for pumping tunable dye lasers. Dye lasers contain solutions of organic compounds that exhibit fluorescence in the UV, visible, or infrared regions. They can generally be tuned over a range of wavelengths of 20 to 50 nm. Tuned lasers are also useful as sources in absorption spectrometry because they provide good resolution (about 1 nm) and high throughput, although they tend to be less stable than continuum sources. Tunable lasers are available from about 265 to 800 nm. Several dyes are needed to cover a wide wavelength range.

We shall see below how spectrometric instruments can be adjusted to account for the variations in source intensity with wavelength as well as for the variation in detector sensitivity with wavelength.

MONOCHROMATORS

A monochromator consists chiefly of lenses or mirrors to focus the radiation, entrance and exit slits to restrict unwanted radiation and help control the spectral purity of the radiation emitted from the monochromator, and a dispersing medium to "separate" the wavelengths of the polychromatic radiation from the source. There are two basic types of dispersing elements, the prism and the diffraction grating. Various types of optical filters may also be used to select specific wavelengths.

Dispersion by prisms is good at short wavelengths, poor at long wavelengths (IR).

1. Prisms. When electromagnetic radiation passes through a prism, it is refracted because the index of refraction of the prism material is different from that in air. The index of refraction depends on the wavelength and, therefore, so does the degree of refraction. Shorter wavelengths are refracted more than longer wavelengths. The effect of refraction is to "spread" the wavelengths apart into different wavelengths (Figure 16.14). By rotation of the prism, different wavelengths of the spectrum can be made to pass through an exit slit and through the sample. A prism works satisfactorily in the ultraviolet and visible regions and can also be used in the infrared region. However, because of its **nonlinear dispersion**, it works more effectively for the shorter wavelengths. Glass prisms and lenses can be used in the visible region, but quartz or fused silica must be used in the ultraviolet region. The latter can also be used in the visible region.

In the infrared region, glass and fused silica transmit very little, and the prisms and other optics must be made from large crystals of alkali or alkaline earth halides, which are transparent to infrared radiation. Sodium chloride (rock salt) is used in most instruments and is useful for the entire region from 2.5 to 15.4 μm (4000 to 650 cm^{-1}). For longer wavelengths, KBr (10 to 25 μm) or CsI (10 to 38 μm) can be used. These (and the monochromator compartment) must be kept dry.

Dispersion by gratings is independent of wavelength, but the intensity varies with wavelength.

2. Diffraction Gratings. These consist of a large number of parallel lines (grooves) ruled on a highly polished surface such as aluminum, about 15,000 to 30,000 per inch for the ultraviolet and visible regions and 1500 to 2500 per inch for the infrared region. The grooves act as scattering centers for rays impinging on the grating. The result is equal dispersion of all wavelengths of a given order, that is, **linear dispersion** (Figure 16.15). The resolving power depends on the number

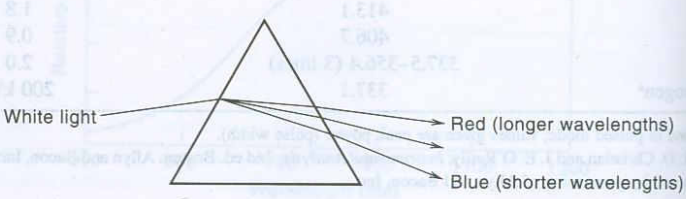


Fig. 16.14. Dispersion of polychromatic light by prism.

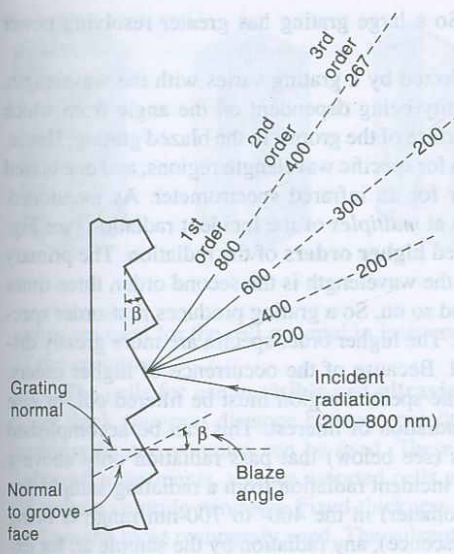


Fig. 16.15. Diffraction of radiation from grating.

of ruled grooves, but generally the resolving power of gratings is better than that of prisms, and they can be used in all regions of the spectrum. They are particularly well suited for the infrared region because of their equal dispersion of the long wavelengths. Gratings are difficult to prepare and original gratings are expensive. However, many **replica gratings** can be prepared from an original grating. This is done by coating the grating with a film of an epoxy resin that, after setting, is stripped off to yield the replica. It is made reflective by aluminizing the surface. These replica gratings are much less expensive and are even used in small inexpensive instruments.

An incident beam of radiation strikes the grating face at an angle i relative to the grating normal (Figure 16.15) and is reflected at an angle θ on the other side of the grating normal. The distance between grooves is d. The path difference between two incoming rays at angle i is $d \sin i$, and the path difference between the corresponding outgoing rays is $d \sin \theta$. The path difference for an incident and reflected ray is $d \sin i - d \sin \theta$. When this difference is equal to one or more wavelengths, fully constructive interference, and no destructive interference, occurs and a bright image results. The corresponding grating equation is

$$n\lambda = d(\sin i - \sin \theta) \tag{16.22}$$

where n is the *diffraction order*, and is an integer. It is apparent that if n is increased and the wavelength decreased by the same multiple, these shorter (higher order) wavelengths will be reflected at the same angle, θ. These have to be filtered before they reach the detector (see below). To disperse light of many wavelengths, the grating is rotated so that the angle i changes.

The *dispersion* of a grating for a given incident angle, i, is given by

$$\frac{d\theta}{d\lambda} = \frac{n}{d \cos \theta} \tag{16.23}$$

that is, it equals the order divided by the product of the grating spacing and cosine of the angle of reflection. The *resolving power* of a grating is the product of the

number of rulings and the order. So a large grating has greater resolving power than a small one.

The intensity of radiation reflected by a grating varies with the wavelength, the wavelength of maximum intensity being dependent on the angle from which the radiation is reflected from the surface of the groove in the blazed grating. Hence, gratings are blazed at specific angles for specific wavelength regions, and one blazed for the blue region would be poor for an infrared spectrometer. As mentioned, gratings also will produce radiation at *multiples* of the incident radiation (see Figure 16.15). These multiples are called **higher orders** of the radiation. The primary order is called the first order, twice the wavelength is the second order, three times the wavelength is the third order, and so on. So a grating produces first-order spectra, second-order spectra, and so on. The higher order spectra are more greatly dispersed and the resolution increased. Because of the occurrence of higher orders, radiation at wavelengths less than the spectral region must be filtered out, or else its higher orders will overlap the radiation of interest. This can be accomplished with various types of optical filters (see below) that pass radiation only above a certain wavelength. For example, if incident radiation from a radiating sample (replaces the source on a spectrophotometer) in the 400- to 700-nm range is being dispersed and measured (e.g., fluorescence), any radiation by the sample at, for example, 325 nm, would have a second order at 650 nm, which would overlap first-order radiation at 650 nm. This can be filtered out by placing a filter between the radiating sample and the grating that blocks radiation of ≤400 nm in the path of the incident beam; then the 325-nm radiation will not reach the grating.

Higher orders are better dispersed.

In fluorescence, higher order radiation from a shorter emitting (primary) wavelength may overlap a longer primary wavelength that is being measured. The shorter primary radiation must be filtered before reaching the grating. See also Section 16.9, single-beam spectrometers.

Ruled gratings have a problem of "ghosting" associated with periodic errors in the ruling engine drive screws, particularly if the gratings are used with high-intensity radiation sources (e.g., in fluorescence instruments—see below). This stray light is greatly reduced with **holographic gratings**. These are manufactured by exposing a photoresist layer, on a suitable substrate, to the interference pattern produced by two monochromatic laser beams, followed by photographic development to produce grooves, and then a reflective coating process. The smoother line profile results in reduced light scatter. Also, these gratings can be produced on curved surfaces and used to collimate light, eliminating mirrors or lenses that result in loss of light. While the cost of these gratings is higher than that of the more conventional type, they are commonly used in spectrometers today, particularly for measurement of radiating samples such as in fluorescence analysis. They have by-and-large replaced prisms in most instruments today.

3. Optical Filters. Various types of optical filters may be used to isolate certain wavelengths of light. There are narrow-bandpass filters, sharp-cut filters, and interference filters. The first two are usually made of glass and contain chemicals (dyes) that absorb all radiation except that desired to be passed. The sharp-cut filters absorb all radiation up to a specified wavelength, and pass radiation at longer wavelengths.

Interference filters consist of two layers of glass on whose inner surfaces a thin semitransparent film of metal is deposited and an inner layer of a transparent material such as quartz or calcium fluoride. Radiation striking the filter exhibits destructive interference, except for a narrow band of radiation for which the filter is designed to transmit. The bandwidth of the filters decreases as the transmitted radiation increases.

SAMPLE CELLS

The cell holding the sample (usually a solution) must, of course, be transparent in the wavelength region being measured. The materials described above for the

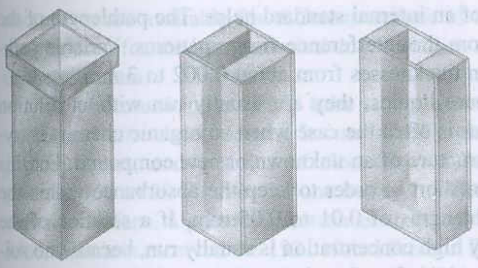


Fig. 16.16. Some typical UV and visible absorption cells.

optics are used for the cell material in instruments designed for the various regions of the spectrum.

Cells for:
UV—quartz
Vis—glass, quartz
IR—salt crystals

The cells for use in **visible** and **ultraviolet** spectrometers are usually cuvets 1 cm thick (*internal* distance between parallel walls), although cells of different pathlengths and volumes can be used. These are illustrated in Figure 16.16. For **infrared** instruments, various assorted cells are used. The most common is a cell of sodium chloride windows. Fixed-thickness cells are available for these purposes and are the most commonly used. The solvent, of course, must not attack the windows of the cell. Sodium chloride cells must be protected from atmospheric moisture (stored in desiccators) and moist solvents. They require periodic polishing to remove "fogging" due to moisture contamination. Silver chloride windows are often used for wet samples or aqueous solutions. These are soft and will gradually darken due to exposure to visible light.

Recommended pathlengths:
UV/Vis: 0.1–1 cm
Near-IR (800–1100 nm): 5–10 cm
(1100–3000 nm): 0.1–2 cm

Table 16.6 lists the properties of several infrared transmitting materials. The short pathlengths required in infrared spectrometry are difficult to reproduce, especially when the windows must be repolished, and so quantitative analysis is not

Table 16.6
Properties of Infrared Materials

Material	Useful Range (cm^{-1})	General Properties
NaCl	40,000–625	Hygroscopic, water soluble, low cost, most commonly used material.
KCl	40,000–500	Hygroscopic, water soluble.
KBr	40,000–400	Hygroscopic, water soluble, slightly higher in cost than NaCl and more hygroscopic.
CsBr	40,000–250	Hygroscopic, water soluble.
CsI	40,000–200	Very hygroscopic, water soluble, good for lower wavenumber studies.
LiF	83,333–1425	Slightly soluble in water, good UV material.
CaF_2	77,000–1110	Insoluble in water, resists most acids and alkalis.
BaF_2	67,000–870	Insoluble in water, brittle, soluble in acids and NH_4Cl.
AgCl	10,000–400	Insoluble in water, corrosive to metals. Darkens upon exposure to short-wavelength visible light. Store in dark.
AgBr	22,000–333	Insoluble in water, corrosive to metals. Darkens upon exposure to short-wavelength visible light. Store in dark.
KRS-5	16,600–285	Insoluble in water, highly toxic, soluble in bases, soft, good for ATR work.
ZnS	50,000–760	Insoluble in water, normal acids and bases, brittle.
ZnSe	20,000–500	Insoluble in water, normal acids and bases, brittle.
Ge	5000–560	Brittle, high index of refraction.
Si	83,333–1430 400–30	Insoluble in most acids and bases.
UV quartz	56,800–3700	Unaffected by water and most solvents.
IR quartz	40,000–3000	Unaffected by water and most solvents.
Polyethylene	625–10	Low-cost material for far-IR work.

Adapted from McCarthy Scientific Co. Catalogue 489, with permission.

as accurate in this region. Use of an internal standard helps. The pathlength of the empty cell can be measured from the interference fringe patterns. Variable pathlength cells are also available in thicknesses from about 0.002 to 3 mm.

When samples exist as pure liquids, they are usually run without dilution ("neat") in the infrared region, as is often the case when an organic chemist is trying to identify or confirm the structure of an unknown or new compound. For this purpose, the cell length must be short in order to keep the absorbance within the optimum region, generally pathlengths of 0.01 to 0.05 mm. If a solution of the sample is to be prepared, a fairly high concentration is usually run, because no solvent is completely transparent in the infrared region, and this will keep the solvent absorbance minimal. So again, short pathlengths are required, generally 0.1 mm or less.

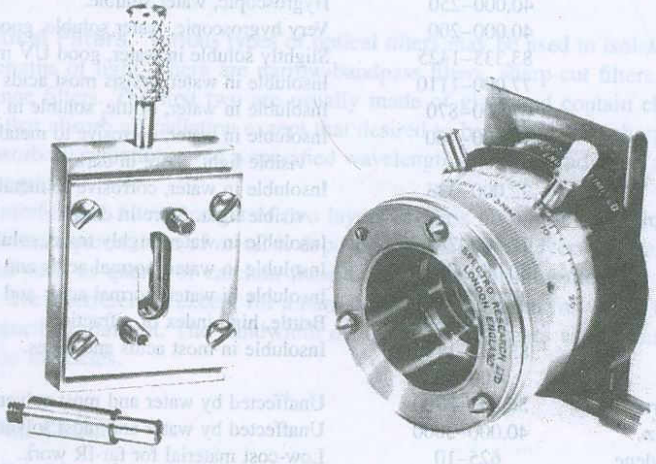
Solids are often not sufficiently soluble in the available solvents to give a high enough concentration to measure in the infrared region. However, powders may be run as a suspension or thick slurry (mull) in a viscous liquid having about the same index of refraction in order to reduce light scattering. The sample is ground in the liquid, which is often Nujol, a mineral oil (see Figure 16.4). Chlorofluorocarbon greases are useful when the Nujol masks any C—H bands present. The mull technique is useful for qualitative analysis, but it is difficult to reproduce for quantitative work. Samples may also be ground with KBr (which is transparent in the infrared region) and pressed into a pellet for mounting for measurement.

Gases may be analyzed by infrared spectrometry, and for this purpose a long-path cell is used, usually 10 cm in length, although cells as long as 20 m and up have been used in special applications. Some typical infrared cells are shown in Figure 16.17.

DETECTORS

Detectors for:
UV—phototube, PM tube, diode array
Vis—phototube, PM tube, diode array
IR—thermocouples; bolometers, thermistors

UV–Vis Detectors. Again, the detectors will also vary with the wavelength region to be measured. A **phototube** (or photocell) is commonly used in the *ultraviolet* and *visible regions*. This consists of a photoemissive cathode and an anode. A high voltage is impressed between the anode and cathode. When a photon enters the window of the tube and strikes the cathode, an electron is emitted and attracted to the anode, causing current to flow that can be amplified and measured. The response of the photoemissive material is wavelength dependent, and different



(a) (b)

Fig. 16.17. Typical infrared cells. (*a*) Fixed-path cell. (Courtesy of Barnes Engineering Co.) (*b*) Variable-pathlength cell. (Courtesy of Wilks Scientific Corporation.)

phototubes are available for different regions of the spectrum. For example, one may be used for the blue and ultraviolet portions and a second for the red portion of the spectrum.

A **photomultiplier (PM) tube** is more sensitive than a phototube for the *visible* and *ultraviolet regions*. It consists of a photoemissive cathode, which the photon strikes, and a series of electrodes (dynodes), each at a more positive potential (50 to 90 V) than the one before it. When an electron strikes the photoemissive surface, a primary electron is emitted (this is the photoelectric effect—Albert Einstein received the 1921 Nobel Prize in Physics for its discovery in 1905, not for the special theory of relativity which he also introduced in 1905—see www.lucidcafe.com/lucidcafe/library/96mar/einstein.html). The primary electron released from the photoemissive surface is accelerated toward the first dynode. The impact of the electron on the dynode surface causes the release of many secondary electrons, which in turn are accelerated to the next electrode where each secondary electron releases more electrons, and so on, up to about 10 stages of amplication. The electrons are finally collected by the anode. The final output of the photomultiplier tube may, in turn, be electronically amplified.

Again, different photomultiplier tubes have different response characteristics, depending on the wavelength. Figure 16.18 illustrates the response characteristics of some typical photomultiplier tubes with different photoemissive cathode surfaces. The 1P28 (S-5 surface) tube is popular because it can be used in both the ultraviolet and visible regions (e.g., in a UV–visible spectrometer). A 1-S surface is needed for the red region. Because of the greater sensitivity of photomultiplier tubes, less intense radiation is required and narrower slit widths can be used for better resolution of the wavelengths.

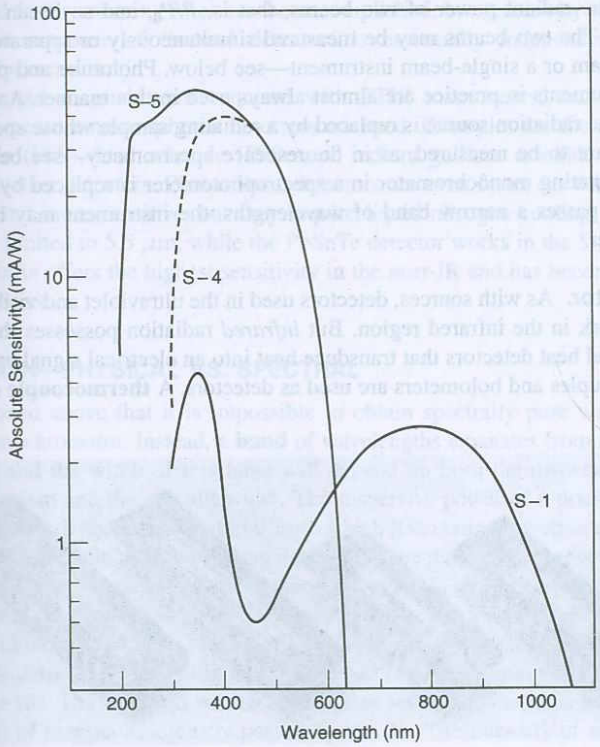


Fig. 16.18. Some spectral responses of photomultipliers. S-5 = RCA 1P28; S-4 = RCA 1P21; S-1 = RCA 7102. (From G. D. Christian and J. E. O'Reilly, *Instrumental Analysis*, 2nd ed. Boston: Allyn and Bacon, Inc., 1986. Reproduced by permission of Allyn and Bacon, Inc.)

Diode arrays can record an entire spectrum at once, from UV to near-IR.

Photomultiplier tubes have also been developed with response limited to the ultraviolet region (160 to 320 nm), the so-called **solar-blind photomultipliers**. They are helpful in reducing stray light effects from visible radiation and are useful as *UV detectors* in nondispersive systems.

Diode array detectors are used in spectrometers that record an entire spectrum simultaneously (see Section 16.10). A **diode array** consists of a series of hundreds of silicon photodiodes positioned side by side on a single silicon crystal or chip. Each has an associated storage capacitor that collects and integrates the photocurrent generated when photons strike the photodiode. They are read by periodical discharging, taking from 5 to 100 ms to read an entire array. If radiation dispersed into its different wavelengths falls on the surface area of the diode array, a spectrum can be recorded. A photograph of diode arrays is shown in Figure 16.19. They consist of 1024 diode elements in a space of a couple of centimeters. The spectral response of a silicon diode array is that of silicon, about 180 to 1100 nm; that is, ultraviolet to near infrared. See Figure 16.20. This range is wider than for photomultiplier tubes and the quantum efficiency is higher. The design of a diode array spectrometer is described in Section 16.10.

Another type of array detector is the charge-coupled device (CCD) detector. Two-dimensional CCDs are used in digital cameras.

Inexpensive silicon diodes or photocells are often used in lower priced instruments. These consist of a chip of pure silicon "doped" with a specific element in which a photon striking it causes an electric impulse (current). which is amplified for readout. Diodes are prepared to be sensitive to specific colors of light.

A spectrophotometer is a double-beam spectrometer that measures absorbance directly.

Spectrometers that use phototubes or photomultiplier tubes (or diode arrays) as detectors are generally called **spectrophotometers**, and the corresponding measurement is called **spectrophotometry**. More strictly speaking, the journal *Analytical Chemistry* defines a spectrophotometer as a spectrometer that measures the *ratio* of the radiant power of two beams, that is, P/P_0, and so it can record absorbance. The two beams may be measured simultaneously or separately, as in a double-beam or a single-beam instrument—see below. Phototube and photomultiplier instruments in practice are almost always used in this manner. An exception is when the radiation source is replaced by a radiating sample whose spectrum and intensity are to be measured, as in fluorescence spectrometry—see below. If the prism or grating monochromator in a spectrophotometer is replaced by an optical filter that passes a narrow band of wavelengths, the instrument may be called a photometer.

Common detectors:
- Photomultiplier tubes: 160–1100 nm
- Silicon-based photodiode arrays: 180–1100 nm
- Charge-coupled devices (CCDs): 180–1100 nm
- Silicon photodiodes: 350–1100 nm
- Indium gallium arsenide (InGaAs): 800–1700 nm
- Lead sulfide (PbS): 1000–3000 nm

IR Detector. As with sources, detectors used in the ultraviolet and visible regions do not work in the infrared region. But *infrared* radiation possesses the property of heat, and heat detectors that transduce heat into an electrical signal can be used. Thermocouples and bolometers are used as detectors. A **thermocouple** consists of

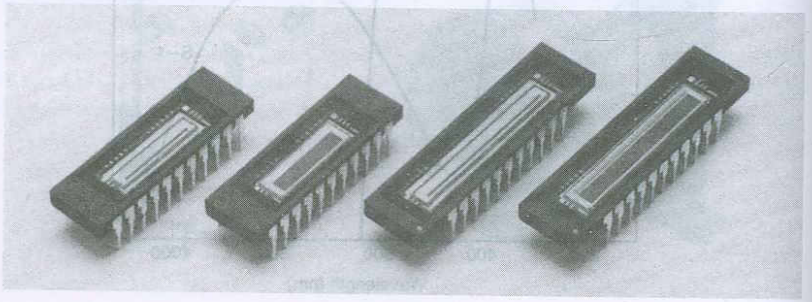


Fig. 16.19. Photo of 1024-element diode arrays. (Courtesy of Hamatsu Photonics, K. K.)

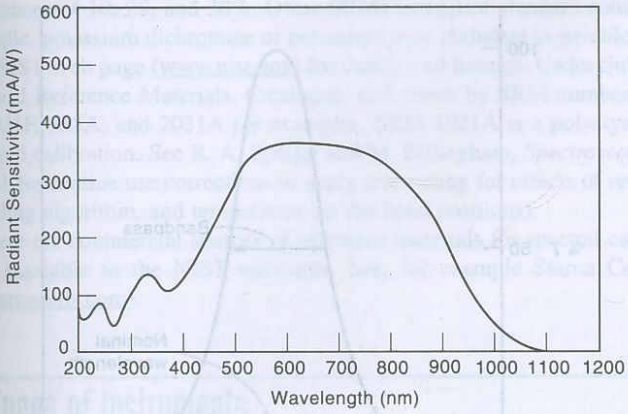


Fig. 16.20. Typical spectral response of diode array. (From M. Kendall-Tobias, *Am Lab.*, March, 1989, p. 102. Reproduced by permission of International Scientific Communications, Inc.)

two dissimilar metal wires, for example, antimony and bismuth, connected at two points. When a temperature difference exists between the two points, a potential difference is developed, which can be measured. One of the junctions, then, is placed in the path of the light from the monochromator. A **thermopile** consists of up to six thermocouples in series, mounted in a vacuum to minimize heat loss by conduction. Half are sensing and half are bonded to a substrate. Thermopiles have response times of about 30 ms. **Balometers** and **thermistors** are materials whose *resistance* is temperature dependent. The thermal resistors are made of sintered oxides of cobalt, manganese, and nickel. Their change in resistance is measured in a Wheatstone bridge circuit. The advantage of these over thermocouples is the more rapid response time (4 ms, compared with 60 ms), and thus improved resolution and faster scanning rates can be accomplished, but sensitivity is compromised. The response of thermal detectors is essentially independent of the wavelengths measured.

For rapid measurements required with FTIR instruments, and for high-sensitivity measurements, photon detectors are used. Examples are the solid-state lead sulfide (PbS), lead selenide (PbSe), or indium gallium arsenide (InGaAs) photoconductive detectors. Photovoltaic detectors are even faster (as fast as 20-ns response) and more sensitive, but they require liquid nitrogen cooling. The InSe detector is limited to 5.5 μm, while the PbSnTe detector works in the 5- to 13-μm range. InGaAs offers the highest sensitivity in the near-IR and has become the detector of choice.

SLIT WIDTH—PHYSICAL VS. SPECTRAL

The radiation passed by a slit is not monochromatic.

We mentioned above that it is impossible to obtain spectrally pure wavelengths from a monochromator. Instead, a **band** of wavelengths emanates from the monochromator and the width of this band will depend on both the dispersion of the grating or prism and the exit slit width. The dispersive power of a prism depends on the wavelength and on the material from which it is made, as well as on its geometrical design, while that of a grating depends on the number of grooves per inch. Dispersion is also increased as the distance to the slit is increased.

After the radiation has been dispersed, a certain portion of it will fall on the exit slit, and the width of this slit determines how broad a band of wavelengths the sample and detector will see. Figure 16.21 depicts the distribution of wavelengths leaving the slit. The **nominal wavelength** is that set on the instrument and is the wavelength of maximum intensity passed by the slit. The intensity of radiation at

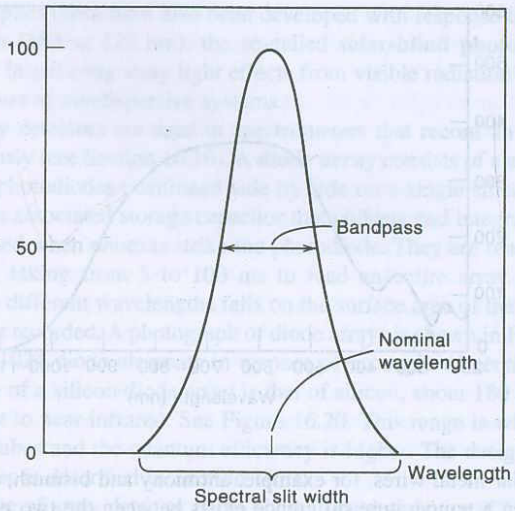


Fig. 16.21. Distribution of wavelengths leaving the slit of monochromator.

wavelengths on each side of this decreases, and the width of the band of wavelengths passed at one-half the intensity of the nominal wavelength is the **spectral bandwidth**, or **bandpass**. The **spectral slit width** is theoretically twice the spectral bandwidth (Figure 16.21 is theoretically an isosceles triangle), and this is a measure of the total wavelength spread that is passed by the slit. Note that the spectral slit width is not the same as the mechanical slit width, which may vary from a few micrometers to a millimeter or more (the spectral slit width is the band of radiation passed by the mechanical slit and is measured in units of wavelength). Seventy-five percent of the radiation intensity is theoretically contained within the wavelengths of the spectral bandwidth.

If the intensity of the source and the sensitivity of the detector are sufficient, the spectral purity can be improved (the bandpass decreased) by decreasing the slit width. The decrease may not be linear, however, and a limit is reached due to aberrations in the optics and diffraction effects caused by the slit at very narrow widths. The diffraction effectively increases the spectral slit width. In actual practice, the sensitivity limit of the instrument is usually reached before diffraction effects become too serious.

The bandwidth varies with wavelength with a prism, but is constant with a grating.

The bandwidth or the spectral slit width is essentially constant with a grating dispersing element for all wavelengths of a given spectral order at a constant slit width setting. This is not so with a prism because of the variation of dispersion with changing wavelength. The bandwidth will be smaller at shorter wavelengths and larger at longer wavelengths.

INSTRUMENTAL WAVELENGTH AND ABSORBANCE CALIBRATION

The wavelength reading of a spectrophotometer can be checked using solutions of known absorbance maxima and minima. Potassium dichromate at pH 2.9 has maximum absorbances at 257 and 350 nm, and minima at 235 and 313 nm. A holmium oxide glass filter absorbs sharply at 279.2, 222.8, 385.8, 446.0, 536.4, and 637.5 nm.

The National Institute of Standards and Technology (NIST) provides standard reference materials (SRMs) to verify the wavelength accuracy and accuracy of absorbance (transmittance) readings. SRM 930E for UV–Vis analysis consists of a set of three neutral density glass filters of standard thickness with nominal

transmittances of 10, 20, and 30%. Other SRMs consist of standard solutions of, for example, potassium dichromate or potassium acid phthalate in perchloric acid. See the NIST Web page (www.nist.gov) for details and listings. Under Subject, go to Standard Reference Materials, Catalogue, and check by SRM number, for example, 931F, 935A, and 2031A for examples. SRM 1921A is a polystyrene film for infrared calibration. See R. A. Spragg and M. Billingham, *Spectroscopy* **10**(1) (1995) 41 for routine use corrections to apply (correcting for effects of resolution, peak-finding algorithm, and temperature on the band positions).

There are commercial sources of reference materials for spectral calibration that are traceable to the NIST standards. See, for example Starna Cells, Inc. (www.starnacells.com).

16.9 Types of Instruments

Although all spectrometric instruments have the basic design presented in Figure 16.12, there are many variations depending on the manufacturer, the wavelength regions for which the instrument is designed, the resolution required, and so on. It is beyond the scope of our discussion to go into these, but we will indicate a few of the important general types of design and the general operation of a spectrometer.

SINGLE-BEAM SPECTROMETERS

These are the most common student spectrometers, since they are less expensive than more sophisticated instruments, and excellent results can be obtained with them. A diagram of the popular Bausch and Lomb Spectronic 20 spectrophotometer (phototube instrument) is shown in Figure 16.22. It consists of a tungsten lamp visible-light source and an inexpensive replica grating of 600 grooves per millimeter to disperse the radiation, ranging in wavelength from 330 to 950 nm. The exit slit allows a band of 20 nm of radiation to pass. If the wavelength is set at 480 nm, for example, radiation from 470 to 490 nm passes through the exit slit. By turning the wavelength cam, the grating is rotated to change the band of 20 nm of wavelengths passing through the exist slit (the path of only one 20-nm band is shown after reflection from the grating in the figure). The filter removes second-order and higher orders of diffraction from the grating that may pass the slit (stray

Higher order radiation from the grating must be filtered.

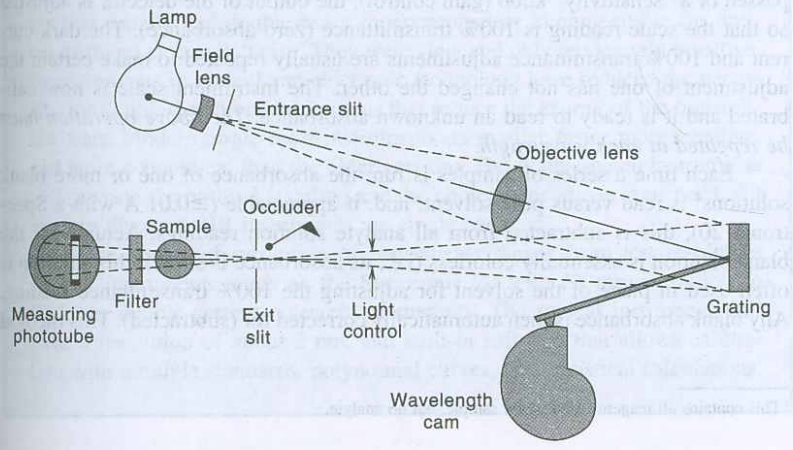


Fig. 16.22. Optical diagram of Bausch and Lomb Spectronic 20 spectrophotometer (top view). (Courtesy of Bausch and Lomb, Inc.)

light). The selection of the filter depends on what radiation must be restricted. For most applications, a cutoff-type filter is used that passes radiation below a certain wavelength where measurements are to be made, but not longer wavelengths where higher orders may appear. Narrower-range filters may be better for some applications, for example, *a red filter to remove any nonred light so the detector sees essentially pure red* (see below).

Any radiation not absorbed by the sample falls on the detector, where the intensity is converted to an electrical signal that is amplified and read on a meter. The measuring phototube for the visible region has maximum response at 400 nm, with only 5% of this response at 625 nm. Measurements above 625 nm are best made by substituting a red-sensitive phototube (RCA 6953) along with a red filter to remove second-order diffraction from the grating (it passes the desired red radiation but not undesired higher orders).

The Spectronic 20 models are available with either analog (Model 20+) or digital (Model 20D+) readout. The analog model features wavelength selection, a power/zeroing knob and *T/A* control. The absorbance is read on an analog meter. Analog output is adjustable from 0 to 1.0 V dc, and can be recorded on a chart recorder or a digital readout device. The digital instrument digitally displays the wavelength and either % *T*, *A*, or concentration. In the FACTOR mode, it electronically converts absorbance values to concentration units by multiplying the absorbance by this factor that has been established from calibration.

We have illustrated that the spectral intensity of the sources and the spectral response of the detectors are dependent on the wavelength. Therefore, some means must be employed to adjust the electrical output of the detector to the same magnitude at all wavelengths. This can be accomplished by one of two ways: by adjusting the slit width to allow more or less light to fall on the detector, or by adjusting the gain on the detector (the amount of amplification of the signal).

A single-beam instrument will have a shutter that can be placed in front of the detector so that no light reaches it. This is the occluder in the Spectronic 20, and it drops into place whenever there is no measuring cell placed in the instrument. With the shutter in position, a "dark current" adjusting knob is used to set the scale reading to zero percent transmittance (infinite absorbance). The **dark current** is a small current that may flow in the absence of light, owing to thermal emission of electrons from the cathode of the phototube. In the above operation, the dark current is set to zero scale reading by effectively changing the voltage on the tube. Now, the cell filled with solvent is placed in the beam path and the shutter is opened. By means of a slit width control to adjust the amount of radiation passed or a "sensitivity" knob (gain control), the output of the detector is adjusted so that the scale reading is 100% transmittance (zero absorbance). The dark current and 100% transmittance adjustments are usually repeated to make certain the adjustment of one has not changed the other. The instrument scale is now calibrated and it is ready to read an unknown absorbance. *The above operation must be repeated at each wavelength.*

Some current flows in the detector, even when no radiation falls on it. This is the dark current.

Each time a series of samples is run, the absorbance of one or more blank solutions[4] is read versus pure solvent; and, if appreciable ($\geq$0.01 A with a Spectronic 20), this is subtracted from all analyte solution readings. Actually, if the blank solution is essentially colorless (i.e., its absorbance is small), this solution is often used in place of the solvent for adjusting the 100% transmittance reading. Any blank absorbance is then automatically corrected for (subtracted). This method

[4] This contains all reagents used in the sample, but no analyte.

should only be used if the blank reading is small and has been demonstrated to be constant. A large blank reading would be more likely to be variable, and it would require a large gain on the detector, causing an increase in the noise level. An advantage of zeroing the instrument with the blank is that one reading, which always contains some experimental error, is eliminated. If this technique is used, it would be a good practice to check the zero with all the blank solutions to make sure the blank is constant.

DOUBLE-BEAM SPECTROMETERS

Double-beam spectrometers can automatically scan the wavelength and record the spectrum.

These are in practice rather complex instruments, but they have a number of advantages. They are used largely as recording instruments, that is, instruments that automatically vary the wavelength and record the absorbance as a function of wavelength. The instrument has two light paths, one for the sample and one for the blank or reference. In a typical setup, the beam from the source strikes a vibrating or rotating mirror that alternately passes the beam through the reference cell and the sample cell and, from each, to the detector. In effect, the detector alternately sees the reference and the sample beam and the output of the detector is proportional to the ratio of the intensities of the two beams (P/P_0).

The output is an alternating signal whose frequency is equal to that of the vibrating or rotating mirror. An ac amplifier is used to amplify this signal, and stray dc signals are not recorded. The wavelength is changed by a motor that drives the dispersing element at a constant rate, and the slit is continually adjusted by a servomotor to keep the energy from the reference beam at a constant value; that is, it automatically adjusts to 100% transmittance through the reference cell (which usually contains the blank or the solvent).

This is a simplified discussion of a double-beam instrument. There are variations on this design and operation, but it illustrates the utility of these instruments. They are very useful for qualitative work in which the entire spectrum is required, and they automatically compensate for absorbance by the blank, as well as for drifts in source intensity.

Single Beam or Double Beam?

Early UV–Vis and IR spectrophotometers, back in the 1950s, were big clunkers that usually had double-beam monochrometers to compensate for optical drift and electronic noise. They were slow and only moderately sensitive. Improvements in optical and electronic technology have reduced the necessity for double-beam optical systems that reduce the energy of the transmitted beam. Modern single-beam instruments are smaller, faster, more sensitive, and more economical than the older versions. But double-beam instruments still provide the optimal stability, and the choice depends on your need. All modern dispersive IR instruments are single beam.

The choice of resolution of instruments ranges from low-resolution student instruments, such as the Spectronic 20 with 20 nm resolution to 0.05 nm double-grating research instruments. The typical instrument will have a resolution of about 2 nm, and built-in software that allows calibration with multiple standards, polynomial curves, and statistical calculations.

16.10 Diode Array Spectrometers—Getting the Entire Spectrum at Once

In diode array spectrometers, there is no exit slit, and all dispersed wavelengths that fall on the array are recorded simultaneously.

In discussing detectors, we mentioned the use of photodiode array detectors for recording an entire spectrum in a few milliseconds. The basic design of a diode array-based spectrometer is shown in Figure 16.23. In this instance, polychromatic light passes through the sample, and the dispersing element is placed after the sample. The use of an exit slit to isolate a given wavelength is eliminated, and the dispersed light is allowed to fall on the face of the diode array detector. Each diode, in effect, acts as an exit slit of a monochromator. Resolution is limited by the element size of the diode array, but generally, the spatial resolution is about twice the size of a single element.

Diode spectrometers are very useful for the analysis of mixtures of absorbing species whose spectra overlap. The conventional simultaneous equation approach for analyzing mixtures is limited to two or three components (absorbance is measured at two or three wavelengths) in which the spectra are substantially different. With the diode array spectrometer, the absorbance at many points can be measured, using data on the sides of absorption bands as well as at absorption maxima. This method of "overdetermination," in which more measurement points than analytes are obtained, improves the reliability of quantitative measurements, allowing six or more constituents to be determined, or simple mixtures of components with similar spectra. An example of a multicomponent analysis is shown in Figure 16.24 for the simultaneous measurement of five hemoglobins. The five spectra were quantitatively resolved by comparing against standard spectra of each compound stored in the computer memory. Full-spectrum analysis can be performed in a variety of software packages. Mixtures of standards may be used for calibration, and this can compensate for possible interactions between components.

Modern-day instruments have done away with analog strip chart recorders. Instead, spectral scans are displayed on video monitors and printed by the computer.

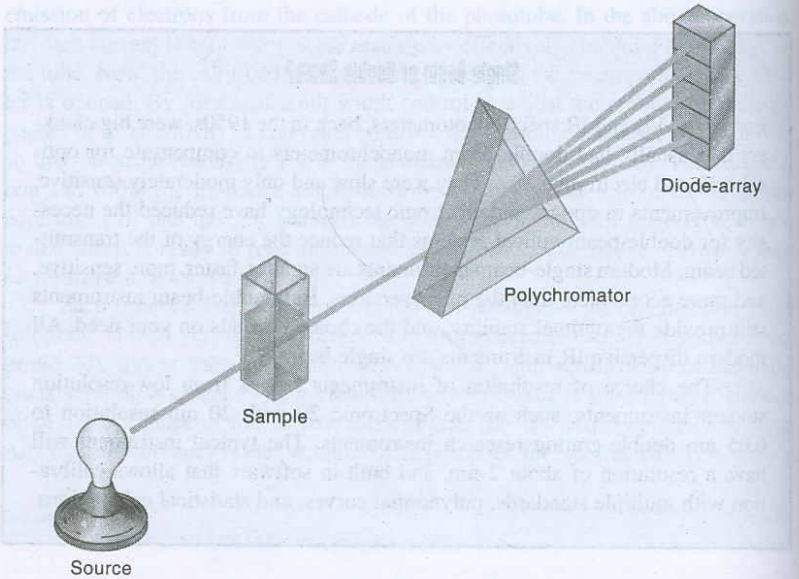
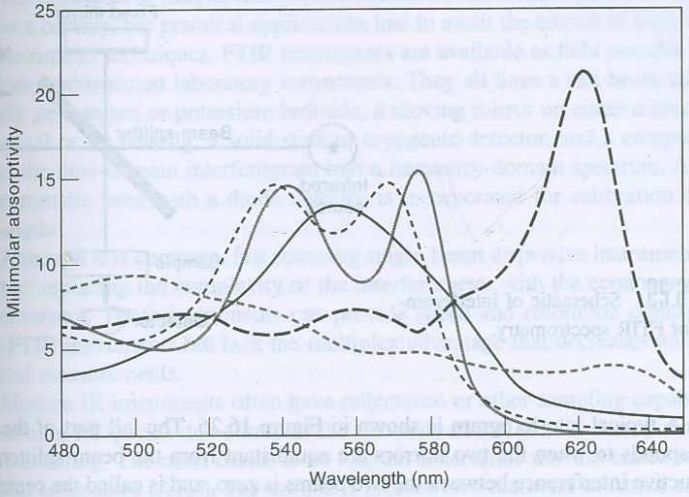


Fig. 16.23. Schematic of diode array spectrometer.



Note: Hb = hemoglobin, HbO_2 = oxyhemoglobin, HbCO = carboxyhemoglobin, Hi = methemoglobin, SHb = sulfhemoglobin.

Fig. 16.24. Millimolar absorptivities in $mmol^{-1}$ L cm^{-1}. [From A. Zwart, A. Buursma, E. J. van Kampen, and W. G. Zijlstra, *Clin. Chem.*, **30** (1984) 373. Reproduced by permission.]

The ability of diode array spectrometers to acquire data rapidly also allows the use of measurement statistics to improve the quantitative data. For example, 10 measurements can be made at each point in one second, from which the standard deviation of each point is obtained. The instrument's computer then weights the data points in a least-squares fit, based on their precisions. This "maximum-likelihood" method minimizes the effect of bad data points on the quantitative calculations.

The measurement precision is improved by averaging many measurements.

16.11 Fourier Transform Infrared Spectrometers

Conventional infrared spectrometers are known as **dispersive instruments**. With the advent of computer- and microprocessor-based instruments, these have been largely replaced by Fourier transform infrared (FTIR) spectrometers, which possess a number of advantages. Rather than a grating monochromator, an FTIR instrument employs an interferometer to obtain a spectrum.

FTIR spectrometers have largely replaced dispersive IR spectrometers.

The basis of an interferometer instrument is illustrated in Figure 16.25. Radiation from a conventional IR source is split into two paths by a beam splitter, one path going to a fixed position mirror, and the other to a moving mirror. When the beams are reflected, one is slightly displaced (out of phase) from the other since it travels a smaller (or greater) distance due to the moving mirror, and they recombine to produce an interference pattern (of all wavelengths in the beam) before passing through the sample. The sample sees all wavelengths simultaneously, and the interference pattern changes with time as the mirror is continuously scanned at a linear velocity. The result of absorption of the radiation by the sample is a spectrum in the **time domain**, called an **interferogram**, that is, absorption intensity as a function of the optical path difference between the two beams.

An interferogram is a spectrum in the time domain. Fourier transformation converts it to the frequency domain.

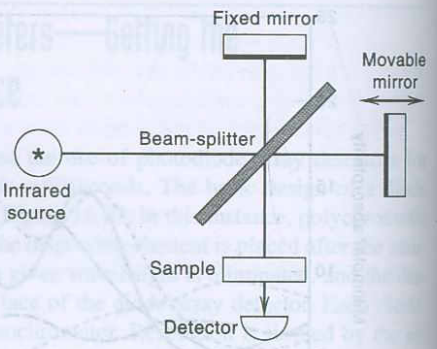


Fig. 16.25. Schematic of interferometer for FTIR spectrometry.

A typical interferogram is shown in Figure 16.26. The tall part of the signal corresponds to when the two mirrors are equidistant from the beam splitter, when destructive interference between the two beams is zero, and is called the centerburst. The intensity drops off rapidly away from this, due to destructive interference. This is converted, using a computer, into the frequency domain via a mathematical operation known as a **Fourier transformation** (hence the name **Fourier transform infrared spectrometer**). A conventional appearing infrared spectrum results.

Advantages of FTIR spectrometers: greater throughput, increased signal-to-noise ratio, simultaneous measurement of all wavelengths.

The advantages of an interferometer instrument is that there is greater throughput (Jacquinot's advantage) since all the radiation is passed. That is, the sample sees all wavelengths at all times, instead of a small portion at a time. This results in increased signal-to-noise ratio. In addition, a *multiplex advantage* (Fellget's advantage) results because the interferometer measures all IR frequencies simultaneously, and so a spectrum with resolution comparable to or better than that with a grating is obtained in a few seconds.

In order to take many interferograms and average them to increase the signal-to-noise level, the computer must average the centerburst at exactly the same position along the mirror's path every time. To achieve this, interferometers have a small red helium–neon (He–Ne) laser whose monochromatic beam passes through the interferometer, the same as the infrared source. Upon recombining, it produces interference fringes separated by the exact wavelength of the laser, 632.8 nm. These fringes serve as a calibration for the moving mirror position, allowing the computer to synchronize all the spectra.

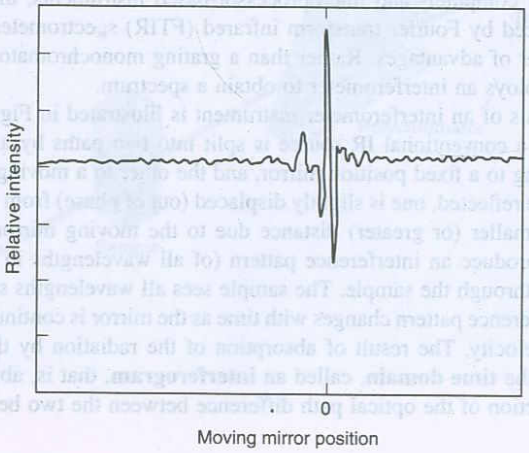


Fig. 16.26. Typical interferogram. Point marked "0" is where both mirrors of the interferometer are the same distance from beam splitter. [From D. W. Ball, *Spectroscopy*, **9**(8) (1994) 24. Reproduced by permission.]

The principles of interferometers and Fourier transformation have been known for over a century, but practical applications had to await the advent of high-speed digital computer techniques. FTIR instruments are available as field portable units and more sophisticated laboratory instruments. They all have a salt beam splitter, typically germanium or potassium bromide, a moving mirror on either a precision mechanical or air bearing, a solid-state or cryogenic detector, and a computer to process the time-domain interferogram into a frequency-domain spectrum. Also, a monochromatic laser with a diode detector is incorporated for calibration of the wavelength.

Although less common, fast scanning single-beam dispersive instruments are available, replacing the complexity of the interferometer with the economy of the monochronator. These instruments can provide speed and resolution comparable to the FTIR instruments but lack the multiplex advantage that decreases noise for low-level measurements.

Modern IR instruments often have reflectance or other sampling capabilities for obtaining IR spectra that eliminate the necessity of salt plate cells and simplify sample handling. The most useful is an internal reflectance method called attenuated total reflectance (ATR). The sample is pressed on a diamond substrate and the infrared radiation penetrates the sample, being reflected internally, and then exits for detection.

16.12 Near-IR Instruments

Radiation sources for near-IR instruments are operated at typically 2500 to 3000 K, compared to 1700 K in the mid-IR region, resulting in about 10 times more intense radiation and improved signal-to-noise ratios. This is possible because the IR radiation of typical sources tails off in the mid-IR region and the maximum intensity shifts further into the near-IR region as the temperature is increased. The higher temperature results in weaker mid-IR radiation, but is beneficial in the near-IR region. A tungsten–halogen lamp provides intense radiation in the 800- to 1100-nm range.

NIR sources are more intense and detectors more sensitive than for the mid-IR region, so noise levels are 1000-fold lower.

A gallium indium arsenide (GaInAs) detector is most commonly used in the near-IR and is roughly 100 times more sensitive than mid-IR detectors. The combination of intense radiation sources and sensitive detectors results in very low noise levels, on the order of microabsorbance units. Glass and quartz are transparent to near-IR radiation, and so the optics and cells are easier to design and use than for the mid-IR region. Near-IR radiation can be sent for long distances over fiber optics, and commercial instruments for process or field (portable) testing often use fiber-optic probes (see below) for nondestructive sample testing.

16.13 Spectrometric Error in Measurements

There will always be a certain amount of error or irreproducibility in reading an absorbance or transmittance scale. Uncertainty in the reading will depend on a number of instrumental factors and on the region of the scale being read, and hence on the concentration.

Because of the logarithmic relationship between transmittance and concentration, small errors in measuring transmittance cause large relative errors in the calculated concentration at low and high transmittances. It is probably obvious to

It is difficult to precisely measure either very small or very large decreases in absorbance.

you that if the sample absorbs only a very small amount of the light, an appreciable *relative* error may result in reading the small decrease in transmittance. At the other extreme, if the sample absorbs nearly all the light, an extremely stable instrument would be required to read the large decrease in the transmittance accurately. There is, therefore, some optimum transmittance or absorbance where the relative error in making the reading will be minimal.

The transmittance for minimum relative error can be derived from Beer's law by calculus, assuming that the error results essentially from the uncertainty in reading the instrument scale and also that the *absolute* error in reading the transmittance is constant, independent of the value of the transmittance. The result is the prediction that the minimum relative error in the concentration theoretically occurs when $T = 0.368$ or $A = 0.434$.

Figure 16.27 illustrates the dependence of the relative error on the transmittance, calculated for a constant error of $0.01T$ in reading the scale. It is evident from the figure that, while the minimum occurs at 36.8% T, a nearly constant minimum error occurs over the range of 20 to 65% T (0.7 to 0.2 A). The percent transmittance should fall within 10 to 80% T ($A = 1$ to 0.1) in order to prevent large errors in spectrophotometric readings. Hence, samples should be diluted (or concentrated), and standard solutions prepared, so that the absorbance falls within the optimal range.

The absorbance should fall in the 0.1 to 1 range.

Figure 16.27 in practice approximates the error only for instruments with **Johnson** or **thermal noise-limited detectors**, such as photoconductive detectors like CdS or PbS detectors (400 to 3500 nm) or thermocouples, bolometers, and Golay detectors in the infrared region. Johnson noise is produced by random thermal motion in resistance circuit elements.

With phototubes and photomultiplier-type detectors (photoemissive detectors, ultraviolet to visible range), thermal noise becomes insignificant as compared to shot noise. **Shot noise** is the random fluctuation of the electron current from an electron-emitting surface (i.e., across a junction from cathode to anode), and in PM tubes that is amplified and becomes the noise-limiting fluctuation. In instruments with these detectors, the absolute error is not constant at all values of T, and the expressions for the spectrophotometric error become more complicated. It has been calculated that, for these cases, the minimal error should occur at $T = 0.136$ or $A = 0.87$. These instruments have a working range of about 0.1 to 1.5 A.

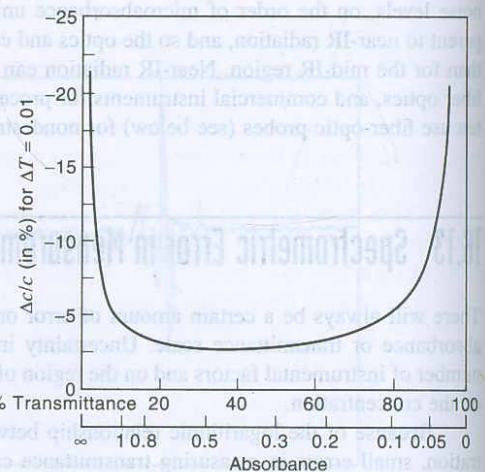


Fig. 16.27. Relative concentration error as function of transmittance for 1% uncertainty in % T.

16.14 Deviation from Beer's Law

Deviations from Beer's law result in nonlinear calibration curves, especially at higher concentrations.

It cannot always be assumed that Beer's law will apply, that is, that a linear plot of absorbance versus concentration will occur. Deviations from Beer's law occur as the result of chemical and instrumental factors. Most "deviations" from Beer's law are really only "apparent" deviations because if the factors causing nonlinearity are accounted for, the true or corrected absorbance-versus-concentration curve will be linear. True deviations from Beer's law will occur when the concentration is so high that the index of refraction of the solution is changed from that of the blank. A similar situation would apply for mixtures of organic solvents with water, and so the blank solvent composition should closely match that of the sample. The solvent may also have an effect on the absorptivity of the analyte.

CHEMICAL DEVIATIONS

Chemical causes for nonlinearity occur when nonsymmetrical chemical equilibria exist. An example is a weak acid that absorbs at a particular wavelength but has an anion that does not:

$$\underset{\text{(absorbs)}}{HA} \rightleftharpoons H^+ + \underset{\text{(transparent)}}{A^-}$$

The ratio of the acid form to the salt form will, of course, depend on the pH (Chapter 7). So long as the solution is buffered or is very acid, this ratio will remain constant at all concentrations of the acid. However, in unbuffered solution, the degree of ionization will increase as the acid is diluted, that is, the above equilibrium will shift to the right. Thus, a smaller fraction of the species exists in the acid form available for absorption for dilute solutions of the acid, causing apparent deviations from Beer's law. The result will be a positive deviation from linearity at higher concentrations (where the fraction dissociated is smaller). If the anion form were the absorbing species, then the deviation would be negative. Similar arguments apply to colored (absorbing) metal ion complexes or chelates in the absence of a large excess of the complexing agent. That is, in the absence of excess complexing agent, the degree of dissociation of the complex will increase as the complex is diluted. Here, the situation may be extremely complicated because the complex may dissociate stepwise into successive complexes that may or may not absorb at the wavelength of measurement. pH also becomes a consideration in these equilibria.

Apparent deviations may also occur when the substance can exist as a dimer as well as a monomer. Again, the equilibrium depends on the concentration. An example is the absorbance by methylene blue, which exhibits a negative deviation at higher concentrations due to association of the methylene blue.

The best way to minimize chemical deviations from Beer's law is by adequate buffering of the pH, adding a large excess of complexing agent, ionic strength adjustment, and so forth. Preparation of a calibration curve over the measurement range will correct for most deviations.

The absorptivity of all species is the same at the isosbestic point.

If both species of a chemical equilibrium absorb, and if there is some overlap of their absorption curves, the wavelength at which this occurs is called the **isosbestic point**, and the molar absorptivity of both species is the same. Such a point is illustrated in Figure 16.28. The spectra are plotted at different pH values since the pH generally causes the shift in the equilibrium. Obviously, the effect of pH could be eliminated by making measurements at the isosbestic point, but the sensitivity is decreased. By making the solution either very acid or very alkaline,

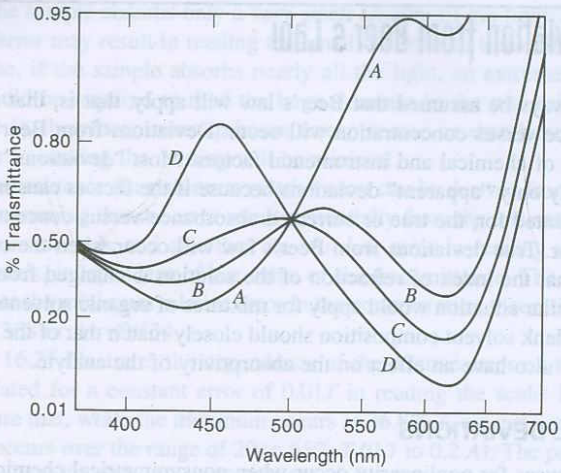


Fig. 16.28. Illustration of isosbestic point of bromthymol blue (501 nm): (*A*) pH 5.45, (*B*) pH 6.95, (*C*) pH 7.50, (*D*) pH 11.60.

one species predominates and the sensitivity is increased by measuring at this condition.

For a two-component system in which the two absorbing species are in equilibrium, all curves intersect at the isosbestic point where they have the same ϵ value. The existence of an isosbestic point is a necessary (although not sufficient) condition to prove that there are only two absorbing substances in equilibrium with overlapping absorption bands. If both of the absorbing species follow Beer's law, then the absorption spectra of all equilibrium mixtures will intersect at a fixed wavelength. For example, the different colored forms of indicators in equilibrium (e.g., the red and yellow forms of methyl orange) often exhibit an isosbestic point, supporting evidence that two and only two colored species participate in the equilibrium.

The existence of an isosbestic point is not proof of the presence of only two components. There may be a third component with $\epsilon = 0$ at this particular wavelength. The absence of an isosbestic point, however, is definite proof of the presence of a third component, provided the possibility of deviation from Beer's law in the two-component system can be dismissed. For a two-component system, the isosbestic point is a unique wavelength for quantitative determination of the total amount of two absorbing species in mutual equilibrium.

INSTRUMENTAL DEVIATIONS

The basic assumption in applying Beer's law is that monochromatic light is used. We have seen in the discussions above that it is impossible to extract monochromatic radiation from a continuum source. Instead, a band of radiation is passed, the width of which depends on the dispersing element and the slit width. In an absorption spectrum, different wavelengths are absorbed to a different degree; that is, the absorptivity changes with wavelength. At a wavelength corresponding to a fairly broad maximum on the spectrum, the band of wavelengths will all be absorbed to nearly the same extent. However, on a steep portion of the spectrum, they will be absorbed to different degrees. The slope of the spectrum increases as the concentration is increased, with the result that the fractions of the amounts of each wavelength absorbed may change, particularly if the instrument setting should drift over the period of the measurement. So a negative deviation in the absorbance-versus-concentration plot will be observed. The greater the slope of the spectrum, the greater is the deviation.

Obviously, it is advantageous to make the measurement on an absorption peak whenever possible, in order to minimize this curvature, as well as to obtain maximum sensitivity. Because a band of wavelengths is passed, the absorptivity at a given wavelength may vary somewhat from one instrument to another, depending on the resolution, slit width, and sharpness of the absorption maximum. Therefore, you should check the absorptivity and linearity on your instrument rather than relying on reported absorptivities. It is common practice to prepare calibration curves of absorbance versus concentration rather than to rely on direct calculations of concentration from Beer's law.

The absorptivity at a given wavelength may vary from instrument to instrument. Therefore, always run a standard.

If there is a second (interfering) absorbing species whose spectrum overlaps with that of the test substance, nonlinearity of the total absorbance as a function of the test substance concentration will result. It may be possible to account for this in preparation of the calibration curve by adding the interfering compound to standards at the same concentration as in the samples. This will obviously work only if the concentration of the interfering compound is essentially constant, and the concentration should be relatively small. Otherwise, simultaneous analysis as described earlier will be required.

Other instrumental factors that may contribute to deviations from Beer's law include stray radiation entering the monochromator and being detected, internal reflections of the radiation within the monochromator, and mismatched cells (in terms of pathlength) used for different analyte solutions or used in double-beam instruments (when there is appreciable absorbance by the blank or solvent in the reference cell). **Stray light** (any detected light that is not absorbed by the sample or is outside the bandwidth of the selected wavelength) becomes especially limiting at high absorbances and eventually causes deviation from linearity. Noise resulting from stray light also becomes a major contributor to the spectrometric error or imprecision at high absorbances. Radiation that does not interact with the sample can originate from light leaks in the instrument, from scattering of light from the optical components, or scattered light through the sample itself. A stray light component equivalent to 0.1% transmittance results in an error of 0.4% for a sample with 1 absorbance unit.

Stray light is the most common cause of negative deviation from Beer's law. For Beer's law, the light falling on the detector goes to zero at infinite concentration (all the light is absorbed). But this is impossible when stray light falls on the detector.

Other chemical and instrumental sources of nonlinearity in absorbance measurements may include hydrogen bonding, interaction with the solvent, nonlinear detector, nonlinear electronics, noncollimated radiation, and high signal levels (saturation).

Nonuniform cell thickness can affect a quantitative analysis. This is potentially a problem, especially in infrared spectrometry, where cell spacers are used. Air bubbles can affect the pathlength and stray light, and it is important to eliminate these bubbles, again especially in the infrared cells.

16.15 Fluorometry

Fluorometric analysis is extremely sensitive and is used widely by biochemists, clinical chemists, and analytical chemists in general.

PRINCIPLES OF FLUORESCENCE

When a molecule absorbs electromagnetic energy, this energy is usually lost as heat, as the molecule is deactivated via collisional processes. With certain molecules (ca. 5 to 10%), however, particularly when absorbing high-energy radiation such as UV radiation, only part of the energy is lost via collisions, and then the

Some molecules that absorb UV radiation lose only part of the absorbed energy by collisions. The rest is reemitted as radiation at longer wavelengths.

electron drops back to the ground state by emitting a photon of lower energy (longer wavelength) than was absorbed. Refer to Figure 16.29.

A molecule at room temperature normally resides in the ground state. The ground state is usually a **singlet state** (S_0), with all electrons paired. Electrons that occupy the same molecular orbital must be "paired," that is, have opposite spins. In a singlet state, the electrons are paired. If electrons have the same spin, they are "unpaired" and the molecule is in a **triplet state**. Singlet and triplet states refer to the **multiplicity** of the molecule. The process leading to the emission of a fluorescent photon begins with the absorption of a photon (a process that takes 10^{-15} s) by the fluorophore, resulting in an electronic transition to a higher-energy (excited) state. In most organic molecules at room temperature, this absorption corresponds to a transition from the lowest vibrational level of the ground state to one of the vibrational levels of the first or second electronic excited state of the same multiplicity (S_1, S_2). The spacing of the vibrational levels and rotational levels in these higher electronic states gives rise to the absorption spectrum of the molecule.

If the transition is to an electronic state higher than S_1, a process of **internal conversion** rapidly takes place. It is thought that the excited molecule passes from the vibrational level of this higher electronic state to a high vibrational level of S_1 that is isoenergetic with the original excited state. Collision with solvent molecules at this point rapidly removes the excess energy from the higher vibrational level of S_1; this process is called **vibrational relaxation**. These energy degradation processes (internal conversion and vibrational relaxation) occur rapidly ($\sim 10^{-12}$ s). Because of this rapid energy loss, emission fluorescence from higher states than the first excited state is rare.

The wavelengths of emitted radiation are independent of the wavelength of excitation. Their intensities are dependent, though.

Once the molecule reaches the first excited singlet, internal conversion to the ground state is a relatively slow process. Thus, decay of the first excited state by emission of a photon can effectively compete with other decay processes. This emission process is **fluorescence**. Generally, fluorescence emission occurs very rapidly after excitation (10^{-6} to 10^{-9} s). Consequently, it is not possible for the eye to perceive fluorescence emission after removal of the excitation source. Because fluorescence occurs from the lowest excited state, the fluorescence spectrum, that is, the wavelengths of emitted radiation, is independent of the wavelength of

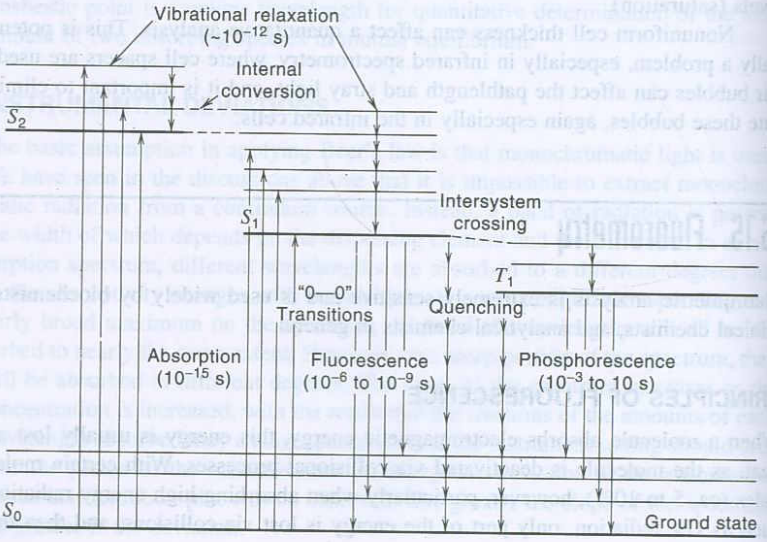


Fig. 16.29. Energy level diagram showing absorption processes, relaxation processes, and their rates.

excitation. The intensity of emitted radiation, however, will be proportional to the intensity of incident radiation (i.e., the number of photons absorbed).

Another feature of excitation and emission transitions is that the longest wavelength of excitation corresponds to the shortest wavelength of emission. This is the "0–0" band, corresponding to the transitions between the 0 vibrational level of S_0 and the 0 vibrational level of S_1 (Figure 16.29).

While the molecule is in the excited state, it is possible for one electron to reverse its spin, and the molecule transfers to a lower-energy triplet state by a process called **intersystem crossing**. Through the processes of internal conversion and vibrational relaxation, the molecule then rapidly attains the lowest vibrational level of the first excited triplet (T_1). From here, the molecule can return to the ground state S_0 by emission of a photon. This emission is referred to as **phosphorescence**. Since transitions between states of different multiplicity are "forbidden," T_1 has a much longer lifetime than S_1 and phosphorence is much longer-lived than fluorescence ($>10^{-4}$ s). Consequently, one can quite often perceive an "afterglow" in phosphorescence when the excitation source is removed. In addition, because of its relatively long life, radiationless processes can compete more effectively with phosphorescence than fluorescence. For this reason, phosphorescence is not normally observed from solutions due to collisions with the solvent or with oxygen. Phosphorescence measurements are made by cooling samples to liquid nitrogen temperature (−196°C) to freeze them and minimize collision with other molecules. Solid samples will also phosphoresce, and many inorganic minerals exhibit long-lived phosphorescence. Studies have been made in which molecules in solution are adsorbed on a solid support from which they can phosphoresce. Phosphorescence may be observed with minerals.

Phosphorescence is longer lived than fluorescence, and it may continue after the excitation source is turned off.

A typical excitation and emission spectrum of a fluorescing molecule is shown in Figure 16.30. The excitation spectrum usually corresponds closely in shape to the absorption spectrum of the molecule. There is frequently (but not necessarily) a close relationship between the structure of the excitation spectrum and the structure of the emission spectrum. In many relatively large molecules, the vibrational spacings of the excited states, especially S_1, are very similar to those in S_0. Thus, the form of the emission spectrum resulting from decay to the various S_0 vibrational levels tends to be a "mirror image" of the excitation spectrum arising from excitation

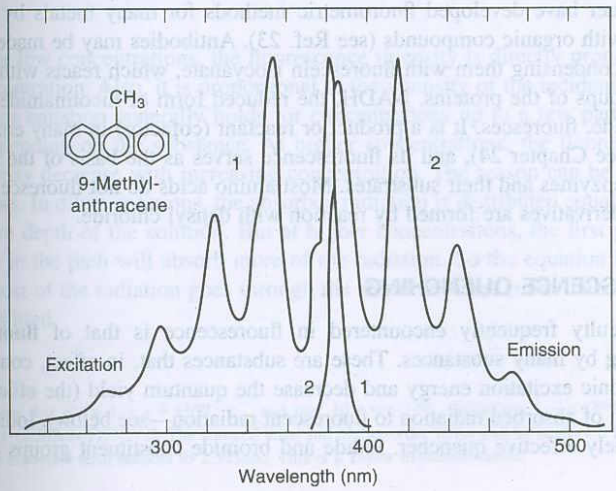


Fig. 16.30. Excitation and emission spectra of fluorescing molecule.

to the various vibrational levels in the excited state, such as S_1. Substructure, of course, results also from different rotational levels at each vibrational level.

The longest wavelength of absorption and the shortest wavelength of fluorescence tend to be the same (the 0–0 transition in Figure 16.29). More typically, however, this is not the case due to solvation differences between the excited molecule and the ground-state molecule. The heats of solvation of each are different, which results in a decrease in the energy of the emitted photon by an amount equal to these two heats of solvation.

Only those molecules that will absorb radiation, usually ultraviolet radiation, can fluoresce, and of those that do absorb, only about 5 to 10% fluoresce. This is an advantage when considering possible interference in fluorescence. The emitted radiation may be in the ultraviolet region, especially if the compound absorbs at less than 300 nm, but it is usually in the visible region. It is the emitted radiation that is measured and related to concentration.

CHEMICAL STRUCTURE AND FLUORESCENCE

In principle, any molecule that absorbs ultraviolet radiation could fluoresce. There are many reasons why they do not, but we will not go into these, other than to point out, in general, what types of substances may be expected to fluoresce.

First of all, the greater the absorption by a molecule, the greater its fluorescence intensity. Many aromatic and heterocyclic compounds fluoresce, particularly if they contain certain substituted groups. Compounds with multiple conjugated double bonds are favorable to fluorescence. One or more electron-donating groups such as —OH, —NH_2, and —OCH_3 enhances the fluorescence. Polycyclic compounds such as vitamin K, purines, and nucleosides and conjugated polyenes such as vitamin A are fluorescent. Groups such as —NO_2, —COOH, —CH_2COOH, —Br, —I, and azo groups tend to *inhibit* fluorescence. The nature of other substituents may alter the degree of fluorescence. The fluorescence of many molecules is greatly pH dependent because only the ionized or un-ionized form may be fluorescent. For example, phenol, C_6H_5OH, is fluorescent but its anion, $C_6H_5O^-$, is not.

If a compound is nonfluorescent, it may be converted to a fluorescent derivative. For example, nonfluorescent steroids may be converted to fluorescent compounds by dehydration with concentrated sulfuric acid. These cyclic alcohols are converted to phenols. Similarly, dibasic acids, such as malic acid, may be reacted with β-naphthol in concentrated sulfuric acid to form a fluorescing derivative. White and Argauer have developed fluorometric methods for many metals by forming chelates with organic compounds (see Ref. 23). Antibodies may be made to fluoresce by condensing them with fluorescein isocyanate, which reacts with the free amino groups of the proteins. NADH, the reduced form of nicotinamide adenine dinucleotide, fluoresces. It is a product or reactant (cofactor) in many enzyme reactions (see Chapter 24), and its fluorescence serves as the basis of the sensitive assay of enzymes and their substrates. Most amino acids do not fluoresce, but fluorescent derivatives are formed by reaction with dansyl chloride.

FLUORESCENCE QUENCHING

Quenching of fluorescence is often a problem in quantitative measurements.

One difficulty frequently encountered in fluorescence is that of **fluorescence quenching** by many substances. These are substances that, in effect, compete for the electronic excitation energy and decrease the quantum yield (the efficiency of conversion of absorbed radiation to fluorescent radiation—see below). Iodide ion is an extremely effective quencher. Iodide and bromide substituent groups decrease

the quantum yield. Substances such as this may be determined indirectly by measuring the extent of fluorescence quenching. Some molecules do not fluoresce because they may have a bond whose dissociation energy is less than that of the radiation. In other words, a molecular bond may be broken, preventing fluorescence.

A colored species in solution with the fluorescing species may interfere by absorbing the fluorescent radiation. This is the so-called **inner-filter effect**. For example, in sodium carbonate solution, potassium dichromate exhibits absorption peaks at 245 and 348 nm. These overlap with the excitation (275 nm) and emission (350 nm) peaks for tryptophan and would interfere. The inner-filter effect can also arise from too high a concentration of the fluorophore itself. Some of the analyte molecules will reabsorb the emitted radiation of others (see the discussion of fluorescence intensity and concentration below).

RELATIONSHIP BETWEEN CONCENTRATION AND FLUORESCENCE INTENSITY

Fluorescence intensity is proportional to the intensity of the source. Absorbance, on the other hand, is independent of it.

It can be readily derived from Beer's law (Problem 48) that the fluorescence intensity F is given by

$$F = \phi P_0(1 - 10^{-abc}) \tag{16.24}$$

where ϕ is the **quantum yield**, a proportionality constant and a measure of the fraction of absorbed photons that are converted into fluorescent photons. The quantum yield is, therefore, less than or equal to unity. The other terms in the equation are the same as for Beer's law. It is evident from the equation that if the product abc is large, the term 10^{-abc} becomes negligible compared to 1, and F becomes constant:

$$F = \phi P_0 \tag{16.25}$$

On the other hand, if abc is small (≤ 0.01), it can be shown[5] by expanding Equation 16.18 that as a good approximation,

$$F = 2.303\phi P_0 abc \tag{16.26}$$

For low concentrations, fluorescence intensity becomes directly proportional to the concentration.

Thus, for low concentrations, the fluorescence intensity is directly proportional to the concentration. Also, it is proportional to the intensity of the incident radiation.

This equation generally holds for concentrations up to a few parts per million, depending on the substance. At higher concentrations, the fluorescence intensity may decrease with increasing concentration. The reason can be visualized as follows. In dilute solutions, the absorbed radiation is distributed equally through the entire depth of the solution. But at higher concentrations, the first part of the solution in the path will absorb more of the radiation. So the equation holds only when most of the radiation goes through the solution, when more than about 92% is transmitted.

[5] It is known that $e^{-x} = 1 - x + x^2/2! \ldots$ and that $10^{-x} = e^{-2.303x}$. Therefore, $1 - e^{-2.303abc} = 1 - [1 - 2.303abc + (2.303abc)^2/2! \ldots]$. The squared term and higher-order terms can be neglected if $abc \leq 0.01$, and so the expanded term reduces to $2.303abc$. This is a Taylor expansion series.

FLUORESCENCE INSTRUMENTATION

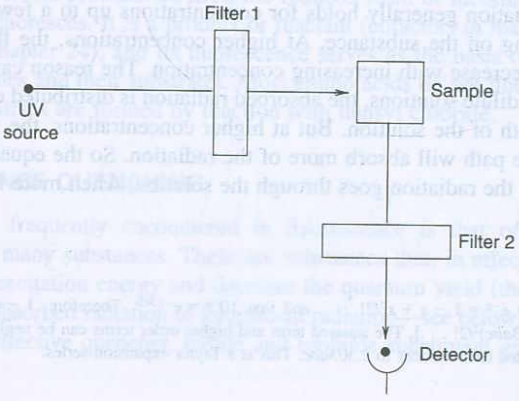
For fluorescence measurements, it is necessary to separate the emitted radiation from the incident radiation. This is most easily done by measuring the fluorescence at right angles to the incident radiation. The fluorescence radiation is emitted in all directions, but the incident radiation passes straight through the solution.

A simple fluorometer design is illustrated in Figure 16.31. An ultraviolet source is required. Most fluorescing molecules absorb ultraviolet radiation over a band of wavelengths, and so a simple line source is sufficient for many applications. Such a source is a mercury vapor lamp. A spark is passed through mercury vapor at low pressure, and principal lines are emitted at 2537, 3650, 5200 (green), 5800 (yellow), and 7800 (red) Å. *Wavelengths shorter than 3000 Å are harmful to the eyes*, and one must never look directly at a short-wavelength UV source. The mercury vapor itself absorbs most of the 2537-Å radiation (self-absorption), and a blue filter in the envelope of the lamp may be added to remove most of the visible light. The 3650-Å line is thus the one used primarily for the activation. A high-pressure xenon arc (a continuum source) is usually used as the source in more sophisticated instruments that will scan the spectrum (spectrofluorometers). The lamp pressure is 7 atm at 25°C and 35 atm at operating temperatures. Take care!

In the simple instrument in Figure 16.31, a primary filter (filter 1) is used to filter out the wavelengths close to the wavelength of the emission because, in practice, some radiation is scattered. The primary filter allows the passage of only the wavelength of excitation. The secondary filter (filter 2) passes the wavelength of emission but not the wavelength of excitation (which may be scattered). Glass will pass appreciable amounts of the 3650-Å line, and so some instruments employ glass cuvets and filters. However, it is better to use quartz (special nonfluorescing grades are available). This simple setup is satisfactory for many purposes.

We can see why fluorometric methods are so sensitive if we compare them with absorption spectrometry. In absorption methods, the difference between two finite signals, P_0 and P, is measured. The sensitivity is then governed by the ability to distinguish between these two, which is dependent on the stability of the instrument, among other factors. In fluorescence, however, we measure the difference between zero and a finite number, and so, in principle, the limit of detection is governed by the intensity of the source and the sensitivity and stability of the detector (the "shot noise"). Also, in fluorescence, the signal depends linearly on concentration, and a much wider dynamic range of concentration can be measured; a dynamic range of 10^3 to 10^4 is not uncommon. In addition to the enhanced

Fluorescence measurements are 1000-fold more sensitive than absorbance measurements. Absorbance is like weighing a ship and captain and subtracting the ship's weight to get the captain's weight ($P = P_0 - p$). In fluorescence, we measure only the captain.



Filter 1 removes wavelengths that would pass filter 2 and appear as fluorescence. Filter 2 removes scattered excitation wavelengths and passes the fluorescence.

Fig. 16.31. Simple fluorometer design.

sensitivity, much wider ranges of concentrations can be measured; a 1000-fold or greater range is not uncommon.

In a **spectrofluorometer**, the measurement is also made at right angles to the direction of the incident radiation. But instead of using filters, the instrument incorporates two monochromators, one to select the wavelength of excitation and one to select the wavelength of fluorescence. The wavelength of excitation from a continuum source can be scanned and the fluorescence measured at a set wavelength to give a spectrum of the excitation wavelengths. This allows the establishment of the wavelength of maximum excitation. Then, by setting the excitation wavelength for maximum excitation, the emission wavelength can be scanned to establish the wavelength of maximum emission. When this spectrum is scanned, there is usually a "scatter peak" corresponding to the wavelength of excitation.

In a spectrofluorometer, the filters are replaced with scanning monochromators. Either the excitation spectrum (similar to the absorbance spectrum) or the emission spectrum may be recorded.

In spectrofluorometers, it is difficult to correct for variations in intensity from the source or response of the detector at different wavelengths, and calibration curves are generally prepared under a given set of conditions. Since the source intensity or detector response may vary from day to day, the instrument is usually calibrated by measuring the fluorescence of a standard solution and adjusting the gain to bring the instrument reading to the same value. A dilute solution of quinine in dilute sulfuric acid is usually used as the calibrating standard.

Sometimes it is desirable to obtain "absolute" spectra of a fluorescing compound to calculate quantum efficiencies for different transitions. This would require point-by-point correction for variations in the recorded signal due to variations in the instrumental parameters. Commercial instruments are available that will provide "corrected spectra." These adjust for variation in the source intensity with wavelength, so the sample is irradiated with constant energy, and they also correct for variations of the detector response. The recorded emission spectrum is presented directly in quanta of photons emitted per unit bandwidth.

16.16 Optical Sensors: Fiber Optics

There has been a great deal of interest in recent years in developing optically based sensors that function much as electrochemical sensors (Chapter 15) do. These have been made possible with the advent of fiber-optic cables that transmit light along a flexible cable (waveguide) or "light pipe." Optical fibers were developed for the communications industry and are capable of transmitting light over long distances, but they have proven valuable for transmitting light to spectrometers and for developing analyte-selective sensors by coupling appropriate chemistries to the fibers. Through the use of optical fibers, a sample need not be brought to the spectrometer because light can be transmitted to and returned from the sample via the cables.

Fiber-optic cables allow the sample to be far removed from the spectrometer.

FIBER-OPTIC PROPERTIES

The construction of a fiber-optic cable is illustrated in Figure 16.32. It consists of a cylindrical *core* that acts as the waveguide, surrounded by a *cladding* material of higher index of refraction, and a protective buffer layer. Light is transmitted along the core by total internal reflection at the core–cladding interface. The angle of acceptance, θ_a, is the greatest angle of radiation that will be totally reflected for a given core–cladding refractive index difference. Any light entering at an angle greater than θ will not be transmitted, and θ_a defines the fiber's numerical aperture (NA):

$$\text{NA} = n_{\text{ext}} \sin \theta_a = \sqrt{n_1^2 - n_2^2} \qquad (16.27)$$

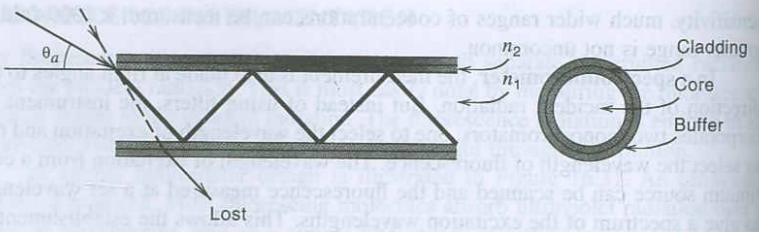


Fig. 16.32. Fiber-optic structure.

where n_2 is the refractive index of the cladding, n_1, is that of the core, and n_{ext} is that of the external medium. The greater NA, the greater the light-gathering ability.

Manufacturers typically provide numerical aperture data for different fibers. Another property usually provided is the light loss per unit length for different wavelengths. A spectral curve is given that shows attenuation versus wavelength. Attenuation is usually expressed in decibels per kilometer (dB/km), and is given by

$$\text{dB} = 10 \log \frac{P_0}{P} \tag{16.28}$$

where P_0 is the input intensity and P the output intensity. Thus, the attenuation for silica-based fibers at 850 nm is in the order of 10 dB/km. Note that dB = 10 × absorbance. So a 10-m (0.01-km) fiber would exhibit an absorbance of 0.01 (0.1 dB attenuation), corresponding to 97.7% transmittance.

Fiber optics may be purchased that transmit radiation from the ultraviolet (190 nm) to the infrared (≥5 μm), but each has a limited range. Plastic and compound glass materials are used for short distances in the visible region, while silica fibers can be used from the UV through the near-IR (2.3 μm) regions, but they are very costly. Fluoride and calcogenide glasses extend farther into the infrared.

In coupling fiber optics to spectrometers, there is a trade-off between increased numerical aperture to collect more light and the collection angle of the spectrometer itself, which is usually limiting. That is, light collected with a numerical aperture greater than that for the spectrometer limit will not be seen by the spectrometer. See Ref. 23 for a discussion of design considerations for fiber optic/spectrometer coupling.

With bifurcated cables, one is used to transmit the source radiation and the other is used to receive the absorbed or fluorescent radiation.

Fiber optics may be used as probes for conventional spectrophotometric and fluorescence measurements. Light must be transmitted from a radiation source to the sample and back to the spectrometer. While there are couplers and designs that allow light to be both transmitted and received by a single fiber, usually a **bifurcated fiber** cable is used. This consists of two fibers in one casing, split at the end that goes to the radiation source and the spectrometer. Often, the cables consist of a bundle of several dozen small fibers, and half are randomly separated from the other at one end. For absorbance measurements, a small mirror is mounted (attached to the cable) a few millimeters from the end of the fiber. The source radiation penetrates the sample solution and is reflected back to the fiber for collection and transmission to the spectrometer. The radiation path length is twice the distance between the fiber and the mirror.

Fluorescence measurements are made in a similar fashion, but without the mirror. Radiation emitting from the end of the fiber in the shape of a cone excites fluorescence in the sample solution, which is collected by the return cable (the

amount depends on the numerical aperture) and sent to the spectrometer. Often, a laser radiation source is used to provide good fluorescence intensity.

FIBER-OPTIC SENSORS

We can convert fiber-optic probes into selective absorbance- or fluorescence-based sensors by immobilizing appropriate reagents on the end of a fiber-optic cable. These possess the advantage over electrochemical sensors in that a reference electrode (and salt bridge) is not needed, and electromagnetic radiation will not influence the response. For example, a fluorometric pH sensor may be prepared by chemically immobilizing the indicator fluorescein isothiocyanate (FITC) on a porous glass bead and attaching this to the end of the fiber with epoxy. The FITC fluorescence spectrum changes with pH (Figure 16.33) over the range of about pH 3 to 7, centered around pK of the indicator. The fluorescence intensity measured at the fluorescence maximum is related to the pH via a calibration curve. The calibration curve will be sigmoid-shaped since it in effect represents a titration of the indicator. See Refs. 31 and 32 for a discussion of the limitations of fiber-optic sensors for measuring pH and ionic activity.

Optical sensors do not have the requirement and associated difficulties of a reference electrode.

If an enzyme, for example, penicillinase, is immobilized along with an appropriate indicator, then the sensor is converted into a biosensor for measuring penicillin. The enzyme catalyzes the hydrolysis of penicillin to produce penicilloic acid, which causes a pH decrease.

Fiber-optic sensors have been developed for oxygen, CO_2, alkali metals, and other analytes. In order for these to function, the indicator chemistry must be reversible.

MINIATURE FIBER-OPTIC SPECTROMETERS

There are inexpensive spectrometers that utilize fiber optics for light transmission, either through a cuvet cell or as fiber-optic probes. An example is the Ocean Optics S2000 spectrometer (www.oceanoptics.com). It has a 2048-element charge-coupled device (CCD)—an array silicon detector that accepts light energy transmitted through a single-strand optical fiber, and disperses it via a fixed grating across the array. Figure 16.34 illustrates this compact spectrometer. It can be

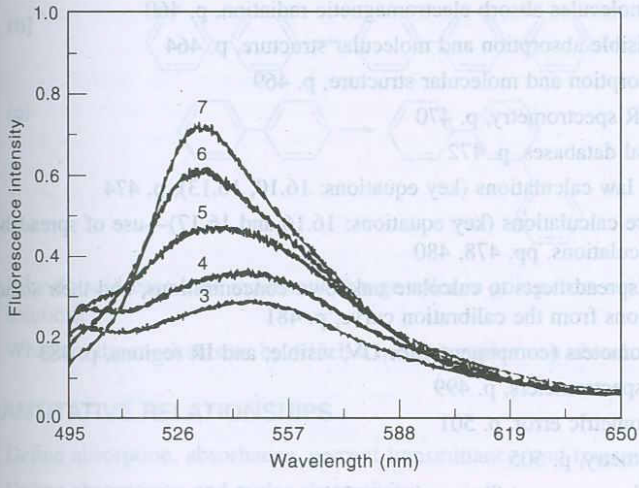


Fig. 16.33. Fluorescence spectra of FITC immobilized on porous glass bead at pH 3, 4, 5, 6, and 7. [From M.-R. S. Fuh, L. W. Burgess, T. Hirschfeld, G. D. Christian, and F. Wang, *Analyst*, **112** (1987) 1159. Reproduced by permission.]

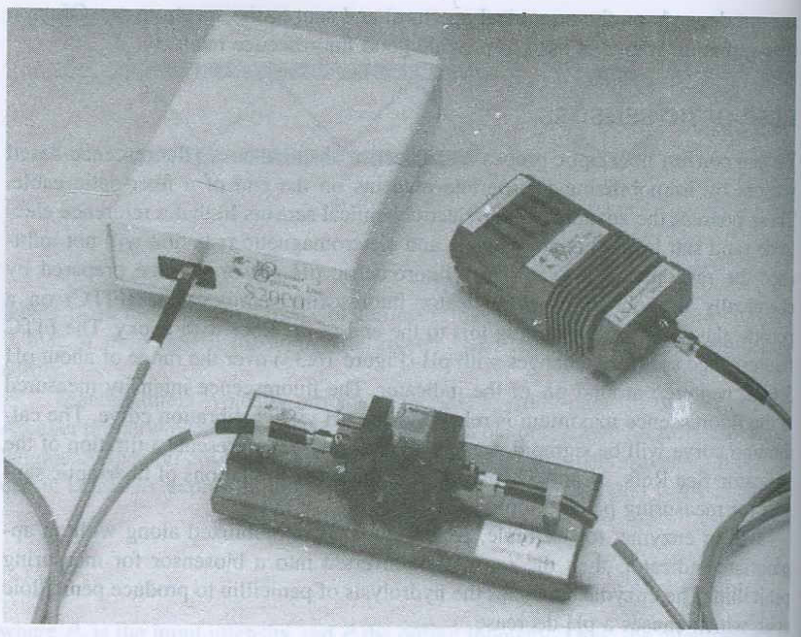


Fig. 16.34. Miniature fiber-optic spectrometer. Box is the spectrometer. Light source is to right, and fiber-optic cable guides light to cuvet. Second cable takes transmitted radiation to spectrometer. (Photo courtesy of Ocean Optics, Inc.)

configured for UV, visible, and NIR applications, from 200 to 1100 nm, using different sources, gratings, fiber optics, and detectors. The instrument provides the entire spectrum over the wavelength of operation and has software for analyzing the spectra.

Learning Objectives

WHAT ARE SOME OF THE KEY THINGS WE LEARNED FROM THIS CHAPTER?

- Wavelength, frequencies, and photon energy (key equations: 16.1 to 16.3), p. 458
- How molecules absorb electromagnetic radiation, p. 460
- UV–visible absorption and molecular structure, p. 464
- IR absorption and molecular structure, p. 469
- Near-IR spectrometry, p. 470
- Spectral databases, p. 472
- Beer's law calculations (key equations: 16.10, 16.13), p. 474
- Mixture calculations (key equations: 16.16 and 16.17)—use of spreadsheets for calculations, pp. 478, 480
- Using spreadsheets to calculate unknown concentrations, and their standard deviations from the calibration curve, p. 481
- Spectrometers (components) for UV, visible, and IR regions, p. 483
- FTIR spectrometers, p. 499
- Spectrometric error, p. 501
- Fluorometry, p. 505
- Optical sensors and fiber optics, p. 511

Questions

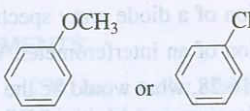
ABSORPTION OF RADIATION

1. Describe the absorption phenomena taking place in the far-infrared, mid-infrared, and visible–ultraviolet regions of the spectrum.
2. What types of electrons in a molecule are generally involved in the absorption of UV or visible radiation?
3. What are the most frequent electronic transitions during absorption of electromagnetic radiation? Which results in more intense absorption? Give examples of compounds that exhibit each.
4. What is a necessary criterion for absorption to occur in the infrared region?
5. What types of molecular vibration are associated with infrared absorption?
6. What distinguishes near-infrared absorption from mid-infrared absorption? What are its primary advantages?
7. Define the following terms: chromophore, auxochrome, bathochromic shift, hypsochromic shift, hyperchromism, and hypochromism.
8. Which of the following pairs of compounds is likely to absorb radiation at the longer wavelength and with greater intensity?

(a) $CH_3CH_2CO_2H$ or $CH_2{=}CHCO_2H$

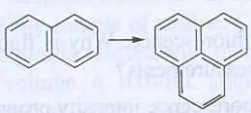
(b) $CH_3CH{=}CHCH{=}CHCH_3$ or $CH_3C{\equiv}C{-}C{\equiv}CCH_3$

(c)

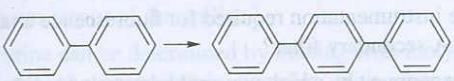


9. In the following pairs of compounds, describe whether there should be an increase in the wavelength of maximum absorption and whether there should be an increase in absorption intensity in going from the first compound to the second:

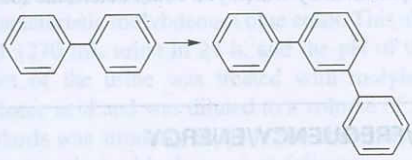
(a)



(b)



(c)



10. Why do acid-base base indicators change color in going from acid to alkaline solution?
11. What are the mechanisms by which a metal complex can absorb radiation?

QUANTITATIVE RELATIONSHIPS

12. Define absorption, absorbance, percent transmittance, and transmittance.
13. Define absorptivity and molar absorptivity.

14. Why is a calibration curve likely to be linear over a wider range of concentrations at the wavelength of maximum absorption compared to a wavelength on a shoulder of the absorption curve?
15. List some solvents that can be used in the ultraviolet, visible, and infrared regions, respectively. Give any wavelength restrictions.
16. What is an isosbestic point?
17. Describe and compare different causes for deviations from Beer's law. Distinguish between real and apparent deviations.

INSTRUMENTATION

18. Describe radiation sources and detectors for the ultraviolet, visible, and infrared regions of the spectrum.
19. Distinguish between the two types of monochromators (light dispersers) used in spectrophotometers and list the advantages and disadvantages of each.
20. Discuss the effect of the slit width on the resolution of a spectrophotometer and the adherence to Beer's law. Compare it with the spectral slit width.
21. Compare the operations of a single-beam spectrophotometer and a double-beam spectrophotometer.
22. Given the weak absorption in the near-infrared region, why do near-infrared instruments function with reasonable sensitivity?
23. Describe the operation of a diode array spectrometer.
24. Describe the operation of an interferometer. What are its advantages?
25. Referring to Figure 16.28, what would be the color of an acid solution and an alkaline solution at maximum absorption? What color filter would be most applicable for the analysis of each in a filter colorimeter? (A filter replaces the prism and slit arrangement).

FLUORESCENCE

26. Describe the principles of fluorescence. Why is fluorescence generally more sensitive than absorption measurements?
27. Under what conditions is fluorescence intensity proportional to concentration?
28. Describe the instrumentation required for fluorescence analysis. What is a primary filter? A secondary filter?
29. Suggest an experiment by which you could determine iodide ion by fluorescence.

Problems

WAVELENGTH/FREQUENCY/ENERGY

30. Express the wavelength 2500 Å in micrometers and nanometers.
31. Convert the wavelength 4000 Å into frequency (Hz) and into wavenumbers (cm^{-1}).
32. The most widely used wavelength region for infrared analysis is about 2 to 15 μm. Express this range in angstroms and in wavenumbers.
33. One mole of photons (Avogadro's number of photons) is called an *einstein* of radiation. Calculate the energy, in calories, of one einstein of radiation at 3000 Å.

BEER'S LAW

34. Several spectrophotometers have scales that are read either in absorbance or in percent transmittance. What would be the absorbance reading at 20% T? At 80% T? What would the transmittance reading be at 0.25 absorbance? At 1.00 absorbance?

35. A 20-ppm solution of a DNA molecule (unknown molecular weight) isolated from *Escherichia coli* was found to give an absorbance of 0.80 in a 2-cm cell. Calculate the absorptivity of the molecule.

36. A compound of formula weight 280 absorbed 65.0% of the radiation at a certain wavelength in a 2-cm cell at a concentration of 15.0 μg/mL. Calculate its molar absorptivity at the wavelength.

37. Titanium is reacted with hydrogen peroxide in 1 M sulfuric acid to form a colored complex. If a 2.00×10^{-5} M solution absorbs 31.5% of the radiation at 415 nm, what would be (a) the absorbance and (b) the transmittance and percent absorption for a 6.00×10^{-5} M solution?

38. A compound of formula weight 180 has an absorptivity of 286 cm^{-1} g^{-1} L. What is its molar absorptivity'?

39. Aniline, $C_6H_5NH_2$, when reacted with picric acid gives a derivative with an absorptivity of 134 cm^{-1} g^{-1} L at 359 nm. What would be the absorbance of a 1.00×10^{-4} M solution of reacted aniline in a 1.00-cm cell?

QUANTITATIVE MEASUREMENTS

40. The drug tolbutamine (f wt = 270) has a molar absorptivity of 703 at 262 nm. One tablet is dissolved in water and diluted to a volume of 2 L. If the solution exhibits an absorbance in the UV region at 262 nm equal to 0.687 in a 1-cm cell, how many grams tolbutamine are contained in the tablet?

41. Amines (weak base) form salts with picric acid (trinitrophenol), and all amine picrates exhibit an absorption maximum at 359 nm with a molar absorptivity of 1.25×10^4. A 0.200-g sample of aniline, $C_6H_5NH_2$, is dissolved in 500 mL water. A 25.0-mL aliquot is reacted with picric acid in a 250-mL volumetric flask and diluted to volume. A 10.0-mL aliquot of this is diluted to 100 mL and the absorbance read at 359 nm in a 1-cm cell. If the absorbance is 0.425, what is the percent purity of the aniline?

42. Phosphorus in urine can be determined by treating with molybdenum(VI) and then reducing the phosphomolybdo complex with aminonaphtholsulfonic acid to give the characteristic molybdenum blue color. This absorbs at 690 nm. A patient excreted 1270 mL urine in 24 h, and the pH of the urine was 6.5. A 1.00-mL aliquot of the urine was treated with molybdate reagent and aminonaphtholsulfonic acid and was diluted to a volume of 50.0 mL. A series of phosphate standards was similarly treated. The absorbance of the solutions at 690 nm, measured against a blank, were as follows:

Solution	Absorbance
1.00 ppm P	0.205
2.00 ppm P	0.410
3.00 ppm P	0.615
4.00 ppm P	0.820
Urine sample	0.625

(a) Calculate the number of grams of phosphorus excreted per day.

(b) Calculate the phosphate concentration in the urine as millimoles per liter.

(c) Calculate the ratio of HPO_4^{2-} to $H_2PO_4^-$ in the sample:

$$K_1 = 1.1 \times 10^{-2} \qquad K_2 = 7.5 \times 10^{-8} \qquad K_3 = 4.8 \times 10^{-13}$$

43. Iron(II) is determined spectrophotometrically by reacting with 1,10-phenanthroline to produce a complex that absorbs strongly at 510 nm. A stock standard iron(II) solution is prepared by dissolving 0.0702 g ferrous ammonium sulfate, $Fe(NH_4)_2SO_4 \cdot 6H_2O$, in water in a 1-L volumetric flask, adding 2.5 mL H_2SO_4, and diluting to volume. A series of working standards is prepared by transferring 1.00-, 2.00-, 5.00-, and 10.00-mL aliquots of the stock solution to separate 100-mL volumetric flasks and adding hydroxylammonium chloride solution to reduce any iron(III) to iron(II), followed by phenanthroline solution and then dilution to volume with water. A sample is added to a 100-mL volumetric flask and treated in the same way. A blank is prepared by adding the same amount of reagents to a 100-mL volumetric flask and diluting to volume. If the following absorbance readings measured against the blank are obtained at 510 nm, how many milligrams iron are in the sample?

Solution	*A*
Standard 1	0.081
Standard 2	0.171
Standard 3	0.432
Standard 4	0.857
Sample	0.463

44. Nitrate nitrogen in water is determined by reacting with phenoldisulfonic acid to give a yellow color with an absorption maximum at 410 nm. A 100-mL sample that has been stabilized by adding 0.8 mL H_2SO_4/L is treated with silver sulfate to precipitate chloride ion, which interferes. The precipitate is filtered and washed (washings added to filtered sample). The sample solution is adjusted to pH 7 with dilute NaOH and evaporated just to dryness. The residue is treated with 2.0 mL phenol disulfonic acid solution and heated in a hot-water bath to aid dissolution. Twenty milliliters distilled water and 6 mL ammonia are added to develop the maximum color, and the clear solution is transferred to a 50-mL volumetric flask and diluted to volume with distilled water. A blank is prepared using the same volume of reagents, starting with the disulfonic acid step. A standard nitrate solution is prepared by dissolving 0.722 g anhydrous KNO_3 and diluting to 1 L. A standard addition calibration is performed by spiking a separate 100-mL portion of sample with 1.00 mL of the standard solution and carrying through the entire procedure. The following absorbance readings were obtained: blank, 0.032; sample, 0.270; sample plus standard, 0.854. What is the concentration of nitrate nitrogen in the sample in parts per million?

45. Two colorless species, A and B, react to form a colored complex AB that absorbs at 550 nm with a molar absorptivity of 450. The dissociation constant for the complex is 6.00×10^{-4}. What would the absorbance of a solution, prepared by mixing equal volumes of 0.0100 *M* solutions of A and B in a 1.00-cm cell, be at 550 nm?

MIXTURES

(You can use the Solver spreadsheet in your CD to perform the simultaneous equation calculations.)

46. Compounds A and B absorb in the ultraviolet region. Compound A exhibits an absorption maximum at 267 nm ($a = 157$) and a trailing shoulder at 312 nm ($a = 12.6$). Compound B has an absorption maximum at 312 nm ($a = 186$) and does not absorb at 267 m. A solution containing the two compounds exhibits absorbances (using a 1-cm cell) of 0.726 and 0.544 at 267 and 312 nm, respectively. What are the concentrations of A and B in mg/L?

47. Titanium(IV) and vanadium(V) form colored complexes when treated with hydrogen peroxide in 1 *M* sulfuric acid. The titanium complex has an absorption maximum at 415 nm, and the vanadium complex has an absorption maximum at 455 nm. A 1.00×10^{-3} *M* solution of the titanium complex exhibits an absorbance of 0.805 at 415 nm and of 0.465 at 455 nm, while a 1.00×10^{-2} *M* solution of the vanadium complex exhibits absorbances of 0.400 and 0.600 at 415 and 455 nm, respectively. A 1.000-g sample of an alloy containing titanium and vanadium was dissolved, treated with excess hydrogen peroxide, and diluted to a final volume of 100 mL. The absorbance of the solution was 0.685 at 415 nm and 0.513 at 455 nm. What were the percentages of titanium and vanadium in the alloy?

FLUORESCENCE

48. Derive Equation 16.24 relating fluorescence intensity to concentration.

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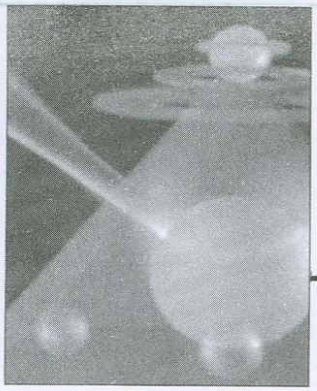

Chapter Seventeen

ATOMIC SPECTROMETRIC METHODS

Chapter 16 dealt with the spectrometric determination of substances in solution, that is, the absorption of energy by molecules, either organic or inorganic. This chapter deals with the spectroscopy of atoms. Since atoms are the simplest and purest form of matter and cannot rotate or vibrate as a molecule does, only electronic transitions within the atom can take place when energy is absorbed. Because the transitions are discrete (quantized), line spectra are observed. There are various ways to obtain free atoms (atomic vapor) and to measure the absorption or emission of radiation by these.

The principal techniques described in this chapter include flame emission spectrometry in which atoms in the form of atomic vapor are created in a flame; a portion of them is thermally and collisionally excited to a higher electronic energy level and then returned to their ground energy state by emitting photons, to create sharp-line emission spectra. Atomic absorption spectrometry is described in which the amount of radiation absorbed by ground-state atoms created in a flame or a minifurnace is measured; the absorption spectrum is sharp-line. Also included is a discussion of the types of flames used for emission or absorption, interferences in flames, and the use of nonflame (electrothermal, minifurnace) atomizers for extremely sensitive atomic absorption measurements.

Atomic spectrometry is widely used in many laboratories, particularly whenever trace element analyses are required. Environmental samples are analyzed for heavy-metal contamination, and pharmaceutical samples may be analyzed for metal impurities. The steel industry needs to determine minor components, as well as major ones. The particular technique used will depend on the sensitivity required, the number of samples to be analyzed, and whether single-element or multielement measurements are needed. The following discussion gives the capabilities of the techniques.

17.1 Flame Emission Spectrometry[1]

In this technique, formerly called flame photometry, the source of excitation energy is a flame. This is a low-energy source, and so the emission spectrum is simple and

[1] See Chapter 16 for the distinction between spectrometry and spectrophotometry.

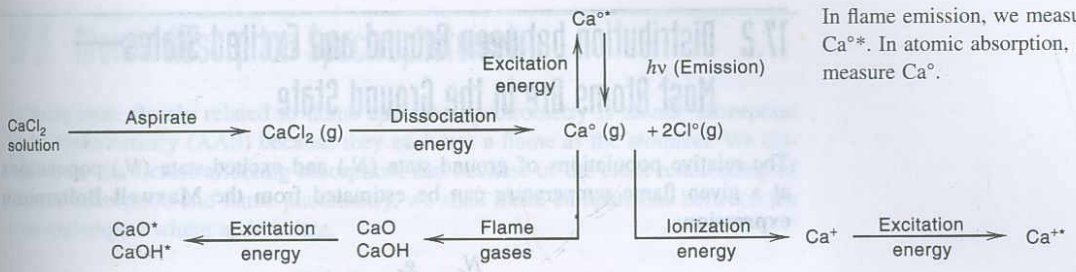

Fig. 17.1. Processes occurring in flame.

In flame emission, we measure $Ca^{\circ *}$. In atomic absorption, we measure Ca°.

there are few emission lines. The sample is introduced into the flame in the form of a solution, and so the technique is very easy to quantify.

There are numerous types of aspirator burners used. Basically, the solution is introduced into the flame as a fine spray. The mechanism of obtaining atomic vapor is complex, but an attempt at explaining the basic processes is illustrated in Figure 17.1. The solvent evaporates, leaving the dehydrated salt. The salt is dissociated into free gaseous atoms in the ground state. A certain fraction of these atoms can absorb energy from the flame and be raised to an excited electronic state. The excited levels have a short lifetime and drop back to the ground state, emitting photons[2] of characteristic wavelengths, with energy equal to $h\nu$. These can be detected with a conventional monochromator–detector setup.

Atoms in the gaseous state exhibit sharp-line spectra since they only undergo electronic transitions.

The intensity of emission is directly proportional to the concentration of the analyte in the solution being aspirated. So a calibration curve of emission intensity as a function of concentration is prepared.

As indicated in the figure, side reactions in the flame may decrease the population of free atoms and hence the emission signal. These will be discussed in Section 17.3.

In the early years of flame photometry, only relatively cool flames were used. We shall see below that only a small fraction of atoms of most elements is excited by flames and that the fraction excited increases as the temperature is increased. Consequently, relatively few elements have been determined routinely by flame emission spectrometry, especially few of those that emit line spectra (several can exist in flames as molecular species, particularly as oxides, which emit molecular band spectra). Only the easily excited alkali metals sodium, potassium, and lithium are routinely determined by flame emission spectrometry in the clinical laboratory. However, with flames such as oxyacetylene and nitrous oxide–acetylene, over 60 elements can now be determined by flame emission spectrometry. This is in spite of the fact that a small fraction of excited atoms is available for emission. Good sensitivity is achieved because, as with fluorescence (Chapter 16), we are, in principle, measuring the difference between zero and a small but finite signal, and so the sensitivity is limited by the response and stability of the detector and the stability (noise level) of the flame aspiration system.

[2] This is in opposition to excited molecules in solution, where there is much greater probability for collisions with solvent and other molecules. In the flame, there is less probability for collision because there are much fewer flame molecules and, therefore, many of the atoms lose their energy of excitation as electromagnetic radiation rather than as heat.

17.2 Distribution between Ground and Excited States—Most Atoms Are in the Ground State

Nearly all the gaseous atoms are in the ground state. Atomic emission is still sensitive, for the same reason that fluorescence spectrometry is. We do not have to measure a small decrease in a signal (which has some noise) as in absorption.

The relative populations of ground-state (N_0) and excited-state (N_e) populations at a given flame temperature can be estimated from the **Maxwell–Boltzmann expression**:

$$\frac{N_e}{N_0} = \frac{g_e}{g_0} e^{-(E_e - E_0)/kT} \tag{17.1}$$

where g_e and g_0 are the *statistical weights* of the excited and ground states, respectively; E_e and E_0 are the energies of the two states ($= h\nu$; E_0 is usually zero); k is the Boltzmann constant (1.3805×10^{-16} erg K^{-1}); and T is the absolute temperature. The statistical weights represent the probability that an electron will reside in a given energy level, and they are available from quantum mechanical calculations.[3] See Problem 21 for an example calculation.

Table 17.1 summarizes the relative population ratios for a few elements at 2000, 3000, and 10,000 K. We see that even for a relatively easily excited element such as sodium, the excited-state population is small except at 10,000 K, as obtained in a plasma. Short-wavelength elements (higher energy, $h\nu$) require much more energy for excitation and exhibit poor sensitivity by flame emission spectrometry where temperatures rarely exceed 3000 K. Those with long-wavelength emissions will exhibit better sensitivity. Measurement of ground-state atoms, as in atomic absorption below, will be less dependent on the wavelength or element. We see also from Table 17.1 that the fraction of excited-state atoms is temperature dependent, whereas the fraction in the ground state is virtually constant (since nearly 100% reside there at all temperatures).

In flame emission methods, we measure the excited-state population; and in atomic absorption methods (below), we measure the ground-state population. Because of chemical reactions that occur in the flame, differences in flame emission and atomic absorption sensitivities above 300 nm are, in practice, not as great as one would predict from the Boltzmann distribution. For example, many elements react partially with flame gases to form metal oxide or hydroxide species, and this reaction detracts from the atomic population equally in either method and is equally temperature dependent in either.

[3] The statistical weight is given by $2J + 1$, where J is the Russel–Saunders coupling and is equal to $L + S$ or $L - S$; L is the total orbital angular momentum quantum number, represented by the sharp (S), principal (P), diffuse (D), and fundamental (F) series ($L = 0$, 1, 2, and 3, respectively); and S is spin ($\pm\frac{1}{2}$). The information is supplied in the form of term symbols, $N^M L_J$, where N is the principal quantum number and M is the multiplicity. For example, the transition for the sodium 589.0-nm line, omitting the principal quantum number N, is $^2S_{1/2} - {}^2P_{1/2}$, and $g_e/g_0 = [2(\frac{1}{2}) + 1]/[2(\frac{1}{2}) + 1] = 2/2 = 1$.

Table 17.1
Values of N_e/N_0 for Different Resonance Lines

Line (nm)	N_e/N_0 2000 K	3000 K	10,000 K
Na 589.0	9.9×10^{-6}	5.9×10^{-4}	2.6×10^{-1}
Ca 422.7	1.2×10^{-7}	3.7×10^{-5}	1.0×10^{-1}
Zn 213.8	7.3×10^{-15}	5.4×10^{-10}	3.6×10^{-3}

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17.3 Atomic Absorption Spectrophotometry

A technique closely related to flame emission spectrometry is atomic absorption spectrophotometry (AAS) because they each use a flame as the atomizer. We discuss here the factors affecting absorption; and because of the close relationship of atomic absorption and flame photometry, we shall make comparisons between the two techniques where appropriate.

PRINCIPLES

The sample solution is aspirated into a flame as in flame emission spectrometry, and the sample element is converted to atomic vapor. The flame then contains atoms of that element. Some are thermally excited by the flame, but most remain in the ground state, as shown dramatically in Table 17.1. These ground-state atoms can absorb radiation of a particular wavelength that is produced by a special source made from that element (see Sources). The wavelengths of radiation given off by the source are the same as those absorbed by the atoms in the flame.

Beer's law is followed in atomic absorption.

Atomic absorption spectrophotometry is identical in principle to absorption spectrophotometry described in the previous chapter. The absorption follows Beer's law. That is, the *absorbance* is directly proportional to the pathlength in the flame and to the concentration of atomic vapor in the flame. Both of these variables are difficult to determine, but the pathlength can be held constant and the concentration of atomic vapor is directly proportional to the concentration of the analyte in the solution being aspirated. The procedure used is to prepare a calibration curve of concentration in the solution versus absorbance.

The major disadvantage of making measurements by atomic absorption, as we shall see below, is that a different source is required for each element.

INSTRUMENTATION

As in regular absorption spectrophotometry, the requirements for atomic absorption spectrophotometry are a light source, a cell (the flame), a monochromator, and a detector. The flame is placed between the source and the monochromator. A schematic diagram of an atomic absorption spectrophotometer is shown in Figure 17.2. This is for a double-beam instrument that measures the ratio of P_0/P. The source beam is alternately sent through the flame and around the flame by the chopper. The detector measures these alternately and the logarithm of the ratio is displayed. The detector amplifier is tuned to receive only radiation modulated at the frequency of the chopper, and so dc radiation emitted by the flame is discriminated against.

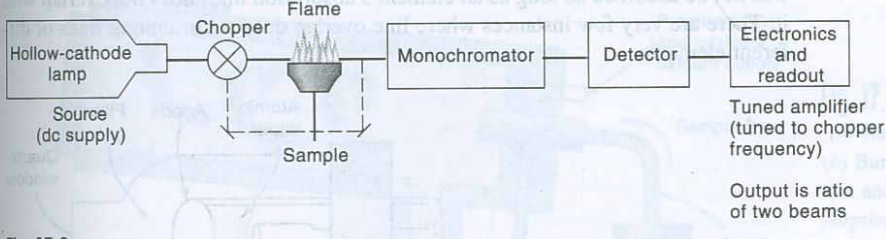

Fig. 17.2. Schematic diagram of atomic absorption instrument. (From G. D. Christian and F. J. Feldman, *Atomic Absorption Spectroscopy. Applications in Agriculture, Biology, and Medicine.* New York: Interscience, 1970. Reproduced by permission of John Wiley & Sons, Inc.)

Double-beam instruments are required for background correction using deuterium continuum lamps (see below). But the beam splitter of a double-beam instrument reduces the radiant energy, causing increased noise levels (decreases signal-to-noise ratios). High-energy source single-beam instruments are available that utilize line-based background corrections (measuring background absorption with a line near the analyte line). These provide good signal-to-noise ratio and are smaller and may even be portable.

The various components of an atomic absorption spectrophotometer are described as follows.

1. Sources. A sharp-line source is required in atomic absorption because the width of the *absorption line* is very narrow, a few thousandths to one-hundredths of a nanometer, at most. Because the absorption line is so narrow, only a small fraction of the radiation from a continuum source passed by the slit and reaching the detector would be absorbed.

A sharp-line source is used in AAS. The source emits the lines of the element to be measured. These possess the precise energies required for absorption by the analyte atoms.

The source used almost exclusively is a **hollow-cathode lamp** (HCL). This is a sharp-line source that emits specific (essentially monochromatic) wavelengths, and the basic construction is illustrated in Figure 17.3. It consists of a cylindrical hollow cathode made of the element to be determined or an alloy of it, and a tungsten anode. These are enclosed in a glass tube usually with a quartz window since the lines of interest are often in the ultraviolet region. The tube is under reduced pressure and filled with an inert gas such as argon or neon. A high voltage is impressed across the electrodes, causing the gas atoms to be ionized at the anode. These positive ions are accelerated toward the negative cathode. When they bombard the cathode, they cause some of the metal to "sputter" and become vaporized. The vaporized metal is excited to higher electronic levels by continued collision with the high-energy gas ions. When the electrons return to the ground state, the characteristic lines that metallic element are emitted. Also emitted are lines of the filler gas, but these are not usually close enough to the element lines to interfere.

These HCL-emitted lines are passed through the flame and can become absorbed by the test element because they possess just the right energy (the right wavelength) to result in the discrete electronic transitions. The most strongly absorbed line is often, but not always, the one corresponding to the most probable electronic transition, usually from the ground state to the lowest excited state. This is called the **resonance line**. The lines from a hollow-cathode lamp are narrower than the absorption line of the element in the flame because of broadening of the absorption line at the higher temperature and pressure of the flame. So the entire source linewidth is absorbed. Greater specificity also results for the reason that, while with a continuum source an element with an absorption line falling anywhere within the spectral slit width would absorb part of the source radiation, a line source will not be absorbed so long as an element's absorption lines does not overlap with it. There are very few instances where line overlap does occur among lines of different elements.

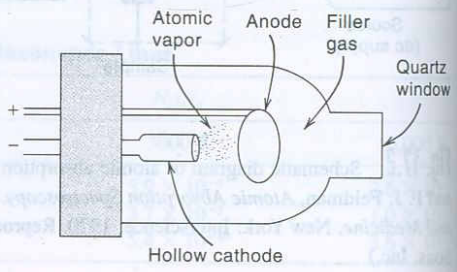

Fig. 17.3. Design of hollow-cathode lamp.

It is sometimes possible to use an alloy of several elements for the hollow cathode, and with such lamps, the lines of all the elements are emitted. These are the so-called multielement hollow-cathode lamps and can be used as a source for usually two or three elements. They may exhibit shorter lifetimes than do single-element lamps due to selective volatilization ("distillation") of one of the elements from the cathode with condensation on the walls of the lamp.

2. Burners. The burner used in most commercial instruments is the **premix chamber burner**, sometimes called the **laminar-flow burner**. This is illustrated in Figure 17.4. The fuel and support gases are mixed in a chamber before they enter the burner head (through a slot) where they combust. The sample solution is aspirated through a capillary by the **Venturi effect** using the support gas, usually air, for the aspiration. The air creates a partial vacuum at the end of the capillary, drawing the sample through the capillary. It is broken into a fine spray at the tip. This is the usual process of **nebulization**.[4] The larger droplets of the resulting aerosol condense and drain out of the chamber. The remaining fine droplets mix with the combustion gases and enter the flame. As much as 90% of the droplets condense out, leaving only 10% to enter the flame.

Premix burners are generally limited to relatively low-burning velocity flames. Although a large portion of the aspirated sample is lost in the chamber, the "atomization efficiency" (efficiency of producing atomic vapor) of that portion of the sample that enters the flame is high because the droplets are finer. Also, the pathlength is long. Combustion with premix burners is very quiet. A popular version of the premix burner is the **Boling** burner. This is a three-slot burner head that results in a broader flame and less distortion of the radiation passing through at the

[4]In atomic absorption spectrophotometry, we often speak of "atomization" in referring to the process of obtaining *atomic vapor*. This is not to be confused with the above process.

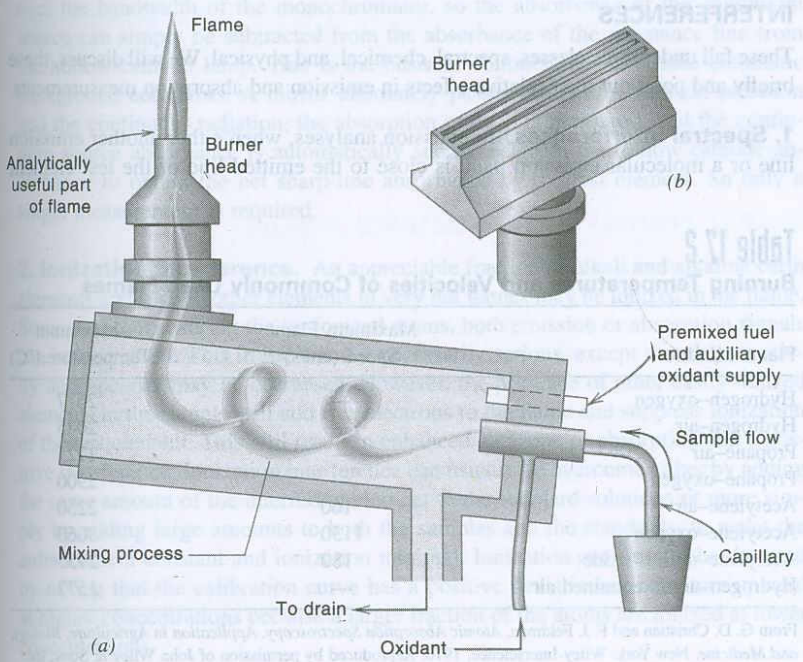

Fig. 17.4. Premix burner. (*a*) Nebulizer, chamber, and burner. (*b*) Burner head. (From G. D. Christian and F. J. Feldman, *Atomic Absorption Spectroscopy. Applications in Agriculture, Biology, and Medicine.* New York: Interscience, 1970. Reproduced by permission of John Wiley & Sons, Inc.)

edges of the flame (see Figure 17.4). This burner warps more easily than others, though, and care must be taken not to overheat it.

The air–acetylene flame is the most popular for AAS. The nitrous oxide–acetylene flame is best for refractory elements.

3. Flames. The chief flames that are used for atomic absorption and emission spectrometry are listed in Table 17.2 together with their maximum burning temperatures. The most widely used flames for atomic absorption are the air–acetylene flame and the nitrous oxide–acetylene flame with premix burners. The latter high-temperature flame is not required and may even be detrimental for many cases in atomic absorption because it will cause ionization of the gaseous atoms (see below). However, it is very useful for those elements that tend to form heat-stable oxides in the air–acetylene flame (the "refractory elements"). The air–acetylene and other hydrocarbon flames absorb a large fraction of the radiation at wavelengths below 200 nm, and an argon–hydrogen-entrained air flame is preferred for this region of the spectrum for maximum detectability. This is a colorless flame, and entrained air is the actual oxidant gas. It is used for elements such as arsenic (193.5 nm) and selenium (197.0 nm), when they are separated from the sample solution by volatilization as their hydrides (AsH_3, H_2Se) and passage of these gases into the flame. This is necessary because this cool flame is more subject to chemical interferences than other flames (see the following paragraphs). A nitrous oxide–acetylene flame offers an advantage in this region of the spectrum when danger of molecular interference exists; the flame absorption is relatively small at short wavelengths.

In flame emission spectrometry, a hot flame is required for the analysis of a large number of elements, and the nitrous oxide–acetylene flame is used. The oxy-acetylene flame has a high burning velocity and cannot be used with a conventional premix burner. The nitrous oxide–acetylene flame can, however, be used with a premix burner. Because of its high temperatures, a special, thick, stainless steel burner head must be used to prevent it from melting. A "cool" air–propane or similar flame is preferred for the flame emission spectrometry of the easily excited elements sodium and potassium because of decreased ionization of these elements.

INTERFERENCES

These fall under three classes, spectral, chemical, and physical. We will discuss, these briefly and point out their relative effects in emission and absorption measurements.

1. Spectral Interferences. In emission analyses, when either another emission line or a molecular emission band is close to the emitted line of the test element

Table 17.2

Burning Temperatures and Velocities of Commonly Used Flames

Flame Mixture	Maximum Flame Speed (cm/s)	Maximum Temperature (°C)
Hydrogen–oxygen	—	2677
Hydrogen–air	—	2045
Propane–air	—	1725
Propane–oxygen	—	2900
Acetylene–air	160	2250
Acetylene–oxygen	1130	3060
Acetylene–nitrous oxide	180	2955
Hydrogen–argon-entrained air	—	1577

From G. D. Christian and F. J. Feldman, *Atomic Absorption Spectroscopy. Application in Agriculture, Biology, and Medicine.* New York: Wiley-Interscience, 1970. Reproduced by permission of John Wiley & Sons, Inc.

and is not resolved from it by the monochromator, spectral interference occurs. The most probable danger is from molecular emission, such as from oxides of other elements in the sample. Similar interference would occur in atomic absorption if a dc instrument were used, but it is eliminated if an ac instrument is used. If, on the other hand, an element or molecule is capable of absorbing the source radiation, a positive interference would occur in atomic absorption. With line sources, this danger is minimized but not eliminated.

Light scatter by particles is a common problem in AAS. Since it is broad band in nature, it can be corrected for in a background absorption measurement.

Light scatter or absorption by solid particles, unvaporized solvent droplets, or molecular species in the flame will cause a positive interference in atomic absorption spectrophotometry. This is especially a problem for wavelengths less than 300 nm, when solutions of high salt content are aspirated because the salt may not be completely desolvated or its molecules dissociated into atoms. Such **background absorption** can be corrected for by measuring the absorbance of a line that is close to the absorption line of the test element but that is not absorbed by the element itself. Since the interfering absorption occurs over a band of wavelengths, the absorbance will be essentially the same at several angstroms removed from the resonance line.

The measurement should be made at least two bandpasses (Chapter 16) away from the absorption line. The line used for correction can be a filler gas line from the hollow-cathode lamp or a "nonresonance" line of the element that is not absorbed, or a nearby line from a second hollow-cathode lamp can be used. A solution of the test element should always be aspirated to check that it does not absorb the background correction line. This technique requires two separate measurements on the sample.

A **background correction** for broadband absorption can also be made in the UV region (where most elements absorb and background absorption is most serious) with a hydrogen or deuterium continuum source. In the visible region, a tungsten continuum source may be used. The monochromator is set at the same wavelength as the resonance line. Sharp-line absorption of the continuum source by the test element is assumed negligible compared to that by the broad background over the bandwidth of the monochromator, so the absorbance of the continuum source can simply be subtracted from the absorbance of the resonance line from the hollow-cathode lamp. This is the basis of commercially available automatic background correctors. A mirror alternately passes the hollow-cathode radiation and the continuum radiation, the absorption of each is measured, and the continuum source absorbance is automatically subtracted from the hollow-cathode absorbance to obtain the net sharp-line absorbance by the test element. So only a single measurement is required.

2. Ionization Interference. An appreciable fraction of alkali and alkaline earth elements and several other elements in very hot flames may be ionized in the flame. Since we are measuring the un-ionized atoms, both emission or absorption signals can be decreased. This in itself is not necessarily serious, except that the sensitivity and linearity may be decreased. However, the presence of other easily ionized elements in the sample will add free electrons to the flame and suppress ionization of the test element. This will result in enhanced emission or absorption and a positive interference. Ionization interference can usually be overcome either by adding the same amount of the interfering element to the standard solutions or more simply by adding large amounts to both the samples and the standards, to make the enhancement constant and ionization minimal. Ionization can usually be detected by noting that the calibration curve has a positive deviation or curvature upward at higher concentrations because a larger fraction of the atoms are ionized at lower concentrations.

Ionization can be suppressed by adding a solution of a more easily ionized element, for example, potassium or cesium.

Refractory compound formation is avoided by chemical competition or by use of a high-temperature flame.

3. Refractory Compound Formation. The sample solution may contain a chemical, usually an anion, that will form a refractory (heat-stable) compound with the test element in the flame. For example, phosphate can react with calcium ions and in the flame to produce calcium pyrophosphate, $Ca_2P_2O_7$. This causes a reduction in the absorbance since the calcium must be in the atomic form to absorb its resonance line. Generally, this type of solution interference can be reduced or eliminated chemically. In the above example, a high concentration (ca. 1%) of strontium chloride or lanthanum nitrate can be added to the solution. Called a releasing agent, the strontium or lanthanum will preferentially combine with the phosphate and prevent its reaction with the calcium. Alternatively, a high concentration of EDTA can be added to the solution to form a chelate with the calcium. This prevents its reaction with phosphate, and the calcium–EDTA chelate is dissociated in the flame to give free calcium vapor. These types of interferences can occur with both atomic absorption and flame emission spectrometry. They may be eliminated also by using a higher-temperature flame such as the nitrous oxide–acetylene flame.

A serious situation occurs when the analyte metal reacts with gases present in the flame. Refractory elements such as aluminum, titanium, molybdenum, and vanadium will react with O and OH species in the flame to produce thermally stable metal oxides and hydroxides. These can be decomposed only by using high-temperature flames. Several of these elements exhibit no appreciable absorption or emission in the conventional air–acetylene flame. A more useful flame for these elements is the nitrous oxide–acetylene flame. It is usually used in the reducing (fuel-rich) condition in which a large red-feather secondary-reaction zone is present. This red zone arises from the presence of CN, NH, and other highly reducing radicals. These (or the lack of oxygen-containing species), combined with the high temperature of the flame, decompose and/or prevent the formation of refractory oxides so that atomic vapor of the metal can be produced.

4. Physical Interferences. Most parameters that affect the rate of sample uptake in the burner and the atomization efficiency can be considered physical interferences. This includes such things as variations in the gas flow rates, variation in sample viscosity due to temperature or solvent variation, high solids content, and changes in the flame temperature. These can generally be accounted for by frequent calibration and use of internal standards. Some instruments offer the capability of using internal standards that can partially compensate for changes in physical parameters. See below.

SAMPLE PREPARATION—SOMETIMES MINIMAL

Sample preparation with flame methods can often be kept to a minimum. As long as chemical or spectral interferences are absent, essentially all that is required is to obtain the sample in the form of a diluted and filtered (for particulates) solution. It often makes no difference what the chemical form of the analyte is because it will be dissociated to the free elemental vapor in the flame. Thus, several elements can be determined in blood, urine, cerebral spinal fluid, and other biological fluids by direct aspiration of the sample. Usually, dilution with water will be required to prevent clogging of the burner.

Note that in the preparation of standards, the matrix of the analyte must always be matched. Thus, if lead in gasoline is to be determined, a simulated solvent matrix must be used for standards, not water.

Chemical interferences can often be overcome by simple addition of (dilution with) a suitable reagent solution. Thus, serum is diluted 1:20 with a solution

containing EDTA for the determination of calcium in order to prevent interference from phosphate. Sodium and potassium, in concentrations equal to those in serum, are added to calcium standards to prevent ionization interference.

Reference 9 gives a review of applications of atomic absorption spectrophotometry to biological samples. This technique is widely used for metal analysis in biological fluids and tissues, in environmental samples such as air and water, and in occupational health and safety areas. Routine applications of flame emission spectrometry to biological samples are generally limited to the alkali and alkaline earth metals. Ion-selective electrode measurements (Chapter 13) have largely replaced the flame emission measurements in the clinical chemistry laboratory.

RELATIVE DETECTABILITIES OF ATOMIC ABSORPTION AND FLAME EMISSION SPECTROMETRY

Table 17.3 lists some representative detection limits of various elements by atomic absorption and flame emission spectrometry. We should distinguish here between the sensitivity and detection limits in atomic absorption. The former term is frequently used in the atomic absorption literature. **Sensitivity** is defined as the concentration required to give 1% absorption (0.0044 *A*). It is a measure of the slope of the analytical calibration curve and says nothing of the signal-to-noise ratio (*S/N*). **Detection limit** is generally defined as the concentration required to give a signal equal to three times the standard deviation of the baseline (blank)—see Chapter 3.

Generally speaking, atomic absorption shows superior detectability for those elements that emit below 300 nm because of the high thermal energy required to excite the atoms for emission at these wavelengths. But at wavelengths between 300 and 400 nm, either method may exhibit comparable detectability, while flame emission is generally superior in the visible region.

Table 17.3
Representative Detection Limits by Atomic Absorption (AAS) and Flame Emission (FES) Spectrometry

		Detection Limit (ppm)	
Element	Wavelength (nm)	AAS[a]	FES[b]
Ag	328.1	0.001(A)	0.01
Al	309.3	0.1(N)	
	396.2		0.08
Au	242.8	0.03(N)	
	267.7		3
Ca	422.7	0.003(A)	0.0003
Cu	324.8	0.006(A)	0.01
Eu	459.4	0.06(N)	0.0008
Hg	253.6	0.8(A)	15
K	766.5	0.004(A)	0.00008
Mg	285.2	0.004(A)	0.1
Na	589.0	0.001(A)	0.0008
Tl	276.8	0.03(A)	
	535.0		0.03
Zn	213.9	0.001(A)	15

[a]Fuel is acetylene. Letter in parentheses indicates oxidant: A = air, N = nitrous oxide.
[b]Nitrous oxide–acetylene flame.

ELECTROTHERMAL ATOMIZERS—ULTRASENSITIVE

Electrothermal atomization is nearly 100% efficient, compared to 0.1% for flame atomization. Only a few microliters of sample are required.

Although aspiration into a flame is the most convenient and reproducible means of obtaining atomic vapor, it is one of the least efficient in terms of converting all the sample elements to atomic vapor and presenting this to the optical path. The overall efficiency of atomic conversion and measurement of ions present in aspirated solutions has been estimated to be as little as 0.1%. Also, aspiration methods usually require several milliliters of solution for analysis.

Electrothermal atomizers are generally a type of minifurnace in which a drop of the sample is dried and then decomposed at a high temperature to produce an atomic vapor cloud.

Electrothermal atomizers have conversion efficiencies approaching 100%, so absolute detection limits are often 100 to 1000 times improved over those of flame aspiration methods. Our discussion will center on resistively heated atomizers (graphite furnace). Although these are not generally useful for emission measurements, they are well suited for atomic absorption measurements. A schematic of a typical electrothermal atomizer is shown in Figure 17.5.

In most of the electrothermal techniques, a few microliters of sample is placed in a horizontal graphite tube or on a carbon rod or tantalum ribbon. The tube or rod is heated resistively by passing a current through it. The sample is first dried at a low temperature for a few seconds (~100 to 200°C), followed by pyrolysis at 500 to 1400°C to destroy organic matter that produces smoke and scatters the light source during measurement; the smoke from pyrolysis is flushed out by flowing argon gas. Finally, the sample is rapidly thermally atomized at a high temperature, up to 3000°C.

The light path passes over the atomizer (or through the tube). A sharp peak of absorbance versus time is recorded as the atomic cloud passes through the light path (Figure 17.5). Either the height of the observed peak or its area is directly related to the quantity of metal vaporized. The heating is done in an inert atmosphere (e.g., argon gas) to prevent oxidation of the graphite or carbon at the high temperatures involved, and also to prevent formation of refractory metal oxides.

A major difficulty with electrothermal atomization methods is that interelement effects are generally much more pronounced than in flames. The interferences can sometimes be compensated for by using a standard additions method for calibration in which the standard is added to a separate aliquot of the sample and the

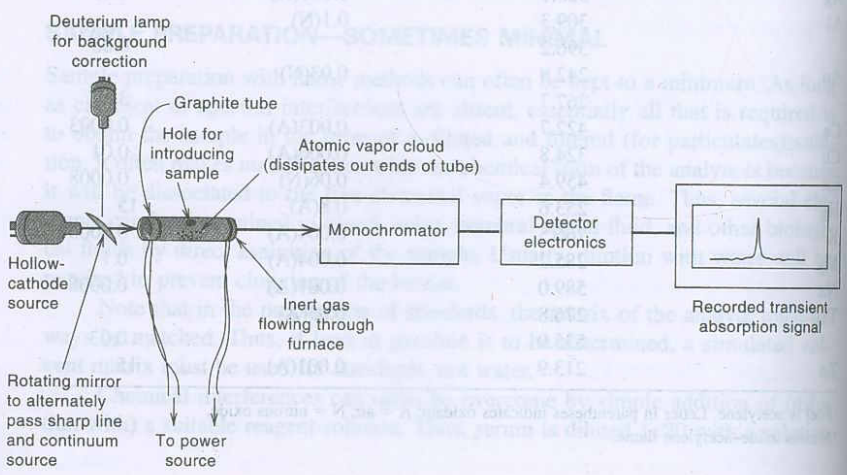

Fig. 17.5. Electrothermal atomization.

increase in the measured signal is proportional to the concentration added. In this manner, the standard is subjected to the same matrix as the sample (see below).

Often when the matrix concentration is changed, there is a change in the peak height and in the *shape* of the analytical peak. In such instances, less influence of the matrix and better accuracy can sometimes be achieved by integrating the signal or measuring its area, rather than measuring its maximum intensity. Of course, this requires more sophisticated instrumentation.

Even with pyrolysis before the atomization step, background absorption in electrothermal methods tends to be more prominent than in flame methods, especially with biological and environmental samples. This is due to residual organic material or vaporized matrix salts. Hence, automatic background correction (see above) is generally required.

Background correction is more critical in electrothermal atomizers.

Detection limits quoted by the manufacturers of electrothermal atomizers are typically in the range of 10^{-10} to 10^{-12} g or even less! The concentrational detection limit will depend on the sample volume. This will depend on the sample matrix composition and the concentration of the test element. Assume that a 10-μL sample is analyzed for an element with a detection limit of 10^{-11} g. Then the concentration detection limit would be 10^{-11} g/0.01 mL or 10^{-9} g/mL. This is equal to 1 ng/mL or 1 part per billion. The extreme sensitivity of these techniques is, therefore, quite apparent, even when dealing with very small sample volumes.

Electrothermal methods are complementary to flame methods. The latter are better suited when the analyte element is at a sufficiently high concentration to measure and adequate solution volume is available. They provide excellent reproducibility, and interferences are usually readily dealt with. The electrothermal techniques, on the other hand, are required when the concentrations are very small or the sample size is limited. It is possible to analyze solid samples directly without preparing a solution. The calibration and use of electrothermal methods generally requires more care. These are among the most sensitive of all analytical methods.

17.4 Internal Standard and Standard Addition Calibration

In atomic spectrometric methods, signals can frequently vary with time due to factors like fluctuations in gas flow rates and aspiration rates. Precision can be improved by the technique of **internal standards**. As an example, a simple flame emission spectrometer designed for simultaneous measurement of sodium and potassium in serum, using fixed wavelengths and two separate detectors, will usually contain a third fixed-wavelength channel-detector channel for lithium. A fixed concentration of lithium is added to all standards and samples. The instrument records and reads out the *ratios* of the K/Li and Na/Li signals. If the aspiration rate, for example, fluctuates, each signal is affected to the same extent and the ratio, at a given K or Na concentration, remains constant. See the spreadsheet exercise in Section 20.5 for an example of how to perform internal standard calibrations from the signals of the analyte and the internal standard.

An internal standard undergoes similar interferences as the analyte. Measurement of the ratio of the analyte to internal standard signals cancels the interferences.

The internal standard element should be chemically similar to the analyte element, and their wavelengths should not be too different. See Ref. 8 for a discussion of the selection of the internal standard.

A second difficulty often encountered in flame-spectrometric methods is a suppression (or sometimes enhancement) of the signal by the sample matrix, for example, due to high viscosity or chemical reaction with the analyte. The technique of **standard addition calibration** can be utilized to minimize errors of this type. The sample is measured in the usual way to produce a given signal. A separate

In standard addition calibration, the standard is added to the sample, and so it experiences the same matrix effects as the analyte.

portion of the sample is taken and spiked with a known amount of standard and followed through the analytical procedure, and a new signal is recorded. The standard, then, is subjected to the same matrix as the unknown analyte. The increase in signal is due to the standard, and the original signal is due to the analyte. It is important to perform blank corrections. A simple proportionality applies, assuming you are in a linear portion of the calibration curve. Two additions of standard are recommended to assure linearity.

Example 17.1

A serum sample is analyzed for potassium by flame emission spectrometry using the method of standard additions. Two 0.500-mL aliquots are added to 5.00-mL portions of water. To one portion is added 10.0 μL of 0.0500 M KCl solution. The net emission signals in arbitrary units are 32.1 and 58.6. What is the concentration of potassium in the serum?

Solution

The amount of standard added is

$$0.0100 \text{ mL} \times 0.0500\ M = 5.00 \times 10^{-4} \text{ mmol}$$

This produces a signal of

$$58.6 - 32.1 = 26.5 \text{ arbitrary units}$$

The millimoles potassium in the sample, then, is

$$5.00 \times 10^{-4} \text{ mmol} \times \frac{32.1 \text{ units}}{26.5 \text{ units}} = 6.06 \times 10^{-4} \text{ mmol}$$

This is contained in 0.500 mL serum, so the concentration is

$$\frac{6.06 \times 10^{-4} \text{ mmol}}{0.500 \text{ mL serum}} = 1.21 \times 10^{-3} \text{ mmol/mL serum}$$

Normally in applying the standard addition method, a calibration curve is constructed, similar to Figure 14.6, where the Y axis would be the atomic emission or absorbance signal. If the volumes of added standards are appreciable, the signals are corrected for dilution by multiplying by $(V_i + v)V_i$, or V_f/V_i, where V_i is the initial volume and v is the added volume, and V_f is the total volume.

SPREADSHEET EXERCISE: MULTIPLE STANDARD ADDITIONS

Calcium is determined in a river water sample using electrothermal AAS, using multiple standard additions. Four 25.0-mL aliquots are taken in 50-mL volumetric flasks, and aliquots of a 2.50 ppm standard are added in the amounts of 0, 1.00, 2.00, and 3.00 mL, followed by dilution to volume. Fixed volumes of each solution are measured, giving net absorbance signals of 0.101, 0.183, 0.238, and 0.310,