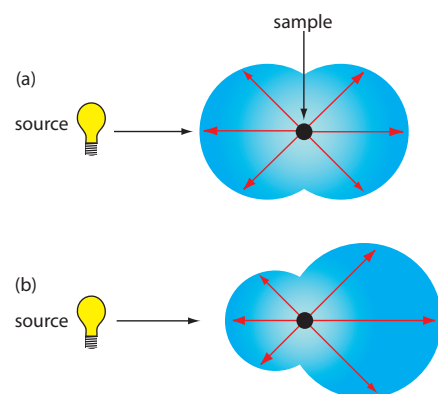
Figure 10.63 Distribution of radiation for (a) Rayleigh, or small-particle scattering, and (b) large-particle scattering.

10H.2 Turbidimetry and Nephelometry

Turbidimetry and nephelometry are two techniques that rely on the elastic scattering of radiation by a suspension of colloidal particles. In TURBIDIMETRY the detector is placed in line with the source and the decrease in the radiation's transmitted power is measured. In NEPHELOMETRY the scattered radiation is measured at an angle of 90º to the source. The similarity of turbidimetry to absorbance spectroscopy and of nephelometry to fluorescence spectroscopy is evident in the instrumental designs shown in Figure 10.64. In fact, we can use a UV/Vis spectrophotometer for turbidimetry and we can use a spectrofluorometer for nephelometry.
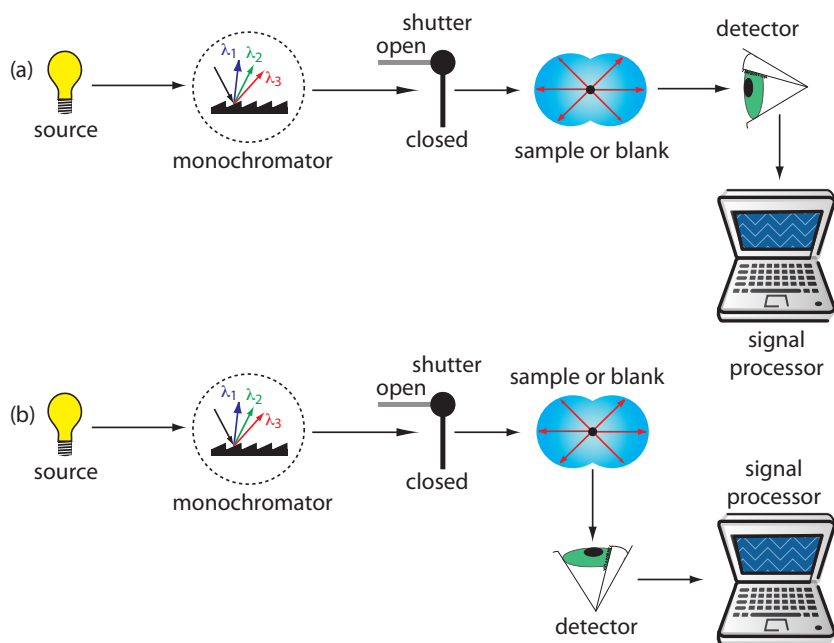
Figure 10.64 Schematic diagrams for (a) a turbimeter, and (b) a nephelometer.

TURBIDIMETRY OR NEPHELOMETRY?

When developing a scattering method the choice between using turbidimetry or using nephelometry is determined by two factors. The most important consideration is the intensity of the scattered radiation relative to the intensity of the source's radiation. If the solution contains a small concentration of scattering particles, then the intensity of the transmitted radiation, I_T, is approximately the same as the intensity of the source's radiation, I_0. As we learned earlier in the section on molecular absorption, there is substantial uncertainty in determining a small difference between two intense signals. For this reason, nephelometry is a more appropriate choice for a sample that contains few scattering particles. Turbidimetry is a better choice when the sample contains a high concentration of scattering particles.

A second consideration in choosing between turbidimetry and nephelometry is the size of the scattering particles. For nephelometry, the intensity of scattered radiation at 90° increases when the particles are small and Rayleigh scattering is in effect. For larger particles, as shown in Figure 10.63, the intensity of scattering decreases at 90°. When using an ultraviolet or a visible source of radiation, the optimum particle size is 0.1–1 μm. The size of the scattering particles is less important for turbidimetry where the signal is the relative decrease in transmitted radiation. In fact, turbidimetric measurements are feasible even when the size of the scattering particles results in an increase in reflection and refraction, although a linear relationship between the signal and the concentration of scattering particles may no longer hold.

DETERMINING CONCENTRATION BY TURBIDIMETRY

For turbidimetry the measured transmittance, T, is the ratio of the intensity of source radiation transmitted by the sample, I_T, to the intensity of source radiation transmitted by a blank, I_0.

$$T = \frac{I_T}{I_0}$$

The relationship between transmittance and the concentration of the scattering particles is similar to that given by Beer's law

$$-\log T = kbC \qquad\qquad 10.32$$

where C is the concentration of the scattering particles in mass per unit volume (w/v), b is the pathlength, and k is a constant that depends on several factors, including the size and shape of the scattering particles and the wavelength of the source radiation. The exact relationship is established by a calibration curve prepared using a series of standards that contain known concentrations of analyte. As with Beer's law, equation 10.32 may show appreciable deviations from linearity.

DETERMINING CONCENTRATION BY NEPHELOMETRY

For nephelometry the relationship between the intensity of scattered radiation, I_S, and the concentration of scattering particles is

$$I_s = kI_0C \qquad\qquad 10.33$$

where k is an empirical constant for the system and I_0 is the intensity of the source radiation. The value of k is determined from a calibration curve prepared using a series of standards that contain known concentrations of analyte.

SELECTING A WAVELENGTH FOR THE INCIDENT RADIATION

The choice of wavelength is based primarily on the need to minimize potential interferences. For turbidimetry, where the incident radiation is transmitted through the sample, a monochromator or filter allow us to avoid wavelengths that are absorbed instead of scattered by the sample. For nephelometry, the absorption of incident radiation is not a problem unless it induces fluorescence from the sample. With a nonfluorescent sample there is no need for wavelength selection and a source of white light may be used as the incident radiation. For both techniques, other considerations in choosing a wavelength including the intensity of scattering, the transducer's sensitivity (many common photon transducers are more sensitive to radiation at 400 nm than at 600 nm), and the source's intensity.

PREPARING THE SAMPLE FOR ANALYSIS

Although equation 10.32 and equation 10.33 relate scattering to the concentration of the scattering particles, the intensity of scattered radiation also is influenced by the size and the shape of the scattering particles. Samples that contain the same number of scattering particles may show significantly different values for $-\log T$ or I_S depending on the average diameter of the particles. For a quantitative analysis, therefore, it is necessary to maintain a uniform distribution of particle sizes throughout the sample and between samples and standards.

Most turbidimetric and nephelometric methods rely on precipitation reaction to form the scattering particles. As we learned in Chapter 8, a precipitate's properties, including particle size, are determined by the conditions under which it forms. To maintain a reproducible distribution of particle sizes between samples and standards, it is necessary to control parameters such as the concentration of reagents, the order of adding reagents, the pH and temperature, the agitation or stirring rate, the ionic strength, and the time between the precipitate's initial formation and the measurement of transmittance or scattering. In many cases a surface-active agent—such as glycerol, gelatin, or dextrin—is added to stabilize the precipitate in a colloidal state and to prevent the coagulation of the particles.
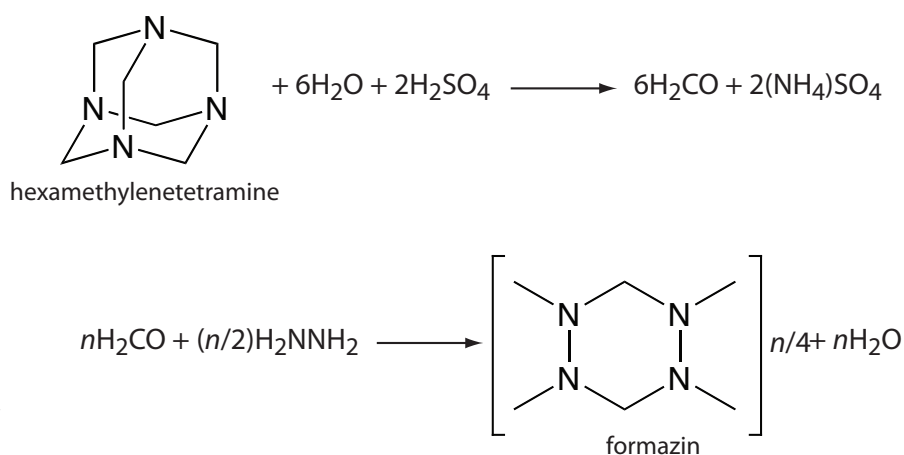
Figure 10.65 Scheme for preparing formazin for use as a turbidity standard.

APPLICATIONS

Turbidimetry and nephelometry are used to determine the clarity of water. The primary standard for measuring clarity is formazin, an easily prepared, stable polymer suspension (Figure 10.65).[20] A stock standard of formazin is prepared by combining a 1 g/100 mL solution of hydrazine sulfate, $N_2H_4 \cdot H_2SO_4$, with a 10 g/100 mL solution of hexamethylenetetramine to produce a suspension of particles that is defined as 4000 nephelometric turbidity units (NTU). A set of external standards with NTUs between 0 and 40 is prepared by diluting the stock standard. This method is readily adapted to the analysis of the clarity of orange juice, beer, and maple syrup.

A number of inorganic cations and anions are determined by precipitating them under well-defined conditions. The transmittance or scattering of light, as defined by equation 10.32 or equation 10.33, is proportional to the concentration of the scattering particles, which, in turn, is related by the stoichiometry of the precipitation reaction to the analyte's concentration. Several examples of analytes determined in this way are listed in Table 10.15.

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20 Hach, C. C.; Bryant, M. "Turbidity Standards," Technical Information Series, Booklet No. 12, Hach Company: Loveland, CO, 1995.

Table 10.15 Examples of Analytes Determined by Turbidimetry or Nephelometry		
analyte	precipitant	precipitate
Ag^+	NaCl	AgCl
Ca^{2+}	$Na_2C_2O_4$	CaC_2O_4
Cl^-	$AgNO_3$	AgCl
CN^-	$AgNO_3$	AgCN
CO_3^{2-}	$BaCl_2$	$BaCO_3$
F^-	$CaCl_2$	CaF_2
SO_4^{2-}	$BaCl_2$	$BaSO_4$

Representative Method 10.5

Turbidimetric Determination of Sulfate in Water

DESCRIPTION OF METHOD

Adding $BaCl_2$ to an acidified sample precipitates SO_4^{2-} as $BaSO_4$. The concentration of SO_4^{2-} is determined either by turbidimetry or by nephelometry using an incident source of radiation of 420 nm. External standards that contain know concentrations of SO_4^{2-} are used to standardize the method.

PROCEDURE

Transfer a 100-mL sample to a 250-mL Erlenmeyer flask along with 20.00 mL of an appropriate buffer. For a sample that contains more than 10 mg SO_4^{2-}/L, the buffer's composition is 30 g of $MgCl_2 \cdot 6H_2O$, 5 g of $CH_3COONa \cdot 3H_2O$, 1.0 g of KNO_3, and 20 mL of glacial CH_3COOH per liter. The buffer for a sample that contain less than 10 mg SO_4^{2-}/L is the same except for the addition of 0.111 g of Na_2SO_4 per L.

Place the sample and the buffer on a magnetic stirrer operated at the same speed for all samples and standards. Add a spoonful of 20–30 mesh $BaCl_2$, using a measuring spoon with a capacity of 0.2–0.3 mL, to precipitate the SO_4^{2-} as $BaSO_4$. Begin timing when the $BaCl_2$ is added and stir the suspension for 60 ± 2 s. When the stirring is complete, allow the solution to sit without stirring for 5.0 ± 0.5 min before measuring its transmittance or its scattering.

Prepare a calibration curve over the range 0–40 mg SO_4^{2-}/L by diluting a stock standard that is 100-mg SO_4^{2-}/L. Treat each standard using the procedure described above for the sample. Prepare a calibration curve and use it to determine the amount of sulfate in the sample.

QUESTIONS

1. What is the purpose of the buffer?

   If the precipitate's particles are too small, I_T is too small to measure reliably. Because rapid precipitation favors the formation of microcrystalline particles of $BaSO_4$, we use conditions that favor the precipitate's growth over the nucleation of new particles. The buffer's high ionic strength and its acidity favor the precipitate's growth and prevent the formation of microcrystalline $BaSO_4$.

2. Why is it important to use the same stirring rate and time for the samples and standards?

   How fast and how long we stir the sample after we add $BaCl_2$ influences the size of the precipitate's particles.

Figure 10.66 Waterfall on the River Swale in Richmond, England. The river, which flows out of the moors in the Yorkshire Dales, is brown in color as the result of organic matter that leaches from the peat found in the moors.

3.   Many natural waters have a slight color due to the presence of humic and fulvic acids, and may contain suspended matter (Figure 10.66). Explain why these might interfere with the analysis for sulfate. For each interferent, suggest a way to minimize its effect on the analysis.

Suspended matter in a sample contributes to scattering and, therefore, results in a positive determinate error. We can eliminate this interference by filtering the sample prior to its analysis. A sample that is colored may absorb some of the source's radiation, leading to a positive determinate error. We can compensate for this interference by taking a sample through the analysis without adding $BaCl_2$. Because no precipitate forms, we use the transmittance of this sample blank to correct for the interference.

4.   Why is Na_2SO_4 added to the buffer for samples that contain less than 10 mg SO_4^{2-}/L?

The uncertainty in a calibration curve is smallest near its center. If a sample has a high concentration of SO_4^{2-}, we can dilute it so that its concentration falls near the middle of the calibration curve. For a sample with a small concentration of SO_4^{2-}, the buffer increases the concentration of sulfate by

$$\frac{0.111\,g\;Na_2SO_4}{L} \times \frac{96.06\;g\;SO_4^{2-}}{142.04\;g\;Na_2SO_4} \times$$

$$\frac{1000\;mg}{g} \times \frac{20.00\;mL}{250.0\;mL} = 6.00\;mg\;SO_4^{2-}/L$$

After using the calibration curve to determine the amount of sulfate in the sample as analyzed, we subtract 6.00 mg SO_4^{2-}/L to determine the amount of sulfate in the original sample.

Example 10.13

To evaluate the method described in Representative Method 10.5, a series of external standard was prepared and analyzed, providing the results shown in the following table.

mg SO_4^{2-}/L	transmittance
0.00	1.00
10.00	0.646
20.00	0.417
30.00	0.269
40.00	0.174

Analysis of a 100.0-mL sample of a surface water gives a transmittance of 0.538. What is the concentration of sulfate in the sample?

SOLUTION

Linear regression of $-\log T$ versus concentration of SO_4^{2-} gives the calibration curve shown on the right, calibration curve shown to the right, which has the following calibration equation.

$$-\log T = -1.04 \times 10^{-5} + 0.0190 \times \frac{\text{mg } SO_4^{2-}}{\text{L}}$$

Substituting the sample's transmittance into the calibration curve's equation gives the concentration of sulfate in sample as 14.2 mg SO_4^{2-} /L.
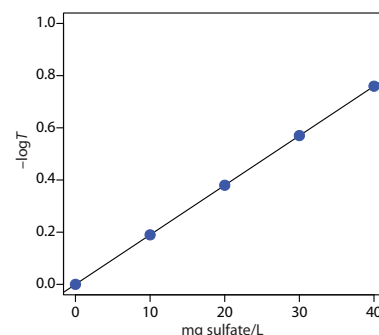
10I   Key Terms

absorbance	absorbance spectrum	absorptivity
amplitude	attenuated total reflectance	atomization
background correction	Beer's law	chemiluminescence
chromophore	continuum source	dark current
double-beam	effective bandwidth	electromagnetic radiation
electromagnetic spectrum	emission	emission spectrum
excitation spectrum	external conversion	Fellgett's advantage
fiber-optic probe	filter	filter photometer
fluorescence	fluorescent quantum yield	fluorimeter
frequency	graphite furnace	interferogram
interferometer	internal conversion	intersystem crossing
ionization suppressor	Jacquinot's advantage	lifetime
line source	method of continuous variations	molar absorptivity
mole-ratio method	monochromatic	monochromator
nephelometry	nominal wavelength	phase angle
phosphorescence	phosphorescent quantum yield	photodiode array
photoluminescence	photon	plasma
polychromatic	protecting agent	radiationless deactivation
relaxation	releasing agent	resolution
self-absorption	signal averaging	signal processor
signal-to-noise ratio	single-beam	singlet excited state
slope-ratio method	spectral searching	spectrofluorometer
spectrophotometer	spectroscopy	stray radiation
transducer	transmittance	triplet excited state
turbidimetry	vibrational relaxation	wavelength
wavenumber		

10J   Chapter Summary

The spectrophotometric methods of analysis covered in this chapter include those based on the absorption, emission, or scattering of electromagnetic radiation. When a molecule absorbs UV/Vis radiation it undergoes a change in its valence shell electron configuration. A change in vibrational energy results from the absorption of IR radiation. Experimentally we measure the fraction of radiation transmitted, T, by the sample. Instrumentation for measuring absorption requires a source of electromagnetic radiation, a means for selecting a wavelength, and a detector for measuring transmittance. Beer's law relates absorbance to both transmittance and to the concentration of the absorbing species ($A = -\log T = \varepsilon b C$).

In atomic absorption we measure the absorption of radiation by gas phase atoms. Samples are atomized using thermal energy from either a flame or a graphite furnace. Because the width of an atom's absorption band is so narrow, the continuum sources common for molecular absorption are not used. Instead, a hollow cathode lamp provides the necessary line source of radiation. Atomic absorption suffers from a number of spectral and chemical interferences. The absorption or scattering of radiation from the sample's matrix are important spectral interferences that are minimized by background correction. Chemical interferences include the formation of nonvolatile forms of the analyte and ionization of the analyte. The former interference is minimized by using a releasing agent or a protecting agent, and an ionization suppressor helps minimize the latter interference.

When a molecule absorbs radiation it moves from a lower energy state to a higher energy state. In returning to the lower energy state the molecule may emit radiation. This process is called photoluminescence. One form of photoluminescence is fluorescence in which the analyte emits a photon without undergoing a change in its spin state. In phosphorescence, emission occurs with a change in the analyte's spin state. For low concentrations of analyte, both fluorescent and phosphorescent emission intensities are a linear function of the analyte's concentration. Thermally excited atoms also emit radiation, forming the basis for atomic emission spectroscopy. Thermal excitation is achieved using either a flame or a plasma.

Spectroscopic measurements also include the scattering of light by a particulate form of the analyte. In turbidimetry, the decrease in the radiation's transmission through the sample is measured and related to the analyte's concentration through an equation similar to Beer's law. In nephelometry we measure the intensity of scattered radiation, which varies linearly with the analyte's concentration.

10K Problems

1. Provide the missing information in the following table.

wavelength (m)	frequency (s^{-1})	wavenumber (cm^{-1})	energy (J)
4.50×10^{-9}			
	1.33×10^{15}		
		3215	
			7.20×10^{-19}

2. Provide the missing information in the following table.

[analyte] (M)	absorbance	%T	molar absorptivity $(M^{-1}\ cm^{-1})$	pathlength (cm)
1.40×10^{-4}			1120	1.00
	0.563		750	1.00
2.56×10^{-4}	0.225		440	
1.55×10^{-3}	0.167			5.00
		33.3	565	1.00
4.35×10^{-3}		21.2	1550	
1.20×10^{-4}		81.3		10.00

3. A solution's transmittance is 35.0%. What is the transmittance if you dilute 25.0 mL of the solution to 50.0 mL?

4. A solution's transmittance is 85.0% when measured in a cell with a pathlength of 1.00 cm. What is the %T if you increase the pathlength to 10.00 cm?

5. The accuracy of a spectrophotometer is evaluated by preparing a solution of 60.06 ppm $K_2Cr_2O_7$ in 0.0050 M H_2SO_4, and measuring its absorbance at a wavelength of 350 nm in a cell with a pathlength of 1.00 cm. The expected absorbance is 0.640. What is the expected molar absorptivity of $K_2Cr_2O_7$ at this wavelength?

6. A chemical deviation to Beer's law may occur if the concentration of an absorbing species is affected by the position of an equilibrium reaction. Consider a weak acid, HA, for which K_a is 2×10^{-5}. Construct Beer's law calibration curves of absorbance versus the total concentration of weak acid ($C_{total} = [HA] + [A^-]$), using values for C_{total} of 1.0×10^{-5}, 3.0×10^{-5}, 5.0×10^{-5}, 7.0×10^{-5}, 9.0×10^{-5}, 11×10^{-5},

and 13×10^{-5} M for the following sets of conditions and comment on your results:

(a) $\varepsilon_{HA} = \varepsilon_{A^-} = 2000$ M^{-1} cm^{-1}; unbuffered solution.

(b) $\varepsilon_{HA} = 2000$ M^{-1} cm^{-1}; $\varepsilon_{A^-} = 500$ M^{-1} cm^{-1}; unbuffered solution.

(c) $\varepsilon_{HA} = 2000$ M^{-1} cm^{-1}; $\varepsilon_{A^-} = 500$ M^{-1} cm^{-1}; solution buffered to a pH of 4.5.

Assume a constant pathlength of 1.00 cm for all samples.

7. One instrumental limitation to Beer's law is the effect of polychromatic radiation. Consider a line source that emits radiation at two wavelengths, λ' and λ''. When treated separately, the absorbances at these wavelengths, A' and A'', are

$$A' = -\log \frac{P'_T}{P'_0} = \varepsilon' bC \qquad A'' = -\log \frac{P''_T}{P''_0} = \varepsilon'' bC$$

If both wavelengths are measured simultaneously the absorbance is

$$A = -\log \frac{(P'_T + P''_T)}{(P'_0 + P''_0)}$$

(a) Show that if the molar absorptivities at λ' and λ'' are the same ($\varepsilon' = \varepsilon'' = \varepsilon$), then the absorbance is equivalent to

$$A = \varepsilon bC$$

(b) Construct Beer's law calibration curves over the concentration range of zero to 1×10^{-4} M using $\varepsilon' = 1000$ M^{-1} cm^{-1} and $\varepsilon'' = 1000$ M^{-1} cm^{-1}, and $\varepsilon' = 1000$ M^{-1} cm^{-1} and $\varepsilon'' = 100$ M^{-1} cm^{-1}. Assume a value of 1.00 cm for the pathlength and that $P'_0 = P''_0 = 1$. Explain the difference between the two curves.

8. A second instrumental limitation to Beer's law is stray radiation. The following data were obtained using a cell with a pathlength of 1.00 cm when stray light is insignificant ($P_{stray} = 0$).

[analyte] (mM)	absorbance
0.00	0.00
2.00	0.40
4.00	0.80
6.00	1.20
8.00	1.60
10.00	2.00

Calculate the absorbance of each solution when P_{stray} is 5% of P_0, and plot Beer's law calibration curves for both sets of data. Explain any differences between the two curves. (*Hint*: Assume P_0 is 100).

9. In the process of performing a spectrophotometric determination of iron, an analyst prepares a calibration curve using a single-beam spectrophotometer similar to that shown in Figure 10.26. After preparing the calibration curve, the analyst drops and breaks the cuvette. The analyst acquires a new cuvette, measures the absorbance of the sample, and determines the %w/w Fe in the sample. Does the change in cuvette lead to a determinate error in the analysis? Explain.

10. The spectrophotometric methods for determining Mn in steel and for determining glucose use a chemical reaction to produce a colored species whose absorbance we can monitor. In the analysis of Mn in steel, colorless Mn^{2+} is oxidized to give the purple MnO_4^- ion. To analyze for glucose, which is also colorless, we react it with a yellow colored solution of the $Fe(CN)_6^{3-}$, forming the colorless $Fe(CN)_6^{4-}$ ion. The directions for the analysis of Mn do not specify precise reaction conditions, and samples and standards are treated separately. The conditions for the analysis of glucose, however, require that the samples and standards are treated simultaneously at exactly the same temperature and for exactly the same length of time. Explain why these two experimental procedures are so different.

11. One method for the analysis of Fe^{3+}, which is used with a variety of sample matrices, is to form the highly colored Fe^{3+}–thioglycolic acid complex. The complex absorbs strongly at 535 nm. Standardizing the method is accomplished using external standards. A 10.00-ppm Fe^{3+} working standard is prepared by transferring a 10-mL aliquot of a 100.0 ppm stock solution of Fe^{3+} to a 100-mL volumetric flask and diluting to volume. Calibration standards of 1.00, 2.00, 3.00, 4.00, and 5.00 ppm are prepared by transferring appropriate amounts of the 10.0 ppm working solution into separate 50-mL volumetric flasks, each of which contains 5 mL of thioglycolic acid, 2 mL of 20% w/v ammonium citrate, and 5 mL of 0.22 M NH_3. After diluting to volume and mixing, the absorbances of the external standards are measured against an appropriate blank. Samples are prepared for analysis by taking a portion known to contain approximately 0.1 g of Fe^{3+}, dissolving it in a minimum amount of HNO_3, and diluting to volume in a 1-L volumetric flask. A 1.00-mL aliquot of this solution is transferred to a 50-mL volumetric flask, along with 5 mL of thioglycolic acid, 2 mL of 20% w/v ammonium citrate, and 5 mL of 0.22 M NH_3 and diluted to volume. The absorbance of this solution is used to determine the concentration of Fe^{3+} in the sample.

    (a) What is an appropriate blank for this procedure?

(b) Ammonium citrate is added to prevent the precipitation of Al^{3+}. What is the effect on the reported concentration of iron in the sample if there is a trace impurity of Fe^{3+} in the ammonium citrate?

(c) Why does the procedure specify that the sample contain approximately 0.1 g of Fe^{3+}?

(d) Unbeknownst to the analyst, the 100-mL volumetric flask used to prepare the 10.00 ppm working standard of Fe^{3+} has a volume that is significantly smaller than 100.0 mL. What effect will this have on the reported concentration of iron in the sample?

12. A spectrophotometric method for the analysis of iron has a linear calibration curve for standards of 0.00, 5.00, 10.00, 15.00, and 20.00 mg Fe/L. An iron ore sample that is 40–60% w/w is analyzed by this method. An approximately 0.5-g sample is taken, dissolved in a minimum of concentrated HCl, and diluted to 1 L in a volumetric flask using distilled water. A 5.00 mL aliquot is removed with a pipet. To what volume—10, 25, 50, 100, 250, 500, or 1000 mL—should it be diluted to minimize the uncertainty in the analysis? Explain.

13. Lozano-Calero and colleagues developed a method for the quantitative analysis of phosphorous in cola beverages based on the formation of the blue-colored phosphomolybdate complex, $(NH_4)_3[PO_4(MoO_3)_{12}]$.[21] The complex is formed by adding $(NH_4)_6Mo_7O_{24}$ to the sample in the presence of a reducing agent, such as ascorbic acid. The concentration of the complex is determined spectrophotometrically at a wavelength of 830 nm, using an external standards calibration curve.

In a typical analysis, a set of standard solutions that contain known amounts of phosphorous is prepared by placing appropriate volumes of a 4.00 ppm solution of P_2O_5 in a 5-mL volumetric flask, adding 2 mL of an ascorbic acid reducing solution, and diluting to volume with distilled water. Cola beverages are prepared for analysis by pouring a sample into a beaker and allowing it to stand for 24 h to expel the dissolved CO_2. A 2.50-mL sample of the degassed sample is transferred to a 50-mL volumetric flask and diluted to volume. A 250-μL aliquot of the diluted sample is then transferred to a 5-mL volumetric flask, treated with 2 mL of the ascorbic acid reducing solution, and diluted to volume with distilled water.

(a) The authors note that this method can be applied only to noncolored cola beverages. Explain why this is true.

(b) How might you modify this method so that you can apply it to any cola beverage?

21 Lozano-Calero, D.; Martín-Palomeque, P.; Madueño-Loriguillo, S. *J. Chem. Educ.* **1996**, *73*, 1173–1174.

(c) Why is it necessary to remove the dissolved gases?

(d) Suggest an appropriate blank for this method?

(e) The author's report a calibration curve of

$$A = -0.02 + (0.72 \text{ ppm}^{-1}) \times C_{P_2O_5}$$

A sample of Crystal Pepsi, analyzed as described above, yields an absorbance of 0.565. What is the concentration of phosphorous, reported as ppm P, in the original sample of Crystal Pepsi?

Crystal Pepsi was a colorless, caffeine-free soda produced by PepsiCo. It was available in the United States from 1992 to 1993.

14. EDTA forms colored complexes with a variety of metal ions that may serve as the basis for a quantitative spectrophotometric method of analysis. The molar absorptivities of the EDTA complexes of Cu^{2+}, Co^{2+}, and Ni^{2+} at three wavelengths are summarized in the following table (all values of ε are in $M^{-1} \text{ cm}^{-1}$).

metal	$\varepsilon_{462.9}$	$\varepsilon_{732.0}$	$\varepsilon_{378.7}$
Co^{2+}	15.8	2.11	3.11
Cu^{2+}	2.32	95.2	7.73
Ni^{2+}	1.79	3.03	13.5

Using this information determine the following:

(a) The concentration of Cu^{2+} in a solution that has an absorbance of 0.338 at a wavelength of 732.0 nm.

(b) The concentrations of Cu^{2+} and Co^{2+} in a solution that has an absorbance of 0.453 at a wavelength of 732.0 nm and 0.107 at a wavelength of 462.9 nm.

(c) The concentrations of Cu^{2+}, Co^{2+}, and Ni^{2+} in a sample that has an absorbance of 0.423 at a wavelength of 732.0 nm, 0.184 at a wavelength of 462.9 nm, and 0.291 at a wavelength of 378.7 nm.

The pathlength, b, is 1.00 cm for all measurements.

15. The concentration of phenol in a water sample is determined by using steam distillation to separate the phenol from non-volatile impurities, followed by reacting the phenol in the distillate with 4-aminoantipyrine and $K_3Fe(CN)_6$ at pH 7.9 to form a colored antipyrine dye. A phenol standard with a concentration of 4.00 ppm has an absorbance of 0.424 at a wavelength of 460 nm using a 1.00 cm cell. A water sample is steam distilled and a 50.00-mL aliquot of the distillate is placed in a 100-mL volumetric flask and diluted to volume with distilled water. The absorbance of this solution is 0.394. What is the concentration of phenol (in parts per million) in the water sample?

16. Saito describes a quantitative spectrophotometric procedure for iron based on a solid-phase extraction using bathophenanthroline in a

poly(vinyl chloride) membrane.[22] In the absence of Fe^{2+} the membrane is colorless, but when immersed in a solution of Fe^{2+} and I^-, the membrane develops a red color as a result of the formation of an Fe^{2+}–bathophenanthroline complex. A calibration curve determined using a set of external standards with known concentrations of Fe^{2+} gave a standardization relationship of

$$A = (8.60 \times 10^3 \text{ M}^{-1}) \times [Fe^{2+}]$$

What is the concentration of iron, in mg Fe/L, for a sample with an absorbance of 0.100?

17. In the DPD colorimetric method for the free chlorine residual, which is reported as mg Cl_2/L, the oxidizing power of free chlorine converts the colorless amine N,N-diethyl-p-phenylenediamine to a colored dye that absorbs strongly over the wavelength range of 440–580 nm. Analysis of a set of calibration standards gave the following results.

mg Cl_2/L	absorbance
0.00	0.000
0.50	0.270
1.00	0.543
1.50	0.813
2.00	1.084

A sample from a public water supply is analyzed to determine the free chlorine residual, giving an absorbance of 0.113. What is the free chlorine residual for the sample in mg Cl_2/L?

18. Lin and Brown described a quantitative method for methanol based on its effect on the visible spectrum of methylene blue.[23] In the absence of methanol, methylene blue has two prominent absorption bands at 610 nm and 663 nm, which correspond to the monomer and the dimer, respectively. In the presence of methanol, the intensity of the dimer's absorption band decreases, while that for the monomer increases. For concentrations of methanol between 0 and 30% v/v, the ratio of the two absorbance, A_{663}/A_{610}, is a linear function of the amount of methanol. Use the following standardization data to determine the %v/v methanol in a sample if A_{610} is 0.75 and A_{663} is 1.07.

%v/v methanol	A_{663}/A_{610}	%v/v methanol	A_{663}/A_{610}
0.0	1.21	20.0	1.62
5.0	1.29	25.0	1.74
10.0	1.42	30.0	1.84
15.0	1.52		

22 Saito, T. *Anal. Chim. Acta* **1992**, *268*, 351–355.
23 Lin, J.; Brown, C. W. *Spectroscopy* **1995**, *10(5)*, 48–51.

19. The concentration of the barbiturate barbital in a blood sample is determined by extracting 3.00 mL of blood with 15 mL of $CHCl_3$. The chloroform, which now contains the barbital, is extracted with 10.0 mL of 0.45 M NaOH (pH ≈ 13). A 3.00-mL sample of the aqueous extract is placed in a 1.00-cm cell and an absorbance of 0.115 is measured. The pH of the sample in the absorption cell is then adjusted to approximately 10 by adding 0.50 mL of 16% w/v NH_4Cl, giving an absorbance of 0.023. When 3.00 mL of a standard barbital solution with a concentration of 3 mg/100 mL is taken through the same procedure, the absorbance at pH 13 is 0.295 and the absorbance at a pH of 10 is 0.002. Report the mg barbital/100 mL in the sample.

20. Jones and Thatcher developed a spectrophotometric method for analyzing analgesic tablets that contain aspirin, phenacetin, and caffeine.[24] The sample is dissolved in $CHCl_3$ and extracted with an aqueous solution of $NaHCO_3$ to remove the aspirin. After the extraction is complete, the chloroform is transferred to a 250-mL volumetric flask and diluted to volume with $CHCl_3$. A 2.00-mL portion of this solution is then diluted to volume in a 200-mL volumetric flask with $CHCl_3$. The absorbance of the final solution is measured at wavelengths of 250 nm and 275 nm, at which the absorptivities, in ppm^{-1} cm^{-1}, for caffeine and phenacetin are

	a_{250}	a_{275}
caffeine	0.0131	0.0485
phenacetin	0.0702	0.0159

Aspirin is determined by neutralizing the $NaHCO_3$ in the aqueous solution and extracting the aspirin into $CHCl_3$. The combined extracts are diluted to 500 mL in a volumetric flask. A 20.00-mL portion of the solution is placed in a 100-mL volumetric flask and diluted to volume with $CHCl_3$. The absorbance of this solution is measured at 277 nm, where the absorptivity of aspirin is 0.00682 ppm^{-1} cm^{-1}. An analgesic tablet treated by this procedure is found to have absorbances of 0.466 at 250 nm, 0.164 at 275 nm, and 0.600 at 277 nm when using a cell with a 1.00 cm pathlength. Report the milligrams of aspirin, caffeine, and phenacetin in the analgesic tablet.

21. The concentration of SO_2 in a sample of air is determined by the *p*-rosaniline method. The SO_2 is collected in a 10.00-mL solution of $HgCl_4^{2-}$, where it reacts to form $Hg(SO_3)_2^{2-}$, by pulling air through the solution for 75 min at a rate of 1.6 L/min. After adding *p*-rosaniline and formaldehyde, the colored solution is diluted to 25 mL in a volumetric flask. The absorbance is measured at 569 nm in a 1-cm cell, yielding a value of 0.485. A standard sample is prepared by substituting

24 Jones, M.; Thatcher, R. L. *Anal. Chem.* **1951**, *23*, 957–960.

a 1.00-mL sample of a standard solution that contains the equivalent of 15.00 ppm SO_2 for the air sample. The absorbance of the standard is found to be 0.181. Report the concentration of SO_2 in the air in mg SO_2/L. The density of air is 1.18 g/liter.

22. Seaholtz and colleagues described a method for the quantitative analysis of CO in automobile exhaust based on the measurement of infrared radiation at 2170 cm^{-1}.[25] A calibration curve is prepared by filling a 10-cm IR gas cell with a known pressure of CO and measuring the absorbance using an FT-IR, giving a calibration equation of

$$A = -1.1 \times 10^{-4} + (9.9 \times 10^{-4}) \times P_{CO}$$

Samples are prepared by using a vacuum manifold to fill the gas cell. After measuring the total pressure, the absorbance at 2170 cm^{-1} is measured. Results are reported as %CO (P_{CO}/P_{total}). The analysis of five exhaust samples from a 1973 coupe gives the following results.

P_{total} (torr)	absorbance
595	0.1146
354	0.0642
332	0.0591
233	0.0412
143	0.0254

Determine the %CO for each sample, and report the mean and the 95% confidence interval.

23. Figure 10.32 shows an example of a disposable IR sample card made using a thin sheet of polyethylene. To prepare an analyte for analysis, it is dissolved in a suitable solvent and a portion of the sample placed on the IR card. After the solvent evaporates, leaving the analyte behind as a thin film, the sample's IR spectrum is obtained. Because the thickness of the polyethylene film is not uniform, the primary application of IR cards is for a qualitative analysis. Zhao and Malinowski reported how an internal standardization with KSCN can be used for a quantitative IR analysis of polystyrene.[26] Polystyrene is monitored at 1494 cm^{-1} and KSCN at 2064 cm^{-1}. Standard solutions are prepared by placing weighed portions of polystyrene in a 10-mL volumetric flask and diluting to volume with a solution of 10 g/L KSCN in methyl isobutyl ketone. A typical set of results is shown here.

g polystyrene	0.1609	0.3290	0.4842	0.6402	0.8006
A_{1494}	0.0452	0.1138	0.1820	0.3275	0.3195
A_{2064}	0.1948	0.2274	0.2525	0.3580	0.2703

25 Seaholtz, M. B.; Pence, L. E.; Moe, O. A. Jr. *J. Chem. Educ.* **1988**, *65*, 820–823.
26 Zhao, Z.; Malinowski, E. R. *Spectroscopy* **1996**, *11(7)*, 44–49.

When a 0.8006-g sample of a poly(styrene/maleic anhydride) copolymer is analyzed, the following results are obtained.

replicate	A_{1494}	A_{2064}
1	0.2729	0.3582
2	0.2074	0.2820
3	0.2785	0.3642

What is the %w/w polystyrene in the copolymer? Given that the reported %w/w polystyrene is 67%, is there any evidence for a determinate error at $\alpha = 0.05$?

24. The following table lists molar absorptivities for the Arsenazo complexes of copper and barium.[27] Suggest appropriate wavelengths for analyzing mixtures of copper and barium using their Arsenzao complexes.

wavelength (nm)	ε_{Cu} (M^{-1} cm^{-1})	ε_{Ba} (M^{-1} cm^{-1})
595	11900	7100
600	15500	7200
607	18300	7400
611	19300	6900
614	19300	7000
620	17800	7100
626	16300	8400
635	10900	9900
641	7500	10500
645	5300	10000
650	3500	8600
655	2200	6600
658	1900	6500
665	1500	3900
670	1500	2800
680	1800	1500

25. Blanco and colleagues report several applications of multiwavelength linear regression analysis for the simultaneous determination of two-component mixtures.[28] For each of the following, determine the molar concentration of each analyte in the mixture.

(a) Titanium and vanadium are determined by forming complexes with H_2O_2. Results for a mixture of Ti(IV) and V(V) and for standards of 63.1 ppm Ti(IV) and 96.4 ppm V(V) are listed in the following table.

27 Grossman, O.; Turanov, A. N. *Anal. Chim. Acta* **1992**, *257*, 195–202.
28 Blanco, M.; Iturriaga, H.; Maspoch, S.; Tarin, P. *J. Chem. Educ.* **1989**, *66*, 178–180.

wavelength (nm)	absorbance		
	Ti(V) standard	V(V) standard	mixture
390	0.895	0.326	0.651
430	0.884	0.497	0.743
450	0.694	0.528	0.665
470	0.481	0.512	0.547
510	0.173	0.374	0.314

(b) Copper and zinc are determined by forming colored complexes with 2-pyridyl-azo-resorcinol (PAR). The absorbances for PAR, a mixture of Cu^{2+} and Zn^{2+}, and standards of 1.00 ppm Cu^{2+} and 1.00 ppm Zn^{2+} are listed in the following table. Note that you must correct the absorbances for the each metal for the contribution from PAR.

wavelength (nm)	absorbance			
	PAR	Cu standard	Zn standard	mixture
480	0.211	0.698	0.971	0.656
496	0.137	0.732	1.018	0.668
510	0.100	0.732	0.891	0.627
526	0.072	0.602	0.672	0.498
540	0.056	0.387	0.306	0.290

26. The stoichiometry of a metal–ligand complex, ML_n, is determined by the method of continuous variations. A series of solutions is prepared in which the combined concentrations of M and L are held constant at 5.15×10^{-4} M. The absorbances of these solutions are measured at a wavelength where only the metal–ligand complex absorbs. Using the following data, determine the formula of the metal–ligand complex.

mole fraction M	mole fraction L	absorbance
1.0	0.0	0.001
0.9	0.1	0.126
0.8	0.2	0.260
0.7	0.3	0.389
0.6	0.4	0.515
0.5	0.5	0.642
0.4	0.6	0.775
0.3	0.7	0.771
0.2	0.8	0.513
0.1	0.9	0.253
0.0	1.0	0.000

27. The stoichiometry of a metal–ligand complex, ML_n, is determined by the mole-ratio method. A series of solutions are prepared in which the metal's concentration is held constant at 3.65×10^{-4} M and the ligand's concentration is varied from 1×10^{-4} M to 1×10^{-3} M. Using the following data, determine the stoichiometry of the metal-ligand complex.

[ligand] (M)	absorbance	[ligand] (M)	absorbance
1.0×10^{-4}	0.122	6.0×10^{-4}	0.752
2.0×10^{-4}	0.251	7.0×10^{-4}	0.873
3.0×10^{-4}	0.376	8.0×10^{-4}	0.937
4.0×10^{-4}	0.496	9.0×10^{-4}	0.962
5.0×10^{-4}	0.625	1.0×10^{-3}	1.002

28. The stoichiometry of a metal–ligand complex, ML_n, is determined by the slope-ratio method. Two sets of solutions are prepared. For the first set of solutions the metal's concentration is held constant at 0.010 M and the ligand's concentration is varied. The following data are obtained at a wavelength where only the metal–ligand complex absorbs.

[ligand] (M)	absorbance
1.0×10^{-5}	0.012
2.0×10^{-5}	0.029
3.0×10^{-5}	0.042
4.0×10^{-5}	0.055
5.0×10^{-5}	0.069

For the second set of solutions the concentration of the ligand is held constant at 0.010 M, and the concentration of the metal is varied, yielding the following absorbances.

[metal] (M)	absorbance
1.0×10^{-5}	0.040
2.0×10^{-5}	0.085
3.0×10^{-5}	0.125
4.0×10^{-5}	0.162
5.0×10^{-5}	0.206

Using this data, determine the stoichiometry of the metal-ligand complex.

29. Kawakami and Igarashi developed a spectrophotometric method for nitrite based on its reaction with $5, 10, 15, 20$-tetrakis(4-aminophenyl) porphrine (TAPP). As part of their study they investigated the stoichi-

ometry of the reaction between TAPP and NO_2^-. The following data are derived from a figure in their paper.[29]

[TAPP] (M)	[NO_2^-] (M)	absorbance
8.0×10^{-7}	0	0.227
8.0×10^{-7}	4.0×10^{-8}	0.223
8.0×10^{-7}	8.0×10^{-8}	0.211
8.0×10^{-7}	1.6×10^{-7}	0.191
8.0×10^{-7}	3.2×10^{-7}	0.152
8.0×10^{-7}	4.8×10^{-7}	0.127
8.0×10^{-7}	6.4×10^{-7}	0.107
8.0×10^{-7}	8.0×10^{-7}	0.092
8.0×10^{-7}	1.6×10^{-6}	0.058
8.0×10^{-7}	2.4×10^{-6}	0.045
8.0×10^{-7}	3.2×10^{-6}	0.037
8.0×10^{-7}	4.0×10^{-6}	0.034

What is the stoichiometry of the reaction?

30. The equilibrium constant for an acid–base indicator is determined by preparing three solutions, each of which has a total indicator concentration of 1.35×10^{-5} M. The pH of the first solution is adjusted until it is acidic enough to ensure that only the acid form of the indicator is present, yielding an absorbance of 0.673. The absorbance of the second solution, whose pH is adjusted to give only the base form of the indicator, is 0.118. The pH of the third solution is adjusted to 4.17 and has an absorbance of 0.439. What is the acidity constant for the acid–base indicator?

31. The acidity constant for an organic weak acid is determined by measuring its absorbance as a function of pH while maintaining a constant total concentration of the acid. Using the data in the following table, determine the acidity constant for the organic weak acid.

pH	absorbance	pH	absorbance
1.53	0.010	4.88	0.193
2.20	0.010	5.09	0.227
3.66	0.035	5.69	0.288
4.11	0.072	7.20	0.317
4.35	0.103	7.78	0.317
4.75	0.169		

29 Kawakami, T.; Igarashi, S. *Anal. Chim. Acta* **1996**, *333*, 175–180.

32. Suppose you need to prepare a set of calibration standards for the spectrophotometric analysis of an analyte that has a molar absorptivity of $1138 \ M^{-1} \ cm^{-1}$ at a wavelength of 625 nm. To maintain an acceptable precision for the analysis, the %T for the standards should be between 15% and 85%.

    (a) What is the concentration for the most concentrated and for the least concentrated standard you should prepare, assuming a 1.00-cm sample cell.

    (b) Explain how you will analyze samples with concentrations that are 10 μM, 0.1 mM, and 1.0 mM in the analyte.

33. When using a spectrophotometer whose precision is limited by the uncertainty of reading %T, the analysis of highly absorbing solutions can lead to an unacceptable level of indeterminate errors. Consider the analysis of a sample for which the molar absorptivity is $1.0 \times 10^4 \ M^{-1} \ cm^{-1}$ and for which the pathlength is 1.00 cm.

    (a) What is the relative uncertainty in concentration for an analyte whose concentration is $2.0 \times 10^{-4} \ M$ if s_T is ± 0.002?

    (b) What is the relative uncertainty in the concentration if the spectrophotometer is calibrated using a blank that consists of a $1.0 \times 10^{-4} \ M$ solution of the analyte?

34. Hobbins reported the following calibration data for the flame atomic absorption analysis for phosphorous.[30]

mg P/L	absorbance
2130	0.048
4260	0.110
6400	0.173
8530	0.230

    To determine the purity of a sample of Na_2HPO_4, a 2.469-g sample is dissolved and diluted to volume in a 100-mL volumetric flask. Analysis of the resulting solution gives an absorbance of 0.135. What is the purity of the Na_2HPO_4?

35. Bonert and Pohl reported results for the atomic absorption analysis of several metals in the caustic suspensions produced during the manufacture of soda by the ammonia-soda process.[31]

    (a) The concentration of Cu is determined by acidifying a 200.0-mL sample of the caustic solution with 20 mL of concentrated HNO_3,

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30 Hobbins, W. B. "Direct Determination of Phosphorous in Aqueous Matricies by Atomic Absorption," Varian Instruments at Work, Number AA-19, February 1982.

31 Bonert, K.; Pohl, B. "The Determination of Cd, Cr, Cu, Ni, and Pb in Concentrated CaCl₂/NaCl solutions by AAS," AA Instruments at Work (Varian) Number 98, November, 1990.

adding 1 mL of 27% w/v H_2O_2, and boiling for 30 min. The resulting solution is diluted to 500 mL in a volumetric flask, filtered, and analyzed by flame atomic absorption using matrix matched standards. The results for a typical analysis are shown in the following table.

solution	mg Cu/L	absorbance
blank	0.000	0.007
standard 1	0.200	0.014
standard 2	0.500	0.036
standard 3	1.000	0.072
standard 4	2.000	0.146
sample		0.027

Determine the concentration of Cu in the caustic suspension.

(b) The determination of Cr is accomplished by acidifying a 200.0-mL sample of the caustic solution with 20 mL of concentrated HNO_3, adding 0.2 g of Na_2SO_3 and boiling for 30 min. The Cr is isolated from the sample by adding 20 mL of NH_3, producing a precipitate that includes the chromium as well as other oxides. The precipitate is isolated by filtration, washed, and transferred to a beaker. After acidifying with 10 mL of HNO_3, the solution is evaporated to dryness. The residue is redissolved in a combination of HNO_3 and HCl and evaporated to dryness. Finally, the residue is dissolved in 5 mL of HCl, filtered, diluted to volume in a 50-mL volumetric flask, and analyzed by atomic absorption using the method of standard additions. The atomic absorption results are summarized in the following table.

sample	mg Cr_{added}/L	absorbance
blank		0.001
sample		0.045
standard addition 1	0.200	0.083
standard addition 2	0.500	0.118
standard addition 3	1.000	0.192

Report the concentration of Cr in the caustic suspension.

36. Quigley and Vernon report results for the determination of trace metals in seawater using a graphite furnace atomic absorption spectrophotometer and the method of standard additions.[32] The trace metals are first separated from their complex, high-salt matrix by coprecipitating with Fe^{3+}. In a typical analysis a 5.00-mL portion of 2000 ppm Fe^{3+} is added to 1.00 L of seawater. The pH is adjusted to 9 using NH_4OH, and the precipitate of $Fe(OH)_3$ allowed to stand overnight. After isolat-

32 Quigley, M. N.; Vernon, F. *J. Chem. Educ.* **1996**, *73*, 671–673.

ing and rinsing the precipitate, the $Fe(OH)_3$ and coprecipitated metals are dissolved in 2 mL of concentrated HNO_3 and diluted to volume in a 50-mL volumetric flask. To analyze for Mn^{2+}, a 1.00-mL sample of this solution is diluted to 100 mL in a volumetric flask. The following samples are injected into the graphite furnace and analyzed.

sample	absorbance
2.5-μL sample + 2.5 μL of 0 ppb Mn^{2+}	0.223
2.5-μL sample + 2.5 μL of 2.5 ppb Mn^{2+}	0.294
2.5-μL sample + 2.5 μL of 5.0 ppb Mn^{2+}	0.361

Report the ppb Mn^{2+} in the sample of seawater.

37. The concentration of Na in plant materials are determined by flame atomic emission. The material to be analyzed is prepared by grinding, homogenizing, and drying at 103 °C. A sample of approximately 4 g is transferred to a quartz crucible and heated on a hot plate to char the organic material. The sample is heated in a muffle furnace at 550 °C for several hours. After cooling to room temperature the residue is dissolved by adding 2 mL of 1:1 HNO_3 and evaporated to dryness. The residue is redissolved in 10 mL of 1:9 HNO_3, filtered and diluted to 50 mL in a volumetric flask. The following data are obtained during a typical analysis for the concentration of Na in a 4.0264-g sample of oat bran.

sample	mg Na/L	emission (arbitrary units)
blank	0.00	0.0
standard 1	2.00	90.3
standard 2	4.00	181
standard 3	6.00	272
standard 4	8.00	363
standard 5	10.00	448
sample		238

Report the concentration of sodium in the sample of oat bran as μg Na/g sample.

38. Yan and colleagues developed a method for the analysis of iron based its formation of a fluorescent metal–ligand complex with the ligand 5-(4-methylphenylazo)-8-aminoquinoline.[33] In the presence of the surfactant cetyltrimethyl ammonium bromide the analysis is carried out using an excitation wavelength of 316 nm with emission monitored at 528 nm. Standardization with external standards gives the following calibration curve.

33 Yan, G.; Shi, G.; Liu, Y. *Anal. Chim. Acta* **1992**, *264*, 121–124.

$$I_f = -0.03 + (1.594 \text{ mg}^{-1}\text{L}) \times \frac{\text{mg Fe}^{3+}}{\text{L}}$$

A 0.5113-g sample of dry dog food is ashed to remove organic materials, and the residue dissolved in a small amount of HCl and diluted to volume in a 50-mL volumetric flask. Analysis of the resulting solution gives a fluorescent emission intensity of 5.72. Determine the mg Fe/L in the sample of dog food.

39. A solution of 5.00×10^{-5} M 1,3-dihydroxynaphthelene in 2 M NaOH has a fluorescence intensity of 4.85 at a wavelength of 459 nm. What is the concentration of 1,3-dihydroxynaphthelene in a solution that has a fluorescence intensity of 3.74 under identical conditions?

40. The following data is recorded for the phosphorescent intensity of several standard solutions of benzo[a]pyrene.

[benzo[a]pyrene] (M)	emission intensity
0	0.00
1.00×10^{-5}	0.98
3.00×10^{-5}	3.22
6.00×10^{-5}	6.25
1.00×10^{-4}	10.21

What is the concentration of benzo[a]pyrene in a sample that yields a phosphorescent emission intensity of 4.97?

41. The concentration of acetylsalicylic acid, $C_9H_8O_4$, in aspirin tablets is determined by hydrolyzing it to the salicylate ion, $C_7H_5O_2^-$, and determining its concentration spectrofluorometrically. A stock standard solution is prepared by weighing 0.0774 g of salicylic acid, $C_7H_6O_2$, into a 1-L volumetric flask and diluting to volume. A set of calibration standards is prepared by pipeting 0, 2.00, 4.00, 6.00, 8.00, and 10.00 mL of the stock solution into separate 100-mL volumetric flasks that contain 2.00 mL of 4 M NaOH and diluting to volume. Fluorescence is measured at an emission wavelength of 400 nm using an excitation wavelength of 310 nm with results shown in the following table.

mL of stock solution	emission intensity
0.00	0.00
2.00	3.02
4.00	5.98
6.00	9.18
8.00	12.13
10.00	14.96

Several aspirin tablets are ground to a fine powder in a mortar and pestle. A 0.1013-g portion of the powder is placed in a 1-L volumetric flask and diluted to volume with distilled water. A portion of this solution is filtered to remove insoluble binders and a 10.00-mL aliquot transferred to a 100-mL volumetric flask that contains 2.00 mL of 4 M NaOH. After diluting to volume the fluorescence of the resulting solution is 8.69. What is the %w/w acetylsalicylic acid in the aspirin tablets?

42. Selenium (IV) in natural waters is determined by complexing with ammonium pyrrolidine dithiocarbamate and extracting into $CHCl_3$. This step serves to concentrate the Se(IV) and to separate it from Se(VI). The Se(IV) is then extracted back into an aqueous matrix using HNO_3. After complexing with 2,3-diaminonaphthalene, the complex is extracted into cyclohexane. Fluorescence is measured at 520 nm following its excitation at 380 nm. Calibration is achieved by adding known amounts of Se(IV) to the water sample before beginning the analysis. Given the following results what is the concentration of Se(IV) in the sample.

[Se (IV)] added (nM)	emission intensity
0.00	323
2.00	597
4.00	862
6.00	1123

43. Fibrinogen is a protein that is produced by the liver and found in human plasma. Its concentration in plasma is clinically important. Many of the analytical methods used to determine the concentration of fibrinogen in plasma are based on light scattering following its precipitation. For example, da Silva and colleagues describe a method in which fibrinogen precipitates in the presence of ammonium sulfate in a guanidine hydrochloride buffer.[34] Light scattering is measured nephelometrically at a wavelength of 340 nm. Analysis of a set of external calibration standards gives the following calibration equation

$$I_s = -4.66 + 9907.63C$$

where I_s is the intensity of scattered light and C is the concentration of fibrinogen in g/L. A 9.00-mL sample of plasma is collected from a patient and mixed with 1.00 mL of an anticoagulating agent. A 1.00-mL aliquot of this solution is diluted to 250 mL in a volumetric flask and is found to have a scattering intensity of 44.70. What is the concentration of fibrinogen, in gram per liter, in the plasma sample?

34 da Silva, M. P.; Fernandez-Romero, J. M.; Luque de Castro, M. D. *Anal. Chim. Acta* **1996**, *327*, 101–106.

10L   Solutions to Practice Exercises

Practice Exercise 10.1

The frequency and wavenumber for the line are

$$\nu = \frac{c}{\lambda} = \frac{3.00 \times 10^8 \text{ m/s}}{656.3 \times 10^{-9} \text{ m}} = 4.57 \times 10^{14} \text{ s}^{-1}$$

$$\overline{\nu} = \frac{1}{\lambda} = \frac{1}{656.3 \times 10^{-9} \text{ m}} \times \frac{1 \text{ m}}{100 \text{ cm}} = 1.524 \times 10^4 \text{ cm}^{-1}$$

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Practice Exercise 10.2

The photon's energy is

$$E = \frac{hc}{\lambda} = \frac{(6.626 \times 10^{-34} \text{ Js}) (3.00 \times 10^8 \text{ m/s})}{656.3 \times 10^{-9} \text{ m}} = 3.03 \times 10^{-19} \text{ J}$$

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Practice Exercise 10.3

To find the transmittance, T, we begin by noting that

$$A = 1.27 = -\log T$$

Solving for T

$$-1.27 = \log T$$
$$10^{-1.27} = T$$

gives a transmittance of 0.054, or a %T of 5.4%.

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Practice Exercise 10.4

Making appropriate substitutions into Beer's law

$$A = 0.228 = \varepsilon bC = (676 \text{ M}^{-1} \text{ cm}^{-1})(1 \text{ cm})C$$

and solving for C gives a concentration of 3.37×10^{-4} M.

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Practice Exercise 10.5

For this standard addition we write equations that relate absorbance to the concentration of Cu^{2+} in the sample before the standard addition

$$0.118 = \varepsilon bC_{Cu}$$

and after the standard addition

$$0.162 = \varepsilon b\left(C_{Cu} + \frac{20.00 \text{ mg Cu}}{\text{L}} \times \frac{1.00 \text{ mL}}{10.00 \text{ mL}} \right)$$

The value of εb is the same in both equation. Solving each equation for εb and equating

$$\frac{0.162}{C_{Cu} + \dfrac{20.00 \text{ mg Cu}}{L} \times \dfrac{1.00 \text{ mL}}{10.00 \text{ mL}}} = \frac{0.118}{C_{Cu}}$$

leaves us with an equation in which C_{Cu} is the only variable. Solving for C_{Cu} gives its value as

$$\frac{0.162}{C_{Cu} + 2.00 \text{ mg Cu/L}} = \frac{0.118}{C_{Cu}}$$

$$0.162 C_{Cu} = 0.118 C_{Cu} + 0.236 \text{ mg Cu/L}$$

$$0.044 C_{Cu} = 0.236 \text{ mg Cu/L}$$

$$C_{Cu} = 5.4 \text{ mg Cu/L}$$

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Practice Exercise 10.6

Substituting into equation 10.11 and equation 10.12 gives

$$A_{400} = 0.336 = 15.2 C_{Cr} + 5.60 C_{Co}$$

$$A_{505} = 0.187 = 0.533 C_{Cr} + 5.07 C_{Co}$$

To determine C_{Cr} and C_{Co} we solve the first equation for C_{Co}

$$C_{Co} = \frac{0.336 - 15.2 C_{Cr}}{5.60}$$

and substitute the result into the second equation.

$$0.187 = 0.533 C_{Cr} + 5.07 \times \frac{0.336 - 15.2 C_{Cr}}{5.60}$$

$$0.187 = 0.3042 - 13.23 C_{Cr}$$

Solving for C_{Cr} gives the concentration of Cr^{3+} as 8.86×10^{-3} M. Substituting this concentration back into the equation for the mixture's absorbance at 400 nm gives the concentration of Co^{2+} as 3.60×10^{-2} M.

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Practice Exercise 10.7

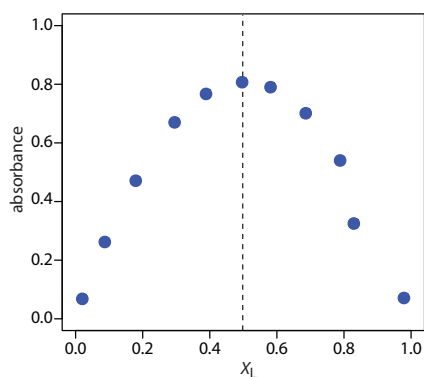Letting X represent MnO_4^{2-} and letting Y represent $Cr_2O_7^{2-}$, we plot the equation

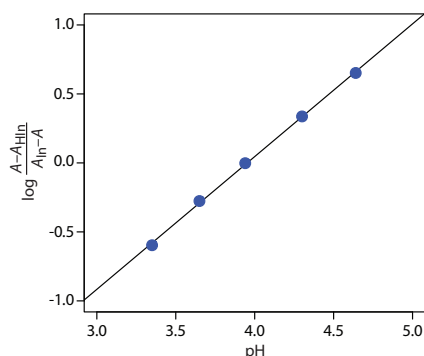$$\frac{A_{mix}}{A_{SX}} = \frac{C_X}{C_{SX}} + \frac{C_Y}{C_{SY}} \times \frac{A_{SY}}{A_{SX}}$$

placing A_{mix}/A_{SX} on the y-axis and A_{SY}/A_{SX} on the x-axis. For example, at a wavelength of 266 nm the value A_{mix}/A_{SX} of is 0.766/0.042, or 18.2, and the value of A_{SY}/A_{SX} is 0.410/0.042, or 9.76. Completing the calculations for all wavelengths and plotting the data gives the result shown in Figure 10.67. Fitting a straight-line to the data gives a regression model of
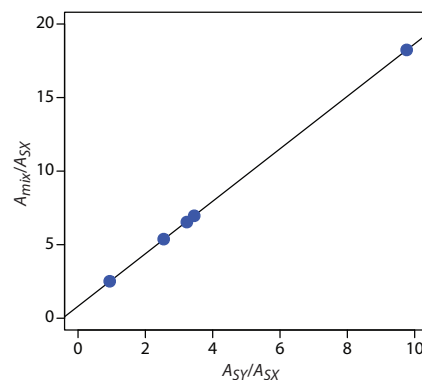
Figure 10.67 Multiwavelength linear regression analysis for the data in Practice Exercise 10.7.

$$\frac{A_{mix}}{A_{SX}} = 0.8147 + 1.7839 \times \frac{A_{SY}}{A_{SX}}$$

Using the y-intercept, the concentration of MnO_4^{2-} is

$$\frac{C_X}{C_{SX}} = 0.8147 = \frac{[MnO_4^-]}{1.0 \times 10^{-4} \text{ M } MnO_4^-}$$

or 8.15×10^{-5} M MnO_4^{2-}, and using the slope, the concentration of $Cr_2O_7^{2-}$ is

$$\frac{C_Y}{C_{SY}} = 1.7839 = \frac{[Cr_2O_7^{2-}]}{1.00 \times 10^{-4} \text{ M } Cr_2O_7^{2-}}$$

or 1.78×10^{-4} M $Cr_2O_7^{2-}$.

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Practice Exercise 10.8

Figure 10.68 Continuous variations plot for the data in Practice Exercise 10.8.

Figure 10.68 shows a continuous variations plot for the data in this exercise. Although the individual data points show substantial curvature—enough curvature that there is little point in trying to draw linear branches for excess metal and excess ligand—the maximum absorbance clearly occurs at $X_L \approx 0.5$. The complex's stoichiometry, therefore, is $Fe(SCN)^{2+}$.

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Practice Exercise 10.9

The value of K_a is

$$K_a = (1.00 \times 10^{-6}) \times \frac{0.225 - 0.000}{0.680 - 0.225} = 4.95 \times 10^{-7}$$

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Practice Exercise 10.10

Figure 10.69 Determining the pK_a of bromothymol blue using the data in Practice Exercise 10.10.

To determine K_a we use equation 10.21, plotting $\log[(A - A_{HIn})/(A_{In^-} - A)]$ versus pH, as shown in Figure 10.69. Fitting a straight-line to the data gives a regression model of

$$\log \frac{A - A_{HIn}}{A_{In^-} - A} = -3.80 + 0.962\text{pH}$$

The y-intercept is $-pK_a$; thus, the pK_a is 3.80 and the K_a is 1.58×10^{-4}.

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