

General principles of spectroscopy and spectroscopic analysis

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5.1 INTRODUCTION

Many of the laws of optics were discovered or rediscovered in the period called the Renaissance. Isaac Newton studied the properties of prisms and their ability to separate white light into what we now call the visible spectrum and also prepared lenses to use in telescopes. Laws of optics such as the law of reflection,

$$\sin \theta_{\text{incident}} = \sin \theta_{\text{reflected}} \quad (5.1)$$

and Snell's Law of refraction,

$$\eta_{\text{incident}} \sin \theta_{\text{incident}} = \eta_{\text{refracted}} \sin \theta_{\text{refracted}} \quad (5.2)$$

where η is the refractive index defined as the ratio of the speed of light in a vacuum to the speed of light in the given medium, date from this period.

In more modern times, infrared, "beyond red," radiation was discovered by William Herschel in 1800. He found that the temperature recorded by a thermometer increased from the violet to the red in the visible region. Going beyond the red he found the temperature continued to increase instead of decreasing if the light ended at the end of the visible spectrum. Recognition of the utility of the infrared spectral region for chemical analysis is credited to W.W. Coblentz and it was not until the mid-1900s that infrared spectroscopy became an established technique. Apparently, noting Herschel's discovery of infrared radiation, Johann Wilhelm Ritter discovered ultraviolet radiation in 1801 by noting that silver chloride was reduced to silver metal when exposed to violet visible light and was even more efficiently reduced by radiation beyond the violet end of the visible spectrum.

James Clerk Maxwell predicted the existence of electromagnetic waves in 1864 and developed the classical sine (or cosine) wave description of the perpendicular electric and magnetic components of these waves. The existence of these waves was demonstrated by Heinrich Hertz 3 years later.

Diffraction of light by transmission and reflection gratings was used to demonstrate the existence of light waves and led to the development of the diffraction equation.

$$n\lambda = d(\sin \theta_{\text{incident}} \pm \sin \theta_{\text{reflected}}) \quad (5.3)$$

where n represents the order of diffraction and d stands for the spacing between grooves in a diffraction grating.

Finally, in the early 20th century Albert Einstein explained the photoelectric effect based on quantized packets of electromagnetic radiation called photons. These quickly led to the familiar relationships of the energy of a photon,

$$E = h\nu = h \frac{c}{\lambda} \quad (5.4)$$

and the particle–wave duality expressed by DeBroglie in 1938

$$\lambda = \frac{h}{m\nu} \quad (5.5)$$

Use of spectroscopy for chemical analysis most probably can be related to alchemists' use of flame tests for the qualitative determination of elemental composition. Comparison of colors of solutions, colorimetry, also emerged at that time. Development of light sources, dispersing devices, optics, detectors, modern transducers and digital technology has led to continuing improvement of spectroscopic instrumentation. Modern instrumentation for spectroscopic analysis in the ultraviolet, visible and infrared spectral regions is based on sophisticated principles and engineering, yet is often simple enough to produce accurate results with a minimum of training.

5.1.1 Spectral regions

This chapter covers ultraviolet, visible and infrared spectroscopies, the most commonly used range of wavelengths employed by chemists today. The range of wavelengths that the human eye can detect varies slightly from individual to individual. Generally, the wavelength region from 350 to 700 nm is defined as the visible region of the spectrum. The energy of a mole of photons ranges from 170 to 340 kJ mol⁻¹ and may be compared

to the approximate bond energy for a C–C bond of 350 kJ mol^{-1} and a C–H bond of 412 kJ mol^{-1} . This amount of energy is sufficient to cause electronic transitions within molecules and in some instances can cause ionization and bond breaking.

Ultraviolet radiation is commonly defined as the wavelengths from 200 to 350 nm. Around 200 nm oxygen absorbs strongly, part of the process to produce ozone in the upper atmosphere, and makes measurements difficult. One solution to the problem is to evacuate the instrument, giving rise to the terminology that wavelengths from 200 to 100 nm are in the “vacuum UV” region. The energies of photons in UV region range from 340 to 595 kJ mol^{-1} . These energies are sufficiently high to cause ionization and bond breaking. As a result, electromagnetic radiation starting at 300 nm or down is often called ionizing radiation.

Technically, the infrared region starts immediately after the visible region at 700 nm. From 700 to 2500 nm is the near infrared, NIR, region and its use is discussed in Chapter 6. The classical infrared region extends from 2500 ($2.5 \mu\text{m}$) to 50,000 nm ($50 \mu\text{m}$). Infrared spectroscopists often use wavenumbers to describe the infrared spectral region. A wavenumber is the reciprocal of the wavelength when the wavelength is expressed in centimeters and has the symbol, ν . As a result 2500 nm is 4000 cm^{-1} and 50,000 nm is 200 cm^{-1} . Multiplication of ν by the speed of light, $3 \times 10^{10} \text{ cm s}^{-1}$, gives the frequency that is directly proportional to the energy. The energies of infrared radiation range from 48 kJ mol^{-1} at 2500 nm to 2.4 kJ mol^{-1} at 50,000 nm. These low energies are not sufficient to cause electron transitions but they are sufficient to cause vibrational changes within molecules. Infrared spectroscopy is often called vibrational spectroscopy.

5.1.2 Spectra

A spectrum is a plot of some measure of the electromagnetic radiation absorbed by a sample versus the wavelength or energy of the electromagnetic radiation. For example, it is common practice to plot the absorbance versus wavelength for spectra in the ultraviolet and visible spectral regions as shown below (Fig. 5.1).

Historically, in the infrared region spectra have been represented as percent transmittance versus wavenumber as shown in Fig. 5.2.

Infrared spectra plotted as absorbance versus wavelength are becoming more common especially with instruments that are computer controlled and can make the change with a few commands.

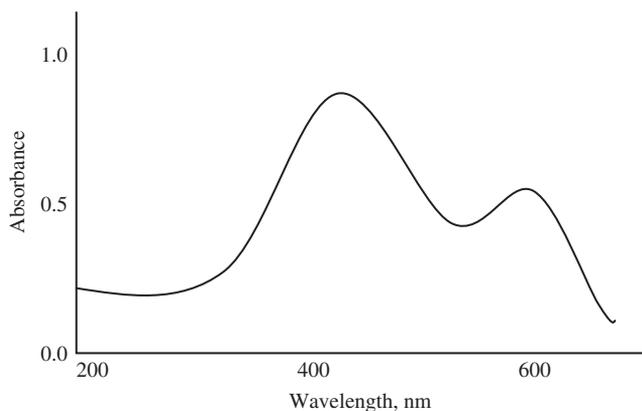


Fig. 5.1. Typical format, absorbance versus wavelength, of ultraviolet and visible spectra. Peaks in these regions tend to be broad.

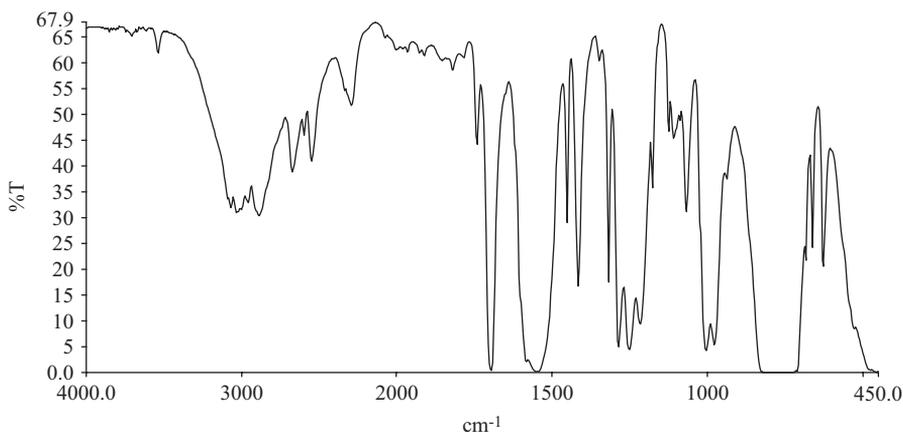


Fig. 5.2. Typical format, percentage transmittance (%T) versus wavenumber, for infrared spectra. This is a spectrum of benzoic acid.

5.1.3 Origin of the spectra

All spectra are due to the absorbance of electromagnetic radiation energy by a sample. Except for thermal (kinetic) energy, all other energy states of matter are quantized. Quantized transitions imply precise energy levels that would give rise to line spectra with virtually no line-width. Most spectral peaks have a definite width that can be explained in several ways. First, the spectral line-width can be related to the

lifetime of the excited state using the Heisenberg uncertainty principle. The width of a peak in terms of ΔE is $h/2\pi\tau$, where τ is the lifetime of the excited state,

$$\Delta E = \frac{h}{2\pi\tau} \quad (5.6)$$

The second contribution to the line-width is Doppler broadening. While the transition energy ΔE may be constant, the frequency and therefore the energy of radiation increases if the molecule is approaching the source and decreases if the molecule is receding from the source. In terms of energy

$$\Delta E = 2E_0 \left(\frac{2kT \ln 2}{mc^2} \right)^{1/2} \quad (5.7)$$

Collisional line broadening can be written in a form similar to Eq. (5.6)

$$\Delta E = \frac{h}{2\pi\tau_c} \quad (5.8)$$

the difference being that τ_c represents the time between collisions and in Eq. (5.6) it represents the excited state lifetime.

Finally, the width of peaks in most spectra is due to the fact that the peak actually represents an “envelope” that describes the outline of a group of closely spaced, unresolved, peaks. In the infrared region the rotational energy levels are superimposed on the vibrational energy levels giving rise to many closely spaced transitions that are generally not resolved. In the visible and ultraviolet regions the vibrational and rotational energy levels are superimposed on the electronic transitions giving rise to very wide absorbance bands.

Rotational transitions from one state to another (e.g., J_0 – J_1) require the least energy and these transitions usually occur in the microwave region of the spectrum. The energy of microwaves ranges from 156 to 325 J. Microwave spectra tend to have very sharp peaks.

Vibrational transitions (e.g., ν_0 – ν_1) require more energy than rotational transitions and this amount of energy is generally found in the infrared region of the spectrum. Infrared spectra have sharp peaks with some width to them.

Each atom within a molecule has three degrees of freedom for its motion in three-dimensional space. If there are N atoms within a molecule there are $3N$ degrees of freedom. However, the molecule as a whole has to move as a unit and the x , y , z translational motion of the entire molecule reduces the degrees of freedom by three. The molecule

also has rotational degrees of freedom. For a non-linear molecule this rotation has three degrees of freedom reducing the number of degrees of freedom to $3N-6$. A linear molecule can be rotated around its axis with no change and only two significant rotations. Therefore, a linear molecule has $3N-5$ degrees of freedom. This calculation indicates the maximum number of transitions a molecule can have.

The number of peaks actually observed in an infrared spectrum is often less than the maximum because some of the vibrations are energetically identical or degenerate. A real molecule will often have two or more vibrations that may differ only by their orientation in space. These will have exactly the same energy and result in one absorption peak. In addition to the degeneracy of vibrational modes, there is also the requirement that a vibration result in a change in the dipole moment of the molecule needs to be observed.

The number of peaks in an IR spectrum may increase due to overtones. Normally, the vibrational level is allowed to change by ± 1 . If the vibrational energy level changes by ± 2 or more (a “forbidden” transition), an overtone results. It is also possible for two normal mode vibrations to combine into a third.

To illustrate the above concept, we consider the possible and observed peaks for H_2S and CS_2 . H_2S is a non-linear molecule and is expected to have $3N-6 = 3$ spectroscopic peaks. The diagram below shows the three possible vibrations as a symmetrical stretch, and asymmetric stretch and a motion called scissoring (Fig. 5.3).

For CS_2 , it is a linear molecule and should have $3N-5 = 4$ vibrational modes. There is a symmetrical stretch, an asymmetrical stretch and a bending in-plane and a bending out-of-plane modes of vibration.

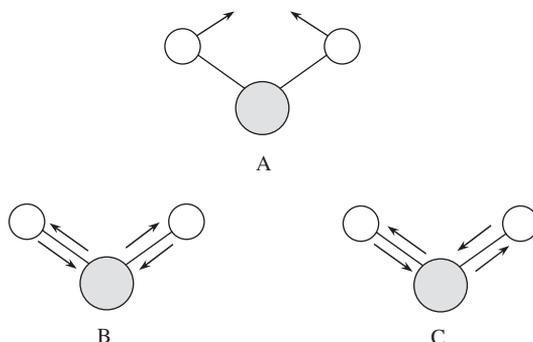


Fig. 5.3. The three vibrational modes of H_2S . (A) Represents the scissoring motion, (B) is the symmetrical stretch and (C) is the asymmetrical stretch.

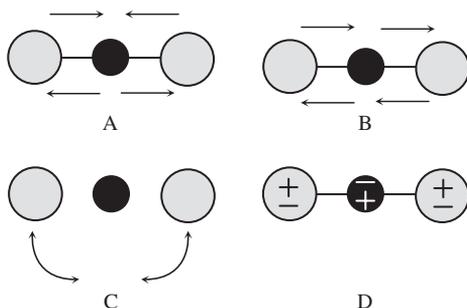


Fig. 5.4. Vibrational modes for CS₂. (A) Represents the symmetrical stretch, (B) is the asymmetrical stretch, (C and D) are the out-of-plane and in-plane bending motions. The + and - symbols indicate motion toward and away from the viewer, respectively.

The two bending motions are identical; they differ only by the direction in space of the motion. Therefore they degenerate and appear as only one peak (Fig. 5.4).

Without derivation, we may consider the bonds between atoms as a spring connecting two atoms in a harmonic oscillator. The energy difference between two vibrational energy levels is

$$E_v = \left(v + \frac{1}{2}\right) \left(\frac{h}{2\pi}\right) \left(\frac{k}{\mu}\right)^{1/2} \quad (5.9)$$

where v is the vibrational quantum number, h represents Planck's constant, k is the Hooke's Law restoring force constant and μ is the reduced mass

$$\text{Reduced mass} = \frac{m_1 m_2}{m_1 + m_2} \quad (5.10)$$

The approximate energy change for vibrational transitions may be calculated using the approximate force constants and subtracting $E_{v=1} - E_{v=0} = \Delta E$ (Table 5.1).

The effect of isotopic substitution on the position of a peak can also be estimated using this relationship.

For ultraviolet and visible spectroscopy the transitions are between electronic energy levels. Certain groupings of atoms, particularly in organic molecules have electrons that can be excited from one energy level to another with the energies found in the ultraviolet and visible regions of the spectrum. These groupings are called chromophores and usually account for most of the absorption of energy by a molecule.

TABLE 5.1

Force constants for bond types useful for estimation vibrational frequencies

Type of bond	Force constant, k (mdyne \AA^{-1})
C-H	5
C-F	6
N-H	6.3
O-H	7.7
C-Cl	3.5
C-C	10
C=C	12
C \equiv C	15.6
C \equiv N	17.7

Source: From Mann, C.K., Vickers, T.J. and Gulick, W.M., *Instrumental Analysis*, Harper & Row, New York, 1974, p. 483.

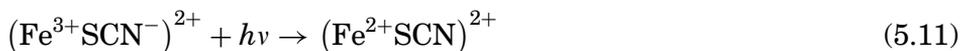
Electrons in an organic compound are usually σ (sigma bond electrons), n (non-bonding electrons) and π (pi bond electrons). These electrons may be excited to their corresponding antibonding levels, σ^* , π^* . The non-bonding electrons may be excited to the σ^* or π^* levels. Of these transitions, only the $\pi^* \rightarrow \pi$ has a large molar absorptivity (ca. $10,000 \text{ l mol}^{-1} \text{ cm}^{-1}$) along with a low enough energy to occur in the ultraviolet or visible regions.

Independent double bonds (i.e., without conjugation) have molar absorptivities that are approximately multiples of the molar absorptivity for one double bond. For instance, 1,4-hexadiene has twice the molar absorptivity of 1-hexene but absorbs at the same wavelength.

For conjugated, non-aromatic substances, both the molar absorptivity and wavelength of maximum absorption increase. An example of this is the comparison of 1-hexene that absorbs at 177 nm with a molar absorptivity of 12,000 while 1,3,5-hexatriene absorbs at 268 nm and has a molar absorptivity of 42,500.

Aromatic compounds have very high molar absorptivities that usually lie in the vacuum ultraviolet region and are not useful for routine analysis. Modest absorption peaks are found between 200 and 300 nm. Substituted benzene compounds show dramatic effects from electron-withdrawing substituents. These substituents are known as auxochromes since they do not absorb electromagnetic radiation but they have a significant effect on the main chromophore. For example, phenol and aniline have molar absorptivities that are six times the molar absorptivity of benzene or toluene at similar wavelengths.

Charge-transfer spectra represent one of the most important classes of spectra for analytical chemistry since the molar absorptivities tend to be very large. Charge-transfer can occur in substances, usually complexes that have one moiety that can be an electron donor and another that can be an electron acceptor. Both the donor and acceptor must have a small difference in their energy levels so that the electron can be readily transferred from the donor to the acceptor orbitals and back again. One example is the well-known, deep-red color of the iron (III) thiocyanate ion. The process appears to be



an electron from the thiocyanate is excited to an orbital of iron, effectively reducing it to iron (II) and the thiocyanate radical. The electron rapidly returns to the thiocyanate to repeat the process.

5.2 SPECTROSCOPIC ANALYSIS

5.2.1 Qualitative relationships

Infrared spectra differ markedly from the typical ultraviolet or visible spectrum. Infrared spectra are marked by many relatively sharp peaks and the spectra for different compounds are quite different. This makes infrared spectroscopy ideal for qualitative analysis of organic compounds.

For qualitative analysis the infrared spectrum is divided roughly into half. The region from 4000 to 2500 cm^{-1} is the group region and 2500 to 200 cm^{-1} is the fingerprint region. In the group region, there are fairly well-defined ranges at which different functional groups absorb. For example, the nitrile group ($-\text{C}=\text{N}$) has a sharp line at 2260–2240 cm^{-1} and the $-\text{OH}$ group has a large broad peak at 3000 cm^{-1} . A brief table of some functional groups is given below (Table 5.2).

The fingerprint region is an area that has many peaks and it allows us to distinguish between different substances that may have the same functional groups. All alcohols will have a large, broad peak at 3000 cm^{-1} , however, each alcohol will have a distinctively different number and position of peaks in the fingerprint region.

Significant tabulations of spectra are available in hardcopy or in electronic databases. In addition, compilations of common absorption bands based on functional group or vibrational mode are also available.

The ultraviolet and visible spectra are usually comprised of a few broad peaks at most. This is due to the fact that electronic transitions

TABLE 5.2

Absorption band ranges for some typical functional groups

Functional group	Frequency range (cm^{-1})
Alcohol $-\text{OH}$	O-H stretch 3550–3200 broad
Ketone $-\text{C}=\text{O}$	C=O stretch 1870–1540 strong
Aldehyde $-\text{C}=\text{O}$	C=O stretch 1740–1720
Acids $-\text{COOH}$ (dimers)	C=O stretch 1700–1750 strong
O-H stretch 3300–2500 broad	
Amines, primary $-\text{NH}_2$	N-H stretch 3500–3400 doublet, weak
Amino acids	NH_3^+ stretch 3100–2600 strong
Esters	C=O stretch 1750–1735
C-O stretch 1300–1000	
Ethers $\text{C}-\text{O}-\text{C}$	C-O-C asym. str. 1170–1050 strong
Halogens $-\text{CH}_2\text{X}$	CH_2 wag 1333–1110

Source: Specific absorption frequencies vary from compound to compound.

dominate these spectral regions. Table 5.3 lists some of the electronic transitions that can occur and their approximate wavelength ranges and molar absorptivities.

Qualitatively, absorbance in the ultraviolet region of the spectrum may be taken to indicate one or more unsaturated bonds present in an organic compound. Other functional groups can also absorb in the UV and visible regions. The portion of a molecule that absorbs the electromagnetic radiation is called a chromophore. Fully saturated compounds only absorb in the vacuum UV region. Unsaturated bonds absorb electromagnetic radiation as a $\pi-\pi^*$ transition. The energy difference is small between these two states and the molar absorptivities are relatively high.

Some salts, particularly of transition metals, are highly colored and absorb in the UV and visible regions. Salts and complexes that have the highest molar absorptivities tend to absorb electromagnetic radiation by a charge-transfer process. In the charge-transfer process, an electron is promoted from one part of a complex to another causing one part of the complex to be oxidized and the other to be reduced as in Eq. (5.11).

5.2.2 Quantitative relationships

Spectroscopic measurements for the UV, visible and infrared regions are most conveniently and reliably made by determining the absorbance of a

TABLE 5.3

Examples of some electronic transitions in the ultraviolet region

Electronic transition	Maximum wavelength (nm)	Maximum molar absorptivity ($\text{l mol}^{-1} \text{ cm}^{-1}$)	Example
$\sigma \rightarrow \sigma^*$	135	—	Ethane
$n \rightarrow \sigma^*$	173	200	Methyl chloride
$n \rightarrow \sigma^*$	259	400	Methyl iodide
$\pi \rightarrow \pi^*$	165	11,000	Ethylene
$\pi \rightarrow \pi^*$	217	21,000	1,3-Butadiene
$\pi \rightarrow \pi^*$	188	900	Acetone
$n \rightarrow \pi^*$	290	17	Acetaldehyde
$n \rightarrow \pi^*$	204	41	Acetic acid
Aromatic $\pi \rightarrow \pi^*$	180	60,000	Benzene
Aromatic $\pi \rightarrow \pi^*$	200	8000	Benzene
Aromatic $\pi \rightarrow \pi^*$	255	215	Benzene
Aromatic $\pi \rightarrow \pi^*$	210	6200	Toluene
Aromatic $\pi \rightarrow \pi^*$	270	1450	Toluene

Source: From Silverstein, R.M., Bassler, G.C. and Morrill, T.C. *Spectrometric Identification of Organic Compounds*, 4th ed., John Wiley and Sons, New York, 1981, p. 312.

solution. The absorbance tends to be a robust measure that is reproducible and only slightly affected by common variables of temperature and trace impurities. The absorbance of a system is determined by measuring the intensity of light at a given wavelength, I_0 and then measuring the intensity with a sample in the same beam of light, I . The ratio of these intensities is the transmittance, T .

$$T = \frac{I}{I_0} \quad (5.12)$$

When using a single-beam spectrometer, I_0 is measured when a reagent blank is used to “zero” the absorbance scale. The value of I is then measured when the sample is inserted into the spectrometer. On the other hand, when using a double-beam instrument both the reagent blank, I_0 , and the sample, I , are measured continuously and the appropriate ratio is determined electronically.

Most infrared measurements are transmittance values plotted as a spectrum. To convert data from an infrared spectrum to usable absorbance values involves the following steps: First, the peak of interest is located and a tangent is drawn from one shoulder to the other to create a baseline. Then a vertical line is constructed from the peak to

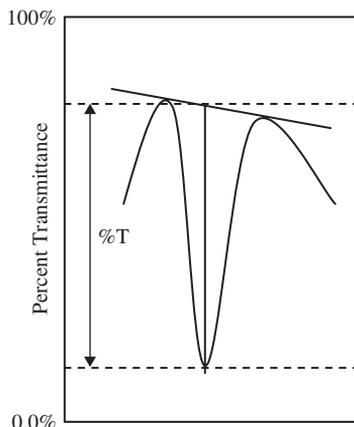


Fig. 5.5. Illustration of the method to determine the percent transmittance of a peak in an infrared spectrum.

the baseline. The percent transmittance of the peak is the difference in transmittance from the baseline intersection to the peak. This procedure is illustrated in Fig. 5.5.

The absorbance is defined as

$$A = -\log T = (\%T/100) \quad (5.13)$$

The Beer–Lambert Law relates the absorbance to concentration in two alternate forms depending on the units used for the concentration:

$$A = a b c \quad \text{or} \quad A = \varepsilon b c \quad (5.14)$$

Modern terminology defines A as the absorbance, a as the absorptivity, b as the optical path length and c as the concentration. In the second equation ε represents the molar absorptivity. Table 5.4 compares these terms.

The Beer–Lambert Law assumes that the electromagnetic radiation being absorbed is monochromatic. In practical instruments it is not a single wavelength but a band of wavelengths that enter the sample. The middle of this band of wavelengths is called the nominal wavelength. It can be shown that as long as a , or ε , is relatively constant over the band of wavelengths, the absorbances of each wavelength can be added to obtain the total absorbance that obeys the Beer–Lambert Law,

$$A_{\text{total}} = A_{\lambda_1} + A_{\lambda_2} + A_{\lambda_3} + \cdots + \quad (5.15)$$

If the absorptivities or molar absorptivities are not approximately equal, the linear relationships of the Beer–Lambert Law will not hold.

TABLE 5.4

Terminology and units used for the Beer–Lambert law

	A	a or ϵ	b	c
$A = abc$	Absorbance dimensionless	Absorptivity ($\text{l g}^{-1} \text{cm}$)	Optical path length (cm)	Concentration (g l^{-1})
$A = \epsilon bc$	Absorbance dimensionless	Molar absorptivity $\text{l mol}^{-1} \text{cm}$	Optical path length (cm)	Concentration mol l^{-1}

In practical situations the absorbance of a sample is determined by making two measurements, the first to determine I_0 and the second to determine I . The determination of I_0 is used to cancel a large number of experimental factors that could affect the result. When measuring I_0 the sample container must closely match the unknown container in all ways except for the analyte content. The cuvettes should be a matched pair if a double beam instrument is used and the same cuvette can be used for both the blank and sample with a single beam instrument. The blank solution filling the cuvette should be identical to the solvent that the sample is dissolved in, except for the sample itself. If done correctly, the least-squares line for the calibration graph will come very close to the 0,0 point on the graph.

5.2.3 Single component analysis

The simplest spectroscopic analysis is of one compound that absorbs electromagnetic radiation strongly at a wavelength where no other substance absorbs. In this case a series of standard solutions, that have absorbances between zero and 1.0 are prepared. Each of the standards is measured and a plot of absorbance versus concentration is drawn. A spreadsheet program can be used to record the data and generate the graph along with a least squares line that has a slope of ϵb . The absorbances of unknown solutions are then determined. The absorbances of the unknown solutions must fall between the highest and lowest absorbances of the standard solutions. Unknowns with absorbances that are too high must be diluted and those with low absorbances must be concentrated. The graph may be used to determine the concentration by drawing a horizontal line from the absorbance of the unknown to the least squares line and then a vertical line to the concentration axis. This method has a drawback that it may not be possible to determine the

concentration to more than two significant figures. Alternatively, the equation for the least squares line may be used to solve for the unknown concentration. The drawback of using the equation is that too many significant figures may be kept and that unknown absorbances far outside the range of the standards may be inappropriately used.

Example. 10.0 ml of an aqueous solution containing the Mn^{2+} ion is reacted with KIO_4 to produce the permanganate ion. The final mixture is diluted to 100 ml. A stock solution of 100 ppm Mn^{2+} is produced by dissolving 0.100 g of manganese metal and diluting to 1.00 l. Standard solutions are prepared by pipeting 1.00, 3.00, 5.00, 7.00 and 10.00 ml of the stock solution into separate flasks and reacting with KIO_4 in the same manner as the unknown. The absorbances of the standards were determined, in a 1.00 cm cuvette, to be 0.075, 0.238, 0.359, 0.533 and 0.745, respectively. The absorbance of the unknown was determined to be 0.443. What is the concentration of the unknown and the molar absorptivity of the MnO_4^- ion under these conditions.

Solution. Calculate the concentration of the standard solutions as:

$$(\text{ppm stock}) (\text{volume stock}) = (\text{ppm std}) (\text{volume std})$$

$$(100 \text{ ppm}) (1.00 \text{ ml}) = \text{ppm std} (10 \text{ ml std})$$

$$\text{ppm std} = 1.00$$

repeat process for remaining standard solutions

Enter data into a spreadsheet and obtain a graph of absorbance versus concentration of the standards. Obtain the least-squares line and its equation (Fig. 5.6).

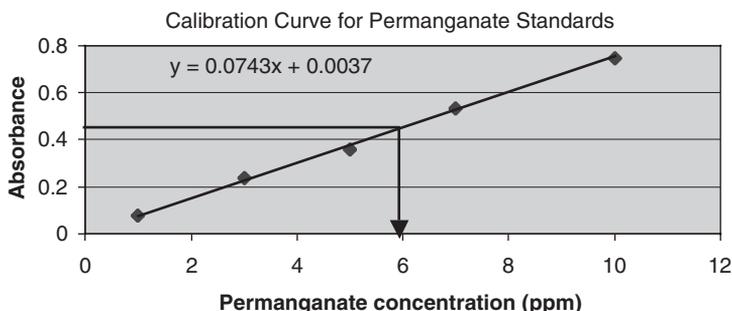


Fig. 5.6. Calibration curve for permanganate standards. Line is a least-squares linear regression for the data. Graphical interpolation is illustrated for an unknown with an absorbance of 0.443.

To determine the concentration using the graphical method draw a horizontal line from $A = 0.443$ to the least-squares line. Then construct a vertical line from that point to the concentration axis and read the value of the unknown. In this case it appears to be slightly less than 6.0 ppm. Using the least-squares equation we get

$$0.443 = 0.0743x + 0.0037 \text{ and the value of } x = 5.91 \text{ ppm}$$

To calculate the absorptivity and molar absorptivity, we see that 0.0743 is the slope of the line and the slope is ab . Since $b = 1.00 \text{ cm}$, $a = 0.743 \text{ cm}^{-1} \text{ ppm}^{-1}$.

We know that $1 \text{ ppm} = 1 \text{ mg l}^{-1}$ therefore $a = 0.743 \text{ l cm}^{-1} \text{ mg}^{-1}$ inserting 10^{-3} for the prefix m results in $a = 743 \text{ l cm}^{-1} \text{ g}^{-1}$. To convert a into ϵ we divide the mass by the molar mass of permanganate ($\text{MnO}_4^- = 118.93 \text{ g mol}^{-1}$) to get $\epsilon = 88,363 \text{ l mol}^{-1} \text{ cm}^{-1}$.

5.2.4 Mixture analysis

If a mixture of two or more substances is absorbing electromagnetic radiation at the same nominal wavelength, their absorbances will be additive,

$$A_{\text{total}} = A_1 + A_2 + \dots + \quad (5.16)$$

If each of the substances in a mixture has different spectra, it will be possible to determine the concentration of each component. In a two-component mixture measurement of the absorbance at two (appropriately chosen) different wavelengths will provide two simultaneous equations that can be easily solved for the concentration of each substance.

$$A_{\lambda_1} = \epsilon_{a\lambda_1}bc_a + \epsilon_{b\lambda_1}bc_b$$

$$A_{\lambda_2} = \epsilon_{a\lambda_2}bc_a + \epsilon_{b\lambda_2}bc_b$$

Since the slope of the calibration curve is ϵb , we need to construct four calibration curves, two at the first wavelength for compounds a and b and two at the second wavelength for compounds a and b . Once the four slopes are determined along with the absorbance of the unknown at the two wavelengths, we have two equations in two unknowns that can be solved algebraically or with simple matrix methods.

Example. A mixture of two compounds, X and Y, needs to be analyzed. The ϵ_{\max} for compound X is at 632 nm, while ϵ_{\max} for compound Y is at 447 nm. Standards are prepared for both X and Y and the calibration curves give the following results:

$$\epsilon_{X632} b = 8879$$

$$\epsilon_{Y632} b = 2210$$

$$\epsilon_{X447} b = 3480$$

$$\epsilon_{Y447} b = 6690$$

The absorbances at the two wavelengths were $A_{632} = 0.771$ and $A_{447} = 0.815$. What are the concentrations of the compounds X and Y?

Substitute the given data into two equations for the total absorbance at 632 and 447 nm to get

$$0.771 = 8879c_x + 2210c_y$$

$$0.815 = 3480c_x + 6690c_y$$

multiply the bottom equation by 8879/3480 to get

$$2.079 = 8879c_x + 17069c_y$$

subtract the first equation from the new second one to get

$$2.079 = 8879c_x + 17069c_y$$

$$-0.771 = -8879c_x - 2210c_y$$

$$1.308 = 0.00c_x + 14859c_y$$

then

$$c_y = 8.80 \times 10^{-5} \text{ mol l}^{-1}$$

$$c_x = (0.771 - 0.194)/8879 = 6.50 \times 10^{-5} \text{ mol l}^{-1}$$

5.2.5 Method of standard additions

In certain circumstances the matrix, defined as everything except the analyte, contributes significantly to the absorbance of a sample and is also highly variable. One method that can be used to improve results is the method of standard additions. The basic idea is to add standard to the analyte so that the standard is subjected to the same matrix effects as the analyte. This method assumes that the system obeys the Beer-Lambert Law.

General principles of spectroscopy and spectroscopic analysis

As an example, consider a sample that contains the dichromate ion. The absorbance of the unknown may be readily determined at 425 nm, which is close to the maximum for the dichromate ion. For this unknown the absorbance measured will be 0.525. Now, we will add 5.00 ml of a 3.00 mM solution of $\text{K}_2\text{Cr}_2\text{O}_7$ to 25.0 ml of the unknown and remeasure the absorbance and find it to be 0.485. The calculations are

$$\text{Original unknown : } A_{\text{unk}} = \epsilon b c_{\text{unk}}$$

$$\text{Unknown with added standard } A_{\text{unk+std}} = \epsilon b c_{\text{unk+std}}$$

The ratio of these two equations is

$$\frac{A_{\text{unk}}}{A_{\text{unk+std}}} = \frac{\epsilon b c_{\text{unk}}}{\epsilon b c_{\text{unk+std}}} = \frac{c_{\text{unk}}}{c_{\text{unk+std}}}$$

For the denominator we can use the dilution equation

$$c_{\text{unk}}v_{\text{unk}} + c_{\text{std}}v_{\text{std}} = c_{\text{unk+std}}v_{\text{unk+std}} = c_{\text{unk+std}}(v_{\text{unk}} + v_{\text{std}})$$

$$c_{\text{unk+std}} = \frac{c_{\text{unk}}v_{\text{unk}} + c_{\text{std}}v_{\text{std}}}{v_{\text{unk}} + v_{\text{std}}}$$

$$\frac{A_{\text{unk}}}{A_{\text{unk+std}}} = \frac{c_{\text{unk}}}{\frac{c_{\text{unk}}v_{\text{unk}} + c_{\text{std}}v_{\text{std}}}{v_{\text{unk}} + v_{\text{std}}}}$$

Looking at this equation we have measured the two absorbances, we know the volumes of the unknown and standard and we know the concentration of the standard. Only one unknown, the concentration of the unknown is left to calculate.

$$\frac{0.525}{0.485} = \frac{c_{\text{unk}}}{\frac{c_{\text{unk}}(25.0 \text{ ml}) + (3.00 \text{ mM})(5.00 \text{ ml})}{(25.0 \text{ ml} + 5.00 \text{ ml})}}$$

$$c_{\text{unk}} = 5.53 \text{ mM dichromate}$$

An alternate method that lends itself to analysis using a database is to add varying amounts of standard to a fixed volume of unknown in separate volumetric flasks. The flasks are all filled to the mark, mixed well and measured. As an example let us take a solution containing an unknown amount of Cu^{2+} ; 25.0 ml of the unknown is pipetted into each of five 50 ml volumetric flasks. Added into these flasks are 0.0 ml, 3.0 ml, 5.0 ml, 7.0 ml and 10.0 ml of a 0.600 ppm solution of Cu^{2+} . Each flask is then filled to the mark with 1.0 M ammonia solution to develop the color. The measured absorbances were 0.326, 0.418, 0.475, 0.545 and 0.635, respectively. The concentrations of each of the standards in the

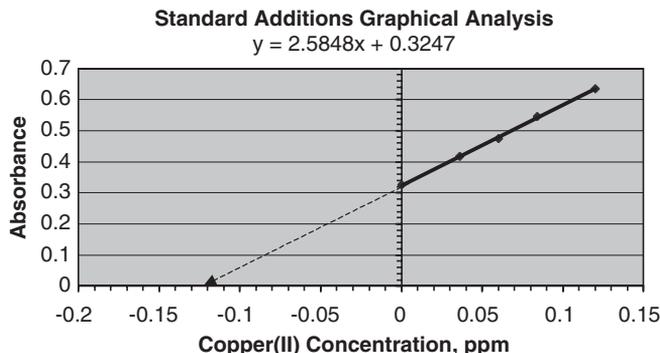


Fig. 5.7. Standard additions graphical analysis.

flasks are 0.0, 0.036, 0.060, 0.084 and 0.12 ppm, respectively. We can write five equations and solve any pair of them

$$A_1 = 0.326 = \epsilon b c_{\text{unk}}$$

$$A_2 = 0.418 = \epsilon b c_{\text{unk}} + \epsilon b(0.036 \text{ ppm})$$

$$A_3 = 0.475 = \epsilon b c_{\text{unk}} + \epsilon b(0.060 \text{ ppm})$$

$$A_4 = 0.545 = \epsilon b c_{\text{unk}} + \epsilon b(0.084 \text{ ppm})$$

$$A_5 = 0.635 = \epsilon b c_{\text{unk}} + \epsilon b(0.12 \text{ ppm})$$

Dividing the second equation by the first equation yields

$$\frac{0.418}{0.326} = \frac{\epsilon b c_{\text{unk}} + \epsilon b(0.036 \text{ ppm})}{\epsilon b c_{\text{unk}}}$$

$$1.282 = 1.0 + \frac{0.036 \text{ ppm}}{c_{\text{unk}}}$$

$$0.282 c_{\text{unk}} = 0.036 \text{ ppm}$$

$$c_{\text{unk}} = 0.128 \text{ ppm Cu}^{2+}$$

We can also plot the data in this problem and extrapolate to the x -axis intercept that will be $-c_{\text{unk}}$. On the graph below the concentration is approximately 0.125 ppm (Fig. 5.7).

5.2.6 Photometric error

The basic measurements of absorbance spectroscopy are actually I_0 and I that determine the transmittance. The uncertainty in the measurement

of the transmittance can be evaluated as a relative uncertainty in the concentration. The Beer-Lambert Law can be rewritten as

$$c = \frac{-1}{\epsilon b} \log T \quad (5.17)$$

converting to natural logarithms results in

$$c = \frac{-0.434}{\epsilon b} \ln T \quad (5.18)$$

Take the partial derivative of this equation yields

$$\delta c = \frac{-0.434}{\epsilon b T} \delta T \quad (5.19)$$

Divide this equation by the first equation to obtain

$$\frac{\delta c}{c} = \frac{-0.434}{\log T} \frac{\delta T}{T} \quad (5.20)$$

This expression is interpreted as the relative uncertainty in the concentration, as related to the relative uncertainty of the transmittance measurements, $\delta T/T$. The graph below illustrates the effect of a 1% uncertainty in transmission measurements on the percent relative uncertainty in the concentration (Fig. 5.8).

The minimum uncertainty (ca. 3%) of photometric error ranges from approximately 20 to 60% transmittance or an absorbance range of 0.2–0.7, a 5% relative error in concentration has a photometric range of 0.1–1.0.

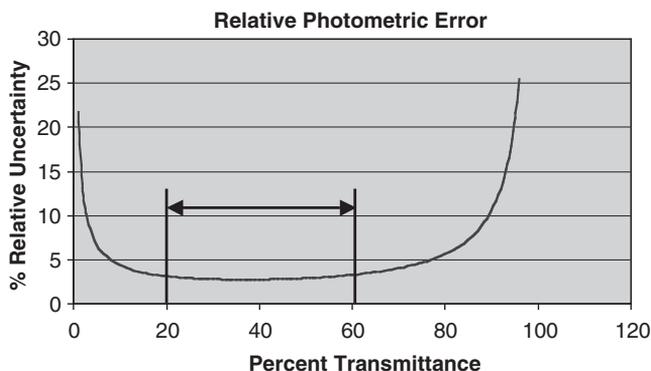


Fig. 5.8. Relative photometric error in concentration as a function of %T. Optimum range of transmittance is shown as 20–60%T for approximately 3% error for a 1% error in T.

5.3 INSTRUMENTATION

All spectrometers have the following basic units: a source of electromagnetic radiation, a dispersion device, sample holder, optical devices for collimating and focusing, a detection device and a data readout or storage system. There are also a variety of ways in which these parts are assembled into the entire spectrometer.

5.3.1 Sources of electromagnetic radiation

High-intensity radiation in the visible region of the spectrum is obtained from a simple tungsten light bulb. This bulb is essentially a black-body emitter and the relative intensity of the wavelengths of light emitted depends on the temperature of the tungsten wire as shown below.

Radiation in the infrared region of the spectrum is obtained from heated ceramic devices such as the Nernst glower or Globar. The Globar is made of silicon carbide and is heated to approximately 800–1500°C to emit black-body radiation in the infrared region of the spectrum. Coils of nichrome wire also emit infrared radiation when electrically heated.

Sources of electromagnetic radiation for the ultraviolet region of the spectrum are high-pressure mercury or xenon lamps or low-pressure deuterium or hydrogen discharge lamps. The mercury and xenon discharge lamps contain a gas that conducts electricity when a high voltage is applied to its electrodes. In the process the gas is excited and emits photons characteristic of the element when returning to the ground state. If the pressure in the tube is low, a characteristic atomic spectrum will be obtained. However, at higher pressures line broadening occurs and a wide distribution of wavelengths will be emitted. The hydrogen and deuterium lamps are low temperature and power lamps that provide a continuous ultraviolet spectrum. The hydrogen or deuterium molecule is excited electrically and then it dissociates to release the energy. The energy of excitation is distributed between the kinetic energies of the hydrogen atom and the photon emitted. Since the kinetic energies of the hydrogen atoms are not quantized, the energy of the photon is also not quantized, resulting in a broad range of energies in the ultraviolet region being emitted.

5.3.2 Optical components

Within the typical spectrometer there is need to collimate electromagnetic radiation into parallel light rays, light needs to be redirected and

it also needs to be focused. All of these operations are done using optical devices of lenses, and mirrors. The thin lens equation

$$\frac{1}{\text{source}} + \frac{1}{\text{focal point}} = \frac{1}{\text{focal length}} \quad (5.21)$$

describes the positioning of a light source and a lens so that the angular emission of a light source can be converted into a collimated beam. When a light source is placed at the focal length from the lens, a collimated beam that has its focal point at an extremely large distance will be produced. A collimated light beam will be focused on a point equal to the focal length to reverse the process. Lenses, and prisms, disperse light because the refractive index of the lens is different from the refractive index of air. If the refractive index of the lens was the same for all wavelengths, then all wavelengths of light in an instrument would be perfectly collimated or focused. However, the refractive index often depends on the wavelength of light (that is why a prism works) and the focal point is wavelength-dependent. The change in refractive index with wavelength is called the dispersion, and substances with large dispersions are valued for preparing prisms. The reflection of a mirror does not depend upon the refractive index, particularly front-coated aluminum mirrors. The result is that parabolic mirrors can achieve the same collimating and focusing functions as lenses. They are superior because they minimize the aberrations due to refractive index effects and also do not decrease the light intensity as much as light passing through a lens. Wherever possible, modern instruments replace lenses with parabolic mirrors.

Dispersion devices. Dispersion of light was first achieved using a glass prism. It was discovered that the prism worked because different wavelengths of light had different refractive indices in glass. The result was that each wavelength was “bent” at a different angle when emerging from the prism, producing the separation of white light into the rainbow of colors. This dispersion was not linear and instrument design was very difficult using prisms. Prisms also had the same disadvantage as lenses in that some light was absorbed passing through the prism, decreasing the overall light intensity. Because of their lack of use in modern instruments, further discussion of prisms is omitted.

Reflection gratings greatly decreased the problems formerly associated with prisms. In a reflection grating light is dispersed linearly from one end of the spectral region to the other. Gratings being reflective devices also minimize losses due to absorption of light.

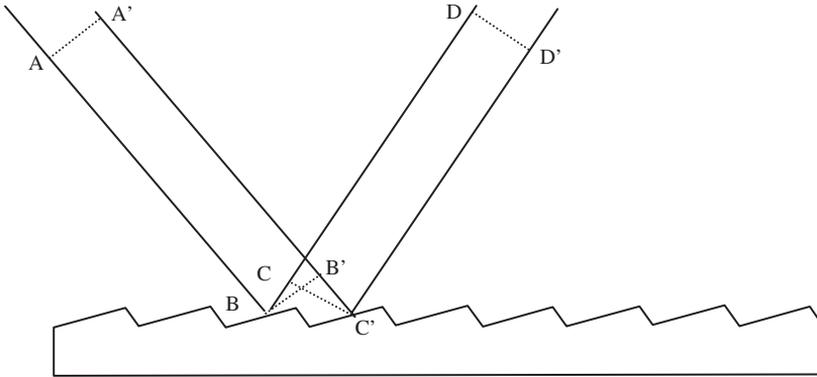


Fig. 5.9. A reflection grating illustrating the relative geometry of two light rays that may result in constructive or destructive reinforcement.

Figure 5.9 shows a reflection grating with rays illustrating just one set of angles for the light source and light output. In this diagram the line segments $\overline{AB} = \overline{A'B'}$ and $\overline{CD} = \overline{C'D'}$ and the line segments \overline{BC} and $\overline{B'C'}$ may or may not be the same length. Geometrically, $\overline{BC} = d \sin \theta_{\text{incident}}$ and $\overline{B'C'} = d \sin \theta_{\text{reflected}}$ the difference between the two segments must be an integer number of wavelengths for constructive reinforcement yielding

$$n\lambda = d(\sin \theta_{\text{incident}} \pm \sin \theta_{\text{reflected}}) \quad (5.22)$$

The incident and reflection angles are measured from the normal (perpendicular) to the grating.

In addition to the important incident and reflected angles for the grating, the blaze angle or the angle of the major reflective surface is important because it helps to concentrate the reflected light in the first order and also determines the usable wavelength range of the grating. The blaze angle, β , is where the angles of incidence and reflection are identical and all wavelengths reinforce and is the angle that the major reflective surface makes with the grating.

$$n\lambda = 2d \sin \beta \quad (5.23)$$

Physically a blaze of white light is observed in the visible region. A grating will typically be useful from approximately one-third of the blaze wavelength to three times the blaze wavelength in the first order.

Gratings also diffract light in the second, third and higher orders. If a grating reinforces light at 600 nm in the first order, it will also reinforce light at 300 nm in the second order and 200 nm in the third order. Gratings are usually paired with simple optical filters to remove unwanted

light. In the above case, ordinary borosilicate glass will absorb virtually all of the ultraviolet radiation from the visible 600 nm light.

Modern gratings may be etched on concave surfaces so that they will serve a dual purpose of diffracting light and also focusing the radiation. This decreases the number of parts in a spectrometer and also decreases losses in intensity by having fewer optical parts.

Creating the master grating that commercial replica gratings are duplicated from is a painstaking task with many possibilities for imperfections. Imperfections in the grating may cause interferences such as stray radiation and unfocused images. Advances in laser technology enable precise layouts of reflection gratings by using the interference patterns of intersecting laser beams. The resulting pattern can be used to sensitize a photoresist that can then be dissolved with an organic solvent and then the exposed surface can be etched to produce the grating. Holographic patterns produce extremely high quality master gratings and the replica gratings are of equal high quality. Another advantage of holographic grating production is that the technology is not limited to flat surfaces. Excellent concave gratings can be formed with the advantages mentioned previously.

As with prisms, there are other devices that have been historically used for dispersing or filtering electromagnetic radiation. These include interference filters and absorption filters. Both of these are used for monochromatic instruments or experiments and find little use compared to more versatile instruments. The interested reader is referred to earlier versions of instrumental analysis texts.

5.3.3 Detectors

Detectors for each region of the spectrum differ because of the unique properties of either the radiation itself or the source of the electromagnetic radiation. Light sources produce plentiful amounts of photons in the visible region and the photon energy is sufficient so that a simple phototube or phototransistor will generate enough electron flow to measure. In the ultraviolet region of the spectrum, the available light sources produce relatively few photons when compared to the visible light sources. Therefore, measurement of ultraviolet photons uses a special arrangement of a phototube called a photomultiplier to obtain a measurable electrical current. It is not difficult to generate sufficient photons in the infrared region but the photons produced are of such low energy that devices to rapidly measure infrared radiation have just been recently developed.

Phototubes are vacuum tubes with a large anode coated with a photoemissive substance such as cadmium sulfide. A positive voltage of approximately 90 V on the cathode attracts electrons dislodged by photons from the cadmium sulfide. The current measured by an ammeter is proportional to the number of photons entering the phototube. Figure 5.10 represents a schematic diagram of a phototube measuring circuit.

A phototransistor or photodiode may also be used to detect visible light. Both devices have p–n junctions. In the photodiode the photon ejects an electron from the p semiconductor to the n semiconductor. The electron cannot cross back across the p–n junction and must travel through the circuitry, an ammeter to return to the p material. In a phototransistor, usually an npn type, the base (p-type semiconductor) is enlarged and photosensitive. Photons dislodge electrons that act as if a potential was applied to the base. This results in an amplified flow of electrons proportional to the number of photons striking the base (Fig. 5.11).

For detection of ultraviolet photons the preferred device is the photomultiplier tube. This device has an electron emissive anode (called a dynode) that photons strike and eject electrons. However, the electrons are attracted toward a second electron emissive surface where each electron generated at the first dynode will eject several electrons toward a third dynode. Approximate 10–12 dynodes are arranged as shown in Fig. 5.12. Each dynode is biased so that it is 90 V more positive than the previous dynode so that the ejected electrons are attracted from one dynode to the next.

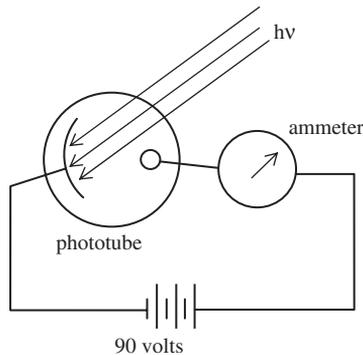


Fig. 5.10. Schematic diagram of a simple phototube circuit. Photons ($h\nu$) strike the CdS-coated anode and electrons are ejected and attracted toward the positive cathode and return through the circuit. The ammeter monitors the flow of electrons that are proportional to the intensity of the photons.

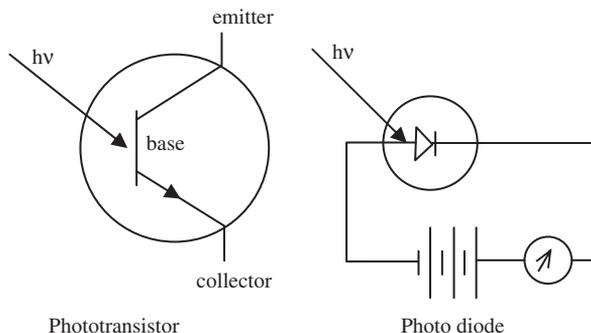


Fig. 5.11. Schematic diagrams of a phototransistor and a photodiode.

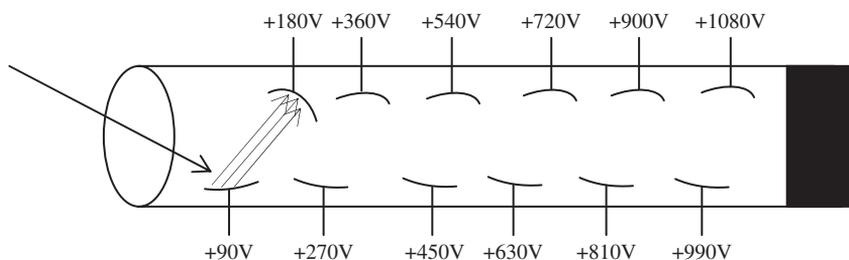


Fig. 5.12. A schematic diagram of a photomultiplier tube.

Infrared radiation has a very low energy and cannot eject electrons from most common photoemissive surfaces. The initial infrared sensors were temperature-sensing devices. Thermocouples and thermistors are forms of bolometers used for detecting infrared radiation.

5.3.4 Transmitting surfaces

Each of the three spectral regions has different requirements for materials that can be used for sample containers, lenses and prisms if used. Visible light is transmitted readily through borosilicate glass and this glass also has good dispersing properties if lenses and prisms are to be used. Disposable plastic cuvettes are also available for spectroscopy in the visible region. There are also a large number of solvents that are useful in the visible region. These include water, liquid alkanes, benzene, toluene, halogenated hydrocarbons, acetonitrile, ethers and esters.

In the ultraviolet region of the spectrum quartz optical materials are required. There are some plastic materials that may also be used in the

ultraviolet region. Solvents usable in the visible region also may not be appropriate for the ultraviolet. Table 5.5 lists some common solvents and their cutoff wavelengths.

The infrared region has a strong, obvious absorbance peak for the -OH group. The surfaces of glass and water are eliminated as useful optical materials. For liquids, sodium chloride crystals are used to construct fixed path-length cells as shown in Fig. 5.13. The path length of the cells is usually between 0.1 mM and 1 mM because most samples are pure compounds. Other transmitting materials used in the infrared are listed, with their usable wavelength range in the table below.

TABLE 5.5

Some common solvents and their UV cutoff wavelengths

Solvent	Lower λ limit (nm)
Water	180
Hexane	195
Cyclohexane	200
Ethanol	210
Diethyl ether	215
Chloroform	245
Carbon tetrachloride	260
Dimethylsulfoxide	268
Toluene	284
Acetone	330
Methyl isobutyl ketone	334

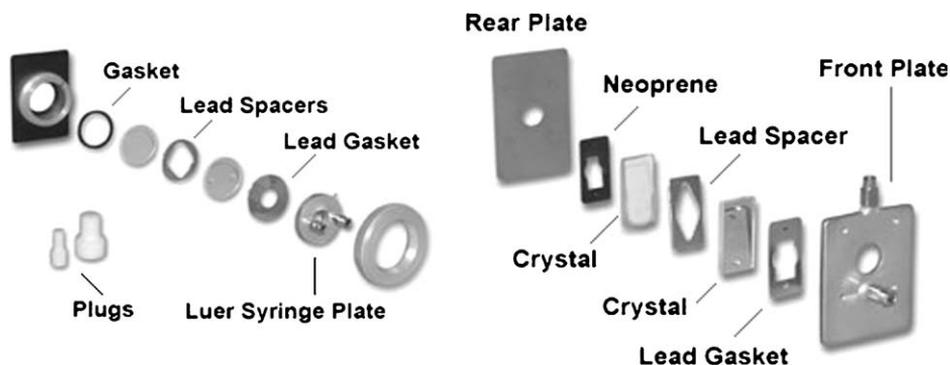


Fig. 5.13. Diagrams of commercial fixed path length infrared cells. Lead, or Teflon[®] spacers provide the cell thickness and also seal the cell from leakage.

5.3.5 Sample handling

Typically, the ultraviolet and visible regions are used for quantitative analysis. By far, the majority of the samples are liquids and are dilute solutions that will achieve the lowest photometric error (between 0.2 and 0.8 absorbance units). A knowledge of the molar absorptivity allows calculation of the usable concentration range for analysis, and samples can be diluted appropriately. Cuvettes having optical path lengths of 1.0, 10.0 and even 100.0 mm are available. Gas samples can also be examined in the ultraviolet and visible regions. Best results are obtained if a reagent blank containing all of the solution components except the analyte is used.

Infrared analysis is usually used as a qualitative method to identify substances. Liquids are usually analyzed as pure substances in cells with very small optical path lengths of 0.1–1.0 mm. Usable spectra can be obtained by placing a drop of relatively non-volatile sample between two sodium chloride plates, allowing them to be held together by capillary action.

It is often necessary to determine the optical path length of salt cells since they are subject to wear and erosion from moisture. To determine the optical path length, b , a spectrum is obtained on the empty cell. Reflections from the internal walls of the cell create an interference pattern that looks like a series of waves in the spectrum. Using as many well-formed waves as possible, the start and ending frequencies (in cm^{-1}) are determined along with the total number of waves. The optical path length is then calculated from the following relationship:

$$b = \frac{\text{number of waves}}{2(\text{wavenumber}_2 - \text{wavenumber}_1)}$$

where the wavenumbers have units of cm^{-1} .

Recently, polyethylene and Teflon[®] mesh sample holders have been used. A drop of sample is placed on the mesh and spread to a relatively uniform thickness for analysis. These holders can often be rinsed and reused. A very convenient alternative to liquid sample holders is the technique called attenuated total reflection or ATR. The ATR cell is a crystal of gallium arsenide, GaAs; and the infrared radiation enters one end of the trapezoidal crystal. With the angles adjusted to obtain total internal reflection, all of the IR radiation passes through the crystal and exits the other end as shown in Fig. 5.14.

However, as the IR waves strike the surfaces to be reflected, part of the wave emerges from the crystal. This can be absorbed by a sample on the other side of the crystal. The GaAs crystal is unaffected by water

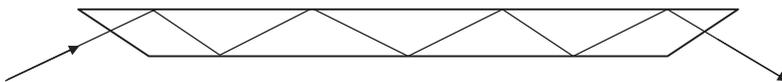


Fig. 5.14. Schematic diagram of an ATR gallium arsenide crystal and the total internal reflection of a light ray. The sample is placed on top of the crystal and interacts with the evanescent wave producing the spectrum.

and aqueous samples can be studied. Solutions can be analyzed providing the solvent does not absorb in the infrared region of interest or if a reference cell can be used to cancel the absorbance of the solvent.

Solid samples can be analyzed in the IR by preparing a solution in a suitable solvent or by preparing a KBr pellet containing the sample. A KBr pellet is prepared by mixing approximately 0.5% sample with very pure and dry KBr (e.g., 1 mg sample and 200 mg KBr). The sample and KBr are ground together to a very fine powder and transferred to a high-pressure press. At approximately 2000 psi the mixture fuses into a solid pellet that can be mounted and scanned in the spectrometer. Presence of water will cloud the pellet and very dry KBr is required, and some presses have the ability to remove water by vacuum while the pellet is being fused. Gaseous samples are readily, and often, analyzed by infrared spectroscopy. Gas cells with optical path lengths of 10 cm fit most IR spectrometers. Additional path length may be had by arranging mirrors in a gas cell to allow the radiation to pass through the cell several times before exiting.

5.4 PUTTING THE PARTS TOGETHER

5.4.1 Spectrometers for the visible region

Simple spectrometers that cover the region from 350 to 1000 nm are available for modest cost and are useful for routine analysis. These spectrometers are usually single beam instruments that are set up according to the block diagram in Fig. 5.15, and Fig. 5.16 illustrates the actual configuration of a commercial instrument.

A single beam instrument requires that a reagent blank be used to determine I_0 (set 0.0 absorbance or 100% T) at each wavelength before measuring I for the sample (that electronically is transmitted to the meter as absorbance or % T). Inserting the reagent blank, zeroing the instrument and then inserting the sample and manually reading the absorbance is time consuming. One solution, made possible by digital electronics, is to measure I_0 for the reagent blank at all desired wavelengths at one time and store the data in memory and then insert the sample and measure I at

General principles of spectroscopy and spectroscopic analysis

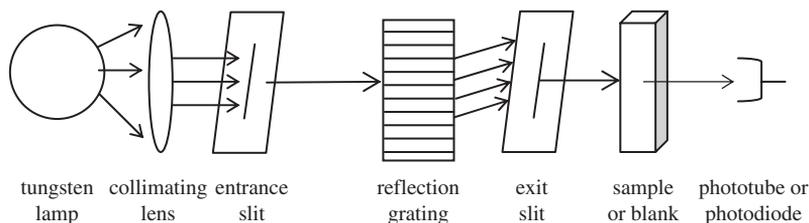


Fig. 5.15. Block diagram of a single beam visible spectrometer.

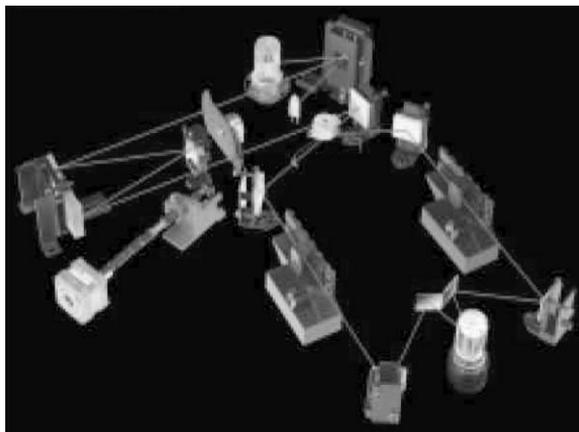


Fig. 5.16. A schematic diagram of a single beam instrument, the Genesis 2000 produced by Thermo Spectra. Layout of parts for a UV-Vis spectrometer (with permission of Thermo inc.).

the same wavelengths. The spectrum is then calculated and displayed on a computer screen. This approach requires very stable electronics to assure that there are minimal system changes between measurement of I_0 and I . The older Beckman DU-7 and many of the FT-IR instruments operate in this manner. While separate recording of the reagent blank and sample intensities is one solution, double beam instruments surmount both problems mentioned above.

A double beam instrument splits the electromagnetic radiation into two separate beams, one for the reagent blank, and the other for the sample. There are two ways to do this. The first method uses a mirror that is half silvered and half transparent. As shown in Fig. 5.17 this results in a continuous beam of light for both the sample and reagent blank.

After passing through the sample and reagent blank, the two beams can be monitored at separate detectors and then combined electronically to obtain the ratio of I/I_0 .

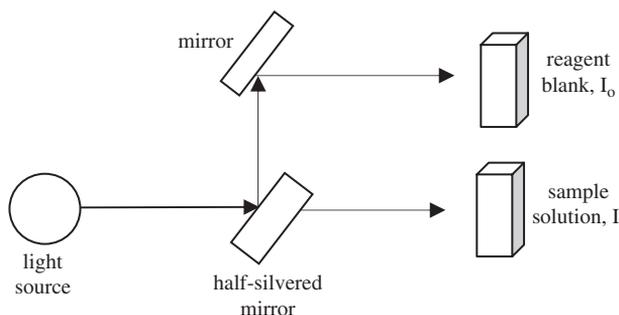


Fig. 5.17. Essentials of a double beam instrument in space. This is characterized by two continuous beams of light.

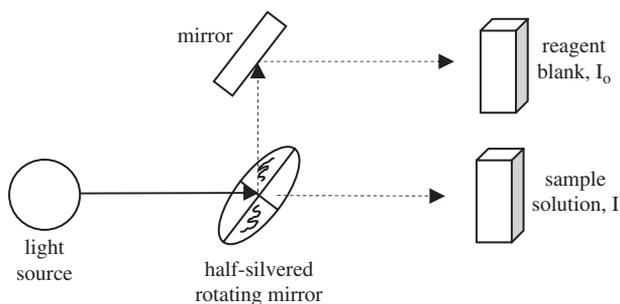


Fig. 5.18. Schematic diagram of a double-beam instrument in space.

The second type of double-beam instrument is one where the light source is divided into two beams by a rotating sector mirror that alternately reflects and transmits the light. This results in a chopped beam of light that alternately passes through the reagent blank and the sample as shown in Fig. 5.18.

The double-beam in-space spectrometer has alternating “segments” of light impinging on the sample and the reagent blank. These beams can be recombined and focused on a single detector. The result will be a square-wave type of signal as shown in Fig. 5.19.

The square wave produced by the double-beam in space spectrometer is preferred since there is only one detector and the signal is a square-wave that is essentially an alternating current. Alternating currents are much easier to manipulate electronically. In particular they can be easily amplified and noise that is either direct current noise or high-frequency noise can be filtered from the signal.

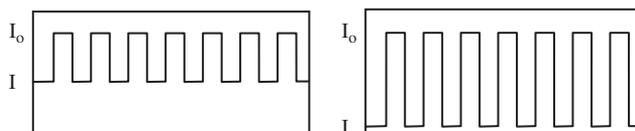


Fig. 5.19. Representations of the output of the detector for a double-beam in-space spectrometer. The first panel shows a system that has a relatively large I/I_0 ratio while panel 2 has a smaller I/I_0 ratio and a larger absorbance.

5.4.2 Rapid spectroscopy

Spectroscopic instruments are used for quantitative and qualitative analyses in stand-alone situations for the most part. However, there are situations where spectroscopic measurements are used as detectors for other instruments, in particular high-performance liquid chromatography (HPLC) discussed in Chapter 15. In addition it is recognized that the signal-to-noise ratio can be increased by repeatedly adding spectra so that the signal increases with each measurement, N , and the noise only increases as the square root of N , $N^{1/2}$. Two types of instrumentation were developed to meet these needs. First is the photodiode array (PDA) spectrometer for the ultraviolet and visible regions of the spectrum and the second is the Fourier transform infrared spectrometer, FT-IR, for the infrared region.

Diode array spectrometers are also known as PDA spectrometers designed to measure the desired band of wavelengths at the same time. This is achieved by placing a linear array of photodiodes in the path of the dispersed beam of UV-Vis radiation. The radiation has passed through the sample, often an HPLC flow cell, prior to dispersion. The grating disperses the radiation so that it is linearly, in terms of wavelength, dispersed at the focal plane. If each of the diodes is of a certain width, then each diode will intercept a given band of radiation. The size of the band of radiation observed is related to the resolution of the instrument. Considering the range from 200 to 700 nm, it would take 500 photodiodes to achieve one nanometer resolution. The speed of a diode array instrument depends on the speed at which a computer can access, sample, measure and discharge the voltage developed on each diode. The number of diodes sampled also has an effect on the rate at which spectra can be obtained. Current photodiode instruments can obtain spectra with resolutions of 1–3 nm (256–1024 diodes/200–1100 nm range) at a rate of up to 2500 spectra sec^{-1} . This makes the PDA ideal as a versatile detector for HPLC applications.

Fourier transform spectroscopy technology is widely used in infrared spectroscopy. A spectrum that formerly required 15 min to obtain on a continuous wave instrument can be obtained in a few seconds on an FT-IR. This greatly increases research and analytical productivity. In addition to increased productivity, the FT-IR instrument can use a concept called Fleggetts Advantage where the entire spectrum is determined in the same time it takes a continuous wave (CW) device to measure a small fraction of the spectrum. Therefore many spectra can be obtained in the same time as one CW spectrum. If these spectra are summed, the signal-to-noise ratio, S/N can be greatly increased. Finally, because of the inherent computer-based nature of the FT-IR system, databases of infrared spectra are easily searched for matching or similar compounds.

The core of the FT-IR is the Michaelson interferometer (Fig. 5.20) and the mathematical relationship between the frequency and time domains of a spectrum that is called the Fourier transform. The Michaelson interferometer is diagrammed below. A beam of infrared radiation from a source as described previously is collimated and directed through the sample to a 50% transmitting mirror, beamsplitter. The split beams reflect off two plane mirrors directly back to the beamsplitter where they recombine. One of the plane mirrors is fixed but the other moves so that the paths that the two light beams travel are not equal. The difference in the distance from the 50% mirror to the moving and fixed mirrors is called the retardation, δ . The difference in distance that one light beam travels compared to another is therefore 2δ . As with a reflection grating, if $2\delta = n\lambda$ then that set of wavelengths (this includes the $n = 2, 3, \dots$ overtones) will be reinforced while all others will be attenuated to some extent. Those wavelengths where 2δ is $(n+0.5)\lambda$ will be 180° out of phase and completely attenuated.

The Fourier transform allows the mathematical conversion between the time domain and the frequency domain of a spectrum. The names for these domains refer to the x -axis of their conventional graphical representations. Figure 5.21 illustrates how these are important in terms of conventional spectra. Importantly, the time and the frequency domains contain exactly the same information.

For the two waves represented in Fig. 5.21 the power at any time t is

$$P(t) = k_A \cos(2\pi\nu_A t) + k_B \cos(2\pi\nu_B t) \quad (5.24)$$

or the algebraic sum of A and B as shown in C of Fig. 5.21.

Frequencies in the infrared region are between 10^{13} and 10^{14} Hz, they are approximately three orders of magnitude greater in the ultraviolet

General principles of spectroscopy and spectroscopic analysis

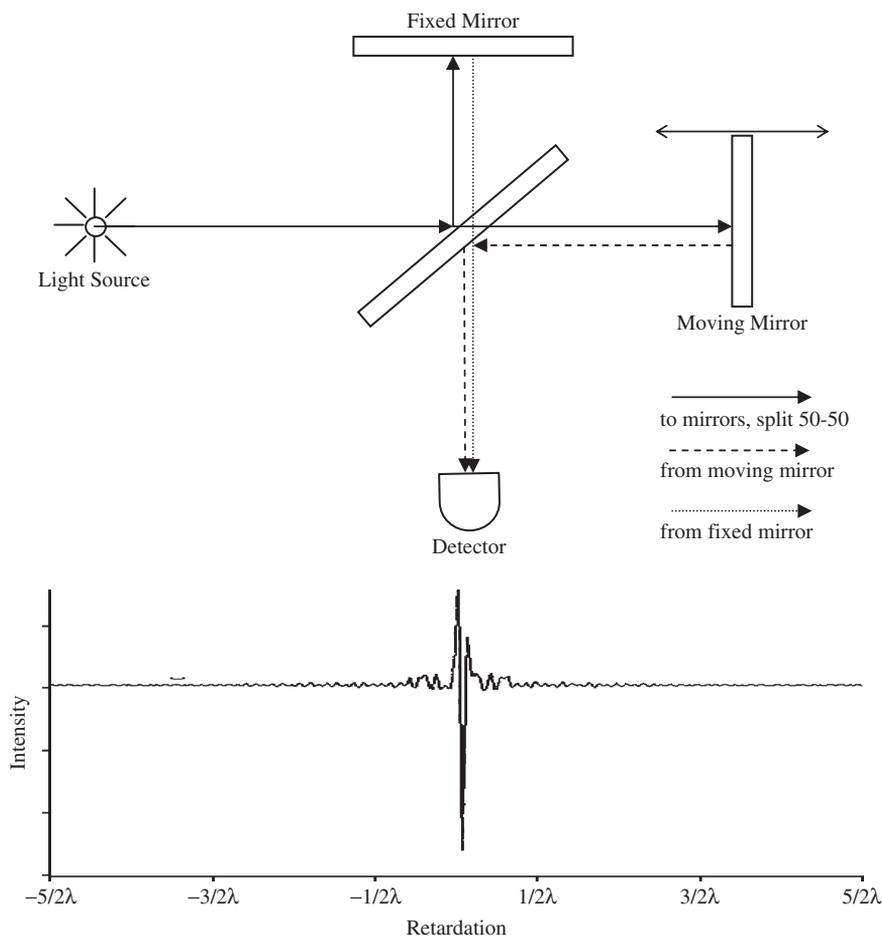


Fig. 5.20. (Top) Schematic diagram of a Michelson interferometer. Retardation determines difference in optical path between fixed mirror and moving mirror. When retardation, δ , is $\lambda/2$ light with a wavelength equal to λ will be reinforced. (Bottom) Interference pattern from the Michelson interferometer. Major peak where $\delta = 0$ is where all wavelengths are reinforced.

and visible regions. There are no detectors available that are fast enough to measure waves at this frequency. Signals that vary in the range of 100–10,000 Hz can be accurately measured with modern electronics. The Michelson interferometer not only produces an interferogram, but the interferogram has a fundamental frequency that can approximately be a factor of 10^{10} lower because of a process called modulation. Modulation is the process of changing a high frequency to a lower one or a low frequency to a higher one, which was developed along with the broadcast

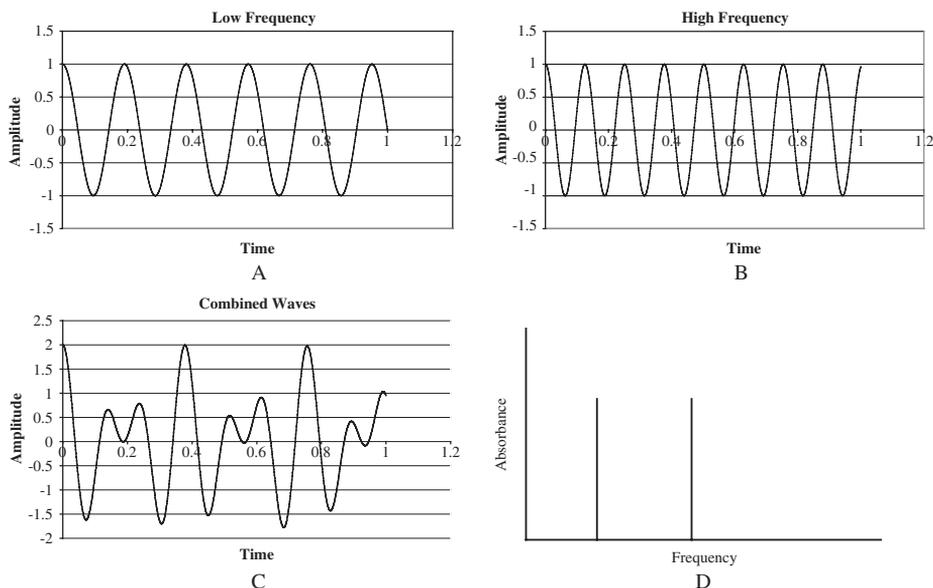


Fig. 5.21. (A) shows a conventional cosine wave of 500 Hz and (B) a cosine wave of 333 Hz, (C) the algebraic sum of (A) and (B), (D) represents the cosine wave in (A) in the frequency domain, (D) the representation of (C) in the frequency domain.

industry to propagate voice and video information at television and radio frequencies. In FT-IR the signal is modulated by the interferometer to a measurable frequency. In order to avoid aliasing, it is necessary to sample an alternating current signal at frequency that is greater than twice the frequency of the signal.

To understand the process, consider the instrument itself. The mirror moves at a constant velocity v_M and the time it takes for the mirror to move $\lambda/2$ is τ then $v_M\tau = \lambda/2$ of mirror movement or λ in total difference traveled by the two light beams. Therefore, τ is the time for one wavelength or $1/\tau$ the frequency of the *light striking the detector*. From this

$$f(\text{s}^{-1}) = \frac{1}{\tau(\text{s})} = \frac{v_M(\text{cm s}^{-1})}{\lambda(\text{cm})/2} = 2v_M(\text{cm s}^{-1})\bar{\nu}(\text{cm}^{-1}) \quad (5.25)$$

If the velocity of the mirror is 0.1 cm s^{-1} and the wavelength is $8.0 \mu\text{m}$ (the center of the IR region) the frequency of the modulated IR radiation is

$$\text{Frequency} = \frac{2(0.1 \text{ cm s}^{-1})}{(8.0 \times 10^{-4} \text{ cm})} = 250 \text{ s}^{-1} \quad (5.26)$$

A similar calculation in the visible region at 400 nm results in a modulated frequency of $125,000 \text{ s}^{-1}$ that is difficult to measure.

Resolution is the ability to distinguish two closely spaced spectral peaks. If the infrared resolution is

$$\Delta\bar{\nu} = \bar{\nu}_2 - \bar{\nu}_1 \quad (5.27)$$

Equation 5.24 will be at a maximum, at zero retardation and reach its maximum again when the waves are in phase when $\nu_A t - \nu_B t$ is unity or

$$1 = \delta\bar{\nu}_2 - \delta\bar{\nu}_1 \quad (5.28)$$

From this

$$\Delta\bar{\nu} = \bar{\nu}_2 - \bar{\nu}_1 = \frac{1}{\delta} \quad (5.29)$$

so that the resolution is approximately equal to the reciprocal of the total retardation. The retardation, δ , is equal to twice the total mirror movement. If the mirror moves a total of 1.0 cm the $\delta = 2.0 \text{ cm}$ and the resolution will be 0.5 cm^{-1} .

5.5 STATISTICS FOR SPECTROSCOPY

5.5.1 Signal averaging

As known from statistics the standard deviation is

$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{N - 1}} \quad (5.30)$$

And the uncertainty or confidence limit will be

$$u - \bar{x} = \frac{ts}{\sqrt{N}} \quad (5.31)$$

where u is the true mean and \bar{x} is the measured mean. As the number of measurements, N , increases, the uncertainty about the value of the mean will decrease with the square root of N . Similarly, the signal-to-noise ratio will increase as the number of measurements increases because the signal is additive with the number of measurements and the noise increases as the square root of N .

$$\frac{\text{Signal}}{\text{Noise}} = \left(\frac{\text{Signal}}{\text{Noise}} \right) \left(\frac{N}{\sqrt{N}} \right) \quad (5.32)$$

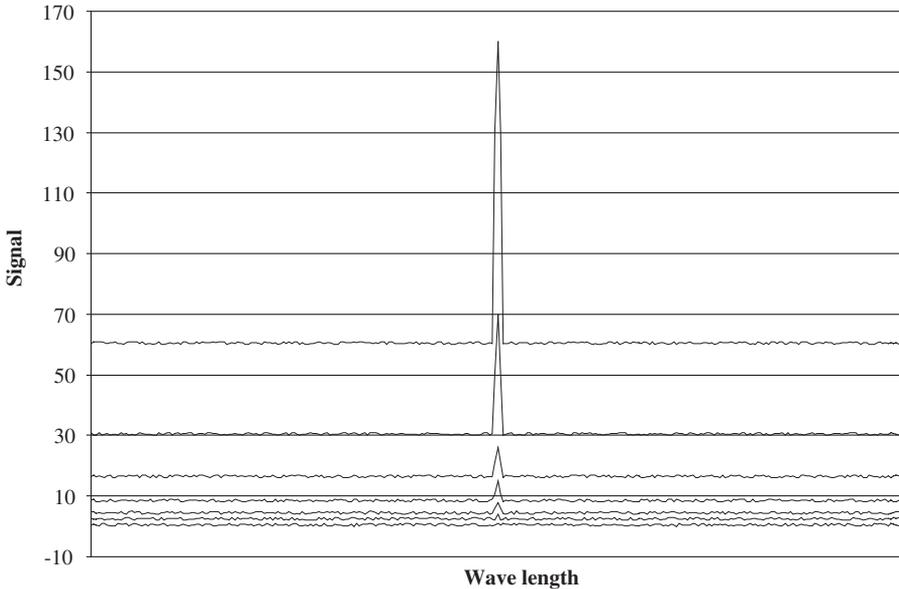


Fig. 5.22. Illustration of the increase in signal to noise ratio when repetitive scans are added. Bottom trace represents a S/N ratio of 1.0. Successive traces represent summation of 4, 16, 50, 100, 1600 and 10,000 repetitive scans.

For a spectrum the same principles occur. Repetitive spectra that are added to each other show the signal emerging from the noise as shown in Fig. 5.22.

5.5.2 Statistics for a calibration curve

Calibration curves are plots of concentration (x_i) versus some response of the instrument (y_i). Concentration values are assumed to be the most precise and all of the error is associated with the response measurement. With those definitions, we write the equation for the least-squares line as $y = mx + b$ and, omitting the derivation, find that

$$m = \frac{n \sum (x_i y_i) - \sum x_i \sum y_i}{n \sum (x_i^2) - (\sum x_i)^2} \quad (5.33)$$

$$b = \frac{n \sum (x_i^2) \sum y_i - \sum x_i y_i \sum x_i}{n \sum (x_i^2) - (\sum x_i)^2} \quad (5.34)$$

Solving the two equations above is easiest using a spreadsheet starting with one column of x_i values and another with y_i values. Another column

can be constructed to obtain $x_i y_i$ values and two more columns to obtain the squares of the x_i and y_i values. Finally, each of these columns can be summed. The appropriate values can be used to determine the slope m and the intercept b . Once m and b have been calculated another column can be added to the spreadsheet to determine the vertical deviation, d_i , of each y_i value from the least-squares line and the square of the deviation d_i^2

$$d_i = (y_i - y) = y_i - (mx_i + b) \quad (5.35)$$

The standard deviation of the y values is calculated as

$$s_y = \sqrt{\frac{\sum d_i^2}{n - 2}} \quad (5.36)$$

From this the standard deviations of the slope, s_m , and intercept, s_b , are

$$s_m = \sqrt{\frac{s_y^2 n}{n \sum (x_i^2) - (\sum x_i)^2}} \quad (5.37)$$

$$s_b = \sqrt{\frac{s_y^2 \sum x_i^2}{n \sum (x_i^2) - (\sum x_i)^2}} \quad (5.38)$$

Preparation of a calibration curve has been described. From the fit of the least-squares line we can estimate the uncertainty of the results. Using similar equations we can determine the standard deviation of the calibration line (similar to the standard deviation of a group of replicate analyses) as

$$s_{\text{line}} = \sqrt{\frac{n \sum (y_i^2) - (\sum y_i)^2 - m^2 (n \sum (x_i^2) - (\sum x_i)^2)}{n(n - 2)}}$$

$$s_{\text{sample}} = \frac{s_{\text{line}}}{m} \sqrt{\left(\frac{1}{M} + \frac{1}{n} + \frac{n (\bar{y}_{\text{sample}} - \bar{y}_{\text{cal}})^2}{m^2 (n \sum (x_i^2) - (\sum x_i)^2)} \right)}$$

The standard deviation for an analytical result will be

$$S_{\text{sample}} = \frac{S_{\text{line}}}{m} \sqrt{\left(\frac{1}{M} + \frac{1}{n} + \frac{n(\bar{y}_{\text{sample}} - \bar{y}_{\text{cal}})^2}{m^2(n \sum x_i^2 - (\sum x_i)^2)} \right)}$$

where M replicates analysis of the sample that have a mean of \bar{y}_{sample} and \bar{y}_{cal} is the mean of the n samples used to construct the calibration curve.

5.5.3 Signal-to-noise ratio

The signal-to-noise ratio is an important parameter that allows us to evaluate the quality of an instrument and spectra. The determination of the signal-to-noise ratio requires a measurement of some quantity of the signal and a measurement of the noise. Measurement of the signal is generally a straightforward difference between a reagent blank and the sample or spectral baseline and peak amplitude. Noise is more difficult. Many instruments appear to have little or no noise. Experiments must be made to expand the scale sufficiently to observe the random fluctuations called noise. Once noise can be observed, its best measure is the root mean square of the noise (rms noise). The term rms means that we have taken the square root of the mean of the squares of a representative number of measurements of the noise. This is not an easy task. Assuming the noise is a sine wave we can estimate

$$N_{\text{rms}} = 0.707 \left(\frac{N_{\text{p-p}}}{2} \right) \quad (5.39)$$

We can measure the peak-to-peak noise, $N_{\text{p-p}}$, rather easily and calculate the equivalent in rms noise.

5.5.4 Limit of detection

Detection limit is the minimum amount of signal that can be observed with some certainty that there is a signal at all. This requires that the signal be at least three times the rms noise of the experiment.

5.5.5 Limit of quantitation

It is recognized that the detection limit is at the extreme of the instrument's capabilities. As such it is very difficult to quantify a signal

that is on the verge of non-existence. For quantitative measurements, most analysts take a value that is 10–20 times as large as the limit of detection as the lower limit for quantitation. If an instrument can detect 15 ppb of a herbicide, the lowest level, it could be used to quantitate that same herbicide is approximately 0.15 ppm.

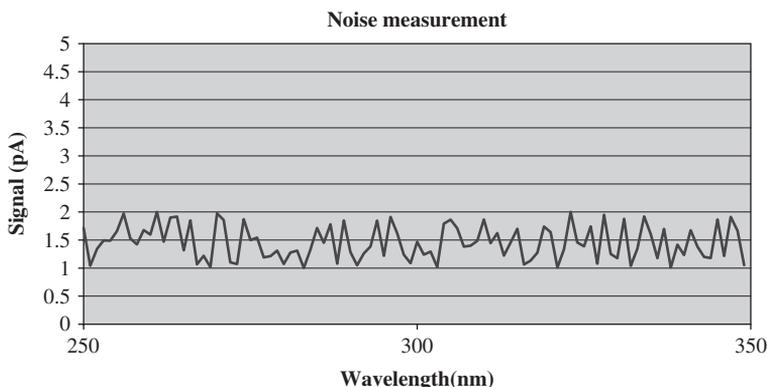
5.5.6 Sensitivity

The sensitivity of a method is a measure of its ability to distinguish one concentration from another. If a particular instrument could be used to determine the concentration of a heavy metal such as lead and could reliably distinguish a 25 ppb solution from a 30 ppb solution, it would be more sensitive than an instrument that could barely tell the difference between a 25 ppb solution and a 50 ppb solution. The best quantitative measure of the sensitivity of an instrument and/or an analytical method is to determine the slope of the calibration curve. The greater the slope, the more sensitive the instrument and/or method.

REVIEW QUESTIONS

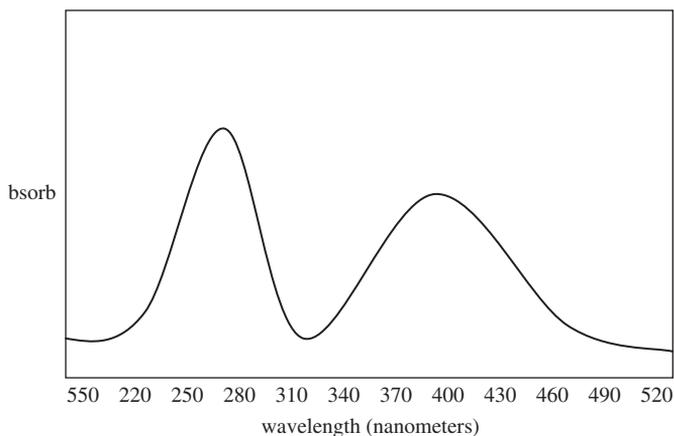
1. Determine the energy in kJ mol^{-1} for electromagnetic radiation with the following wavelengths:
 - a. 225 nm
 - b. 3650 nm
 - c. 450 nm
 - d. 750 nm
 - e. 15 μm
2. Determine the energy in kJ mol^{-1} for electromagnetic radiation with the following wavelengths:
 - a. 180 nm
 - b. 6.0 μm
 - c. 12.0 μm
 - d. 645 nm
 - e. 300 nm
3. Compare the results in problem(s) 1 and/or 2 to the average bond energies for C–C, C=C, C–H and C–Cl bonds. Which can be considered ionizing radiation?

4. Based on minimizing the photometric error, what range of absorbances is optimal for absorbance spectroscopy? What is the relative dynamic range of absorbance measurements?
5. Optimal results will be obtained for what range of concentrations if the molar absorptivity ($l \text{ mol}^{-1} \text{ cm}$) of the analyte is (assume a 1.0 cm optical path length)
 - a. 150
 - b. 1.2×10^5
 - c. 655
 - d. 1025
 - e. 25
6. Optimal results will be obtained for what range of concentrations if the molar absorptivity ($l \text{ mol}^{-1} \text{ cm}$) of the analyte is: (assume a 1.0 mm optical path length)
 - a. 6.2×10^4
 - b. 15
 - c. 575
 - d. 2500
 - e. 125
7. The figure below represents the noise of a spectrometer detector. Estimate the peak-to-peak noise, and the rms noise of this detector. If an analyte produces a signal of 6.3 pA, will it be above or below the limit of detection?

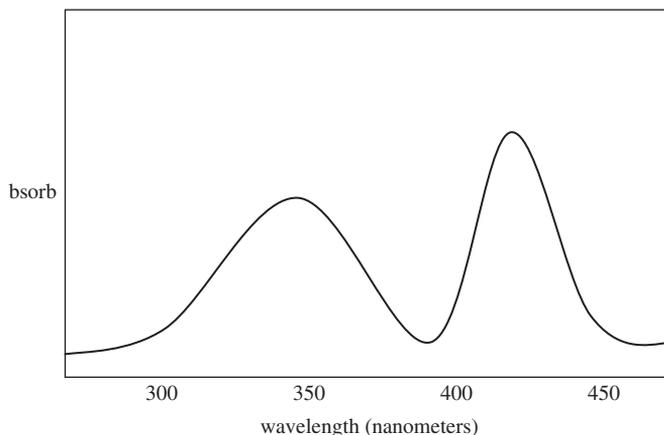


8. The figure below illustrates the visible absorbance spectrum of substance A, what is the appropriate analytical wavelength for determining the concentration of A?

General principles of spectroscopy and spectroscopic analysis



9. The figure below illustrates the visible absorbance spectrum of substance B, what is the appropriate analytical wavelength for determining the concentration of B?



10. A mixture of two substances A and B, shows absorbance peaks at 465 and 720 nm, respectively. The slope of a calibration plot for substance A is 18,350 at 465 nm and 884 at 720 nm. For substance B the slope at 465 nm is 1024 and at 720 nm it is 12,240. What is the concentration of A and B in a sample that has an absorbance of 0.566 at 465 nm and an absorbance of 0.728 at 720 nm?
11. A mixture of two substances X and Y, show absorbance peaks at 365 and 620 nm, respectively. The slope of a calibration plot for substance X is 14,350 at 365 nm and 804 at 620 nm. For substance

Y the slope at 365 nm is 1154 and at 620 nm it is 17,240. What is the concentration of X and Y in a sample that has an absorbance of 0.566 at 365 nm and an absorbance of 0.628 at 720 nm?

12. A 5.00 ml sample containing iron is mixed with hydroxylamine hydrochloride to reduce the iron (III) to iron (II). The solution is then mixed with an excess of phenanthroline and the absorbance is measured and found to be 0.448. A second 5.00 ml solution of the same unknown is mixed with 1.00 ml of 2.0×10^{-4} M Fe^{2+} and is then treated the same way as the original sample. The absorbance is found to be 0.525. What is the concentration of the iron in the sample?
13. A 10.0 ml solution containing proteins is reacted with biuret solution and the absorbance is measured as 0.356. Another 10.0 ml sample of the same protein solution is mixed with 5.0 ml of a protein solution known to contain $1.6 \mu\text{g ml}^{-1}$ of protein. The mixture is reacted in the same way as the original unknown and the absorbance is found to be 0.562. What is the concentration of the protein in the sample?
14. Use a spreadsheet such as EXCEL to obtain a graph of absorbance versus concentration for the following data.

Sample	Absorbance	Concentration (mol l^{-1})
blank	0.00	0.00
1	0.238	0.000200
2	0.455	0.000400
3	0.665	0.000600
4	0.878	0.000800

Also determine the slope and intercept of the least-squares line for this set of data. Determine the concentration and standard deviation of an analyte that has an absorbance of 0.335.

15. Use a spreadsheet such as EXCEL to obtain a graph of absorbance versus concentration for the following data.

Sample	Absorbance	Concentration (mol l^{-1})
blank	0.00	0.00
1	0.165	0.000200
2	0.321	0.000400
3	0.505	0.000600
4	0.687	0.000800

General principles of spectroscopy and spectroscopic analysis

Also determine the slope and intercept of the least-squares line for this set of data. Determine the concentration and standard deviation of an analyte that has an absorbance of 0.335.

16. What wavelength will be observed at a detector that is placed at 23.5° when the light source strikes the reflection grating at an angle of 45.3° and the grating has 1250 lines per mm. Assume that the order of diffraction is first order. What is the second order wavelength observed?
17. What wavelength will be observed at a detector that is placed at 43.5° when the light source strikes the reflection grating at an angle of 38.3° and the grating has 3250 lines per mm. Assume that the order of diffraction is first order. What is the third order wavelength observed?
18. What is the useful wavelength range for a grating with 10,000 lines per mm and a blaze angle of 41.6° ? What spectral region is this?
19. What is the useful wavelength range of a grating with 1560 lines per mm that has 2500 lines per mm. What spectral region is this?
20. How many lines per mm will be needed for a grating that will be able to resolve spectral lines that are 10 nm apart?
21. How many lines per mm are needed to have a resolution of 2.0 cm^{-1} in the infrared?
22. What is the distance that a mirror in a Michaelson interferometer must move to have a resolution of 1.0 cm^{-1} in the infrared?
23. What distance must a mirror move in order to have a resolution of 1.0 nm in the visible region of the spectrum?
24. Fill in the blank spaces in the following table, where needed units are given.

	<i>A</i>	ϵ	<i>b</i>	<i>c</i>	<i>T</i>
1	0.450		1.00 cm	$3.2 \times 10^{-4} \text{ M}$	
2	0.223	2840	dm	$7.8 \times 10^{-5} \text{ M}$	
3		12,100	10.0 cm	$4.2 \times 10^{-6} \text{ M}$	
4		546	0.25 cm		34.2%
5	0.665		1.00 mm	$7.6 \times 10^{-5} \text{ M}$	

25. Fill in the blank spaces in the following table, where needed units are given.

	<i>A</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>T</i>
1	0.650		1.00 cm	7.7×10^{-4} ppm	
2		2,7361 g ⁻¹ cm	1.00 cm	ppb	22.3%
3	0.239		1.0 mM	0.00634 wt%	
4		471 g ⁻¹ cm	10.0 cm	ppm	0.652
5	0.849	7,8451 g ⁻¹ cm	mm	2.35 ppb	

26. Six 25 ml volumetric flasks are filled with 10 ml of the analyte and then 1, 2, 3, 4, 5 and 6 ml of a standard solution containing $6.5 \times 10^{-3} \text{ mol l}^{-1}$ of the same analyte. 5.00 ml of color-developing reagent is added to each flask and enough distilled water is added to bring each flask to exactly 25.0 ml. The absorbances of the five solutions were 0.236, 0.339, 0.425, 0.548, 0.630 and 0.745, respectively. Use a spreadsheet to obtain a graph of the data and extrapolate the data to obtain the information needed to determine the initial concentration of the analyte. From the data, estimate the uncertainty of the result.
27. It is observed that the infrared spectrum obtained with a continuous wave infrared spectrometer has increasing resolution as the scan speed is decreased. Explain this observation.
28. Explain how changing the solvent polarity can be used in certain circumstances to determine the nature of the transition causing an observed absorbance.
29. Explain what types of quantized absorbances are expected in the ultraviolet, visible and infrared spectral regions.
30. Give plausible reasons why Fourier transform techniques are used for the infrared region but not the visible and ultraviolet spectral regions.
31. If the C=O stretch is found at 1856 cm^{-1} what wavenumber would we expect the same stretch to occur at if the oxygen atom was the ¹⁸O isotope?
32. The nitrile stretch frequency is 2354 cm^{-1} . What is the wavenumber of the same stretch if the nitrogen isotope has a mass of 16 rather than 14?
33. The optical path length of an infrared cell can be determined using the method shown in the text. Determine the optical path lengths for the following sodium chloride cells.
- Fifteen interference fringes are observed between 11 and 6 μm.
 - Twenty-two interference fringes are observed between 2500 cm^{-1} and 1000 cm^{-1} .

General principles of spectroscopy and spectroscopic analysis

- c. Twenty-six interference fringes are observed between 14.6 and 8.2 μm .
 - d. Sixteen interference fringes are observed between 2085 and 855 cm^{-1} .
34. Provide plausible reasons why interference fringes are not observed for the typical 1.0 cm quartz cuvette used in the ultraviolet region.