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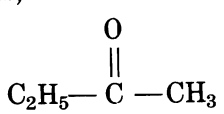
Physical Properties of Drug Molecules*

Electromagnetic Radiation
Atomic Spectra
Molecular Spectra
Ultraviolet and Visible Spectrophotometry
Fluorescence and Phosphorescence
Dielectric Constant and Induced Polarization
Permanent Dipole Moment of Polar Molecules
Infrared Spectroscopy
Electron Spin and Nuclear Magnetic Resonance Spectroscopy
Refractive Index and Molar Refraction
Optical Rotation
Circular Dichroism

A study of the physical properties of drug molecules is a prerequisite for product formulation and often leads to a better understanding of the interrelationship between molecular structure and drug action. These properties may be thought of as either *additive* (derived from the sum of the properties of the individual atoms or functional groups within the molecule) or *constitutive* (dependent on the structural arrangement of the atoms within the molecule). Mass is an additive property, whereas optical rotation may be thought of as a constitutive property.

Many physical properties are constitutive and yet have some measure of additivity. Molar refraction of a compound, for example, is the sum of the refraction (p. 95) of the atoms and groups making up the compound. The arrangements of atoms in each group are different, however, and so the refractive index of two molecules will be different; that is, the individual groups in two different molecules contribute different amounts to the overall refraction of the molecules.

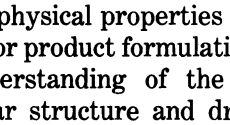
A sample calculation will clarify the principle of additivity and constitutivity. The molar refractions of the two compounds,



and

$$CH_3—CH{=}CH—CH_2—OH,$$

having exactly the same number of carbon, hydrogen, and oxygen atoms, are calculated using Table 4–1.



8H	$8 \times 1.100 =$	8.800
3C (single)	$3 \times 2.418 =$	7.254
1C (double)	$1 \times 1.733 =$	1.733
1O (C=O)	$1 \times 2.211 =$	2.211
		19.998 = 20.0

$CH_3—CH{=}CH—CH_2—OH$

8H	$8 \times 1.100 =$	8.800
2C (single)	$2 \times 2.418 =$	4.836
2C (double)	$2 \times 1.733 =$	3.466
1O (OH)	$1 \times 1.525 =$	1.525
		18.627 = 18.7

Thus, although these two compounds have the same number of atoms of a definite kind, their molar

TABLE 4–1. ***Atomic and Group Contributions to Molar Refraction****

C—(single)	2.418
—C=(double)	1.733
—C≡(triple)	2.398
Phenyl (C_6H_5)	25.463
H	1.100
C (C=O)	2.211
O (O—H)	1.525
O (ether, ester, C—O)	1.643
Cl	5.967
Br	8.865
I	13.900

*These values are reported for the D-line of sodium as the light source. (From *Lange's Handbook*, 12th Edition, J. Dean, Ed., McGraw-Hill, New York, 1979, p. 10–94. See also Bower et al., in *Physical Methods of Organic Chemistry*, 3rd Edition, A. Weissberger, Ed., Vol. 1, Part II, Chapter 28, Wiley-Interscience, New York, 1960.)

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refractions are not the same. The molar refractions of the atoms are additive, but the carbon and oxygen atoms are constitutive in refraction. A single-bonded carbon does not add equally as a double-bonded carbon, and a carbonyl oxygen (C=O) is not the same as a hydroxyl oxygen; therefore, the two compounds exhibit additive–constitutive properties and have different molar refractions.

Additive, constitutive, and additive–constitutive properties, the nature of the interaction between these properties, and the attributes of atoms or groups of atoms will be discussed in this chapter and in other sections of the book, including Chapter 10.

Physical properties encompass specific relations between the molecules and well-defined forms of energy or other external "yardsticks" of measurement. For example, the concept of weight uses the force of gravity as an external measure to compare the mass of objects, while that of optical rotation uses plane-polarized light to describe the optical rotation of molecules. Ideally, a physical property should be easily measured or calculated, and such measurements should be reproducible.

By carefully associating specific physical properties with the chemical nature of closely related molecules, conclusions can be drawn that (1) describe the spatial arrangement of drug molecules, (2) provide evidence for the relative chemical or physical behavior of a molecule, and (3) suggest methods for the qualitative and quantitative analysis of a particular pharmaceutical agent. The first and second of these associations often lead to implications about chemical nature and potential action that are necessary for the creation of new molecules with selective pharmacologic activity. The third provides the researcher with tools for drug design and manufacturing and offers the analyst a wide range of methods for assessing the quality of drug products.

This chapter describes some of the important physical properties of molecules that represent well-defined interactions with electromagnetic energy as found in spectroscopy. Quantities have been expressed in Standard International (SI) units (p. 2) in all practical cases.

ELECTROMAGNETIC RADIATION

Electromagnetic energy can be characterized as a continuous waveform of radiation, the nature of which depends on the size and shape of the wave. As with all forms of radiation, electromagnetic radiation can be described in terms of both a wave model and a field vibrating about a point in space. In either case, the radiation has a characteristic frequency, usually a large number. This frequency, ν, is the number of waves passing a fixed point in 1 second. The wavelength, λ, is the extent of a single wave of radiation, that is, the distance between two successive maxima of the wave, and is related to frequency by

$$\lambda\nu = c \qquad (4\text{–}1)$$

in which c is the speed of light, 3×10^8 m/sec. Wavenumber, $\bar{\nu}$, can be defined as

$$\bar{\nu} = \nu/c \qquad (4\text{–}2)$$

in which the wavenumber (in cm^{-1}) represents the number of wavelengths found in 1 cm of radiation in a vacuum.

The electromagnetic spectrum is classified according to its wavelength, or its corresponding wavenumber, as illustrated in Table 4–2. The wavelength becomes shorter as the corresponding radiant energy increases. According to the elementary quantum theory, the radiant energy absorbed by a chemical species has certain discrete values corresponding to the individual energy transitions that can occur in an atom or molecule. As we shall discuss, the wavelength of the

TABLE 4–2. ***Electromagnetic Spectrum***

Region of the Spectrum	Wavelength	Wavenumber	Frequency	Source
	λ (m)*	$\bar{\nu}$ (cm^{-1})†	Hz‡	
	10^{-13}	10^{11}	3×10^{21}	
Gamma rays	3×10^{-10}	3.3×10^{7}	1×10^{18}	Nuclear transformations
X-rays	3×10^{-8} (= 30 nm)	3.3×10^{5}	1×10^{16}	Inner-shell electron transitions
Vacuum ultraviolet	2×10^{-7} (= 200 nm)	5×10^{4}	1.5×10^{15}	Ionization and valence electron transitions
Near ultraviolet	4×10^{-7}	2.5×10^{4}	7.5×10^{14}	
Visible	7.5×10^{-7} (= 750 nm)	1.3×10^{4}	4×10^{14}	Valence electron transitions
Near infrared (overtone region)	2.5×10^{-6}	4×10^{3}	1.2×10^{14}	
Infrared (fundamental region)	2.5×10^{-5}	4×10^{2}	1.2×10^{13}	Molecular vibrations
Far infrared	10^{-3}	10^{1}	3×10^{11}	Molecular vibrations or rotations
Microwaves	10^{-1}	10^{-1}	3×10^{9}	Electron spin transitions
Radiowaves	10^{3}	10^{-5}	3×10^{5}	Nuclear spin transitions

*m = meter.
†$\bar{\nu}$ = wavenumber.
‡Hz = hertz = waves/sec.

quantized electromagnetic energy determines the molecular or atomic information we receive from the resulting spectra.

ATOMIC SPECTRA

Spectra can be derived from the interactions between electromagnetic radiation of certain wavelengths and the electrons in orbitals of an atom. These interactions produce emission spectra if large amounts of energy, which can be from a flame or some other energy source, excite electrons in the atoms. In losing their excitation energy, some of these atoms emit discrete radiation while returning to a less energetic state. The interactions produce absorption spectra if radiation of a particular wavelength is passed through a sample and the decrease in the intensity of the radiation due to electronic excitation is measured. The absorption or emission of quantized energy corresponds to an electronic orbital transition in an atom or, as we shall presently discuss, a molecule. According to the Bohr model, the energy of an electron in a definite orbital is

$$E = -\frac{2\pi^2 Z^2 m e^4}{n^2 h^2} \tag{4-3}$$

in which Z is the atomic number or effective nuclear charge of the atom, m is the mass of the electron (9.1×10^{-31} kg), n is the principal quantum number of the orbit, e is the charge on the electron (1.602×10^{-19} coulomb or 1.519×10^{-14} $m^{3/2}$ $kg^{1/2}$ s^{-1}), and h is Planck's constant, 6.626×10^{-34} joule second. If we represent E as the energy of a photon of electromagnetic radiation and $c\bar{\nu} = \nu$, the frequency of the radiation

$$E = hc\bar{\nu} \tag{4-4}$$

as suggested by Planck in 1900 as the basis of the quantum theory of atomic structure. Substituting equation (4–4) in equation (4–3), we obtain

$$\bar{\nu} = -\frac{2\pi^2 Z^2 m e^4}{n^2 h^3 c}$$

$$= -\frac{2 \times (3.14)^2 \times (1)^2 \times (9.1 \times 10^{-31} \times (1.519 \times 10^{-14})^4}{n^2 \times (6.626 \times 10^{-34})^3 \times (3 \times 10^8)}$$

$$= \frac{-1.097 \times 10^7}{n^2}\ \text{m}^{-1} = \frac{-109{,}700}{n^2}\ \text{cm}^{-1} \tag{4-5}$$

If $n = 1$, corresponding to the ground state of the hydrogen atom, the numerator, $-109{,}700$ cm^{-1}, represents the energy difference in wavenumber between the quantized energy in the ground state and that in the next electronic orbital (in which $n = 2$). This frequency $109{,}700$ cm^{-1}, is known as the *Rydberg constant*, R_∞, and it is related to the quantized energy of the atom by the simple relation

$$\bar{\nu} = R_\infty \left(\frac{1}{n_1^2} - \frac{1}{n_2^2}\right) \tag{4-6}$$

in which n_1 and n_2 are the principal quantum numbers for the orbital states involved in an electronic transition of the atom.

In general, the difference between electron energy levels, $E_2 - E_1$, having respective quantum numbers, n_2 and n_1, is given by the expression

$$E_2 - E_1 = \frac{2\pi^2 Z^2 m e^4}{h^2}\left(\frac{1}{n_1^2} - \frac{1}{n_2^2}\right) \tag{4-7}$$

Example 4–1. (*a*) What is the energy of a quantum of radiation absorbed to promote the electron in the hydrogen atom from its ground state ($n_1 = 1$) to the second orbital, $n_2 = 2$?

$$E_2 - E_1 = \frac{2 \times (3.14)^2 \times (1)^2 \times (9.1 \times 10^{-31}) \times (1.519 \times 10^{-14})^4}{(6.626 \times 10^{-34})^2} \times \left(\frac{1}{(1)^2} - \frac{1}{(2)^2}\right)$$

$$= 1.63 \times 10^{-18}\ \text{joule}$$

Note that the joule = kg × m^2 × s^{-2} (SI units; see Table 1–3).

(*b*) If we substitute equation (4–6) into equation (4–7), we can obtain

$$E_2 - E_1 = \frac{2\pi^2 Z^2 m e^4}{h^2}\left(\frac{\bar{\nu}}{R_\infty}\right) \tag{4-8}$$

Using this equation, what is the wavelength of the spectral line when an electron passes from the $n = 1$ orbital to the $n = 2$ orbital, where $E_2 - E_1 = 1.63 \times 10^{-18}$ joule?

$$E_2 - E_1 = 1.63 \times 10^{-18} = \frac{2 \times (3.14)^2 \times (1)^2 \times (9.1 \times 10^{-31}) \times (1.519 \times 10^{-14})^4}{(6.626 \times 10^{-34})^2} \times \left(\frac{\bar{\nu}}{109{,}700}\right)$$

$$\bar{\nu} = \frac{(1.63 \times 10^{-18}) \times (1.097 \times 10^5) \times (6.626 \times 10^{-34})^2}{2 \times (3.14)^2 \times (1)^2 \times (9.1 \times 10^{-31}) \times (1.519 \times 10^{-14})^4}$$

$$= 82{,}091\ \text{cm}^{-1}$$

and therefore, from equations (4–1) and (4–2),

$$\lambda = \frac{1}{\bar{\nu}} = \frac{1}{82{,}091} = 1.21 \times 10^{-5}\ \text{cm} = 121\ \text{nm}$$

This is the first line of the Lyman ultraviolet series of the atomic spectra for hydrogen. Note that, from equation (4–3), the electron of the hydrogen atom in the ground state, $n = 1$, has a lower energy (-2.18×10^{-18} joules) than in the next highest electron state, $n = 2(-0.55 \times 10^{-18}$ joules).

When the electron acquires sufficient energy to leave the atom, it is regarded as infinitely distant from the nucleus, and the nucleus is considered no longer to affect the electron. The energy required for this process, which results in the ionization of the nucleus, is known as the *ionization potential*. If we consider this process as occurring when $n = \infty$, then the ionization potential from the ground state ($n = 1$) to $n = \infty$ is

$$E_2 - E_1 = \frac{2\pi^2 Z^2 m e^4}{h^2}\left(\frac{1}{1} - \frac{1}{\infty}\right)$$

TABLE 4–3. *Spectral Wavelengths Associated with Electronic Transitions Used in the Detection of Particular Elements*

Element	Wavelength (nm)
As	193.7
Ca	422.7
Na	589.0
Cu	324.8
Hg	253.7
Li	670.8
Pb	405.8
Zn	213.9
K	766.5

since $1/\infty \cong 0$, then

$$E_2 - E_1 = \frac{2\pi^2 Z^2 me^4}{h^2}$$

This is equivalent to $-E$ for $n = 1$, according to equation (4–3). Thus, the ionization potential exactly equals the negative energy of the electron in the ground state. This is correct according to the definition of ground state energy, which may be thought of as the difference in energy between the $n = 1$ orbital and a distance infinitely far away.

Each element of the periodic table has a characteristic atomic spectrum that can be associated with its electronic transition states. Atomic spectra can be used to identify and quantify specific elements. Some of the more sensitive spectral wavelengths associated with particular atoms are given in Table 4–3.

Atomic spectroscopy has pharmaceutical applications in analyzing for metal ions from drug products and in the quality control of parenteral electrolyte solutions. For example, blood levels of lithium, used to treat bipolar disorder (manic-depression), can be analyzed by atomic spectroscopy to determine overdosing of lithium salts.

MOLECULAR SPECTRA

The absorption of electromagnetic radiation by molecules includes vibrational and rotational transitions, as well as the electronic transitions just described for atoms. These additional transitions make the spectra of molecules more complex than those of atoms. The additional transitions result from energy interactions that produce either vibrations within the molecule associated with the stretching or bending of bonds between the atoms, or the rotation of the molecule about its center of gravity. In the case of vibration, the interatomic bonds may be thought of as springs between atoms (see Fig. 4–10), which can vibrate in various stretching or bending configurations depending on their energy levels, while in rotation, the motion is similar to that of a top spinning according to its energy level. In addition, the molecule may have some kinetic energy associated with its translational (straight-line) motion in a particular direction.

The energy levels associated with these various transitions differ greatly from one another. The energy associated with movement of an electron from one orbital to another (electronic transitions) is typically about 10^{-18} joule, while the energy involved in vibrational changes is about 10^{-19} to 10^{-20} joule depending on the atoms involved, and the energy for rotational change is about 10^{-21} joule. The energy associated with translational change is even smaller, about 10^{-35} joule. The precise energies associated with these individual transitions depend on the atoms and bonds that compose the molecule. Each electronic energy state of a molecule normally has several possible vibrational states, and each of these has several rotational states, as shown in Figure 4–1. The translational states are so numerous and the energy levels between translational states so small that they are normally considered as a continuous form of energy and are not treated as quantized. The total energy of a molecule is the sum of its electronic, vibrational, rotational, and translational energies.

When a molecule absorbs electromagnetic radiation, it can undergo certain transitions that depend on the quantized amount of energy absorbed. In Figure 4–1, the absorption of radiation (line *a*) equivalent to the energy transition ΔE_1 results in the electronic transition from the lowest level of the ground state (S_0) to an excited electronic state (S_1) with a somewhat different rotational energy. Electronic transitions of molecules involve energies corresponding to ultraviolet or visible radiation, whereas purely vibrational transitions (line *b*) involve near-infrared radiation, and rotational transitions (line *c*) are associated with low-energy radiation over the entire infrared wavelength region. The relatively large energy associated with an electronic tran-

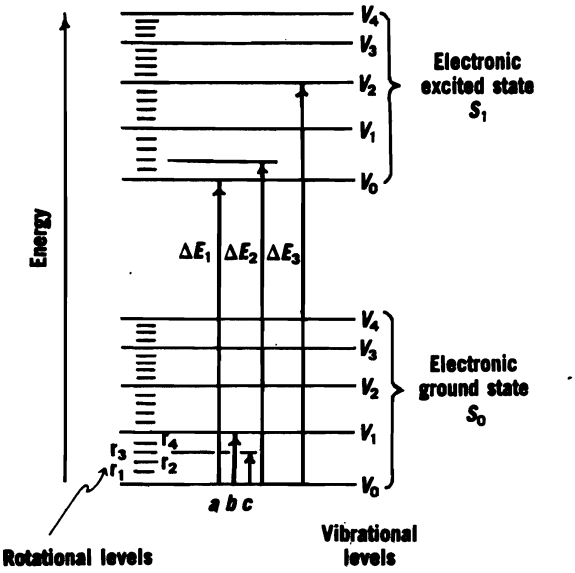


Fig. 4–1. Molecular energy levels and (*a*) electronic, (*b*) vibrational, and (*c*) rotational transitions. Vibrational and rotational energy levels have been exaggerated compared with electronic energy levels in this figure. (Modified from H. H. Bauer, G. D. Christian, and J. E. Reilly, *Instrumental Analysis*, Allyn and Bacon, Boston, 1978.)

sition usually leads to a variety of different vibrational and rotational changes. Slight differences in the vibrational and rotational nature of the excited electronic state complicate the spectrum. These differences lead to broad bands, characteristic of the ultraviolet and visible regions, rather than the sharp, narrow lines characteristic of individual vibrational or rotational changes in the infrared region.

The energy absorbed by a molecule may be found only at a few discrete wavelengths in the ultraviolet, visible, and infrared regions, or the absorptions may be numerous and at longer wavelengths than originally expected. The latter case, involving longer wavelength radiation, is normally found for molecules that have resonance structures, such as benzene, in which the bonds are elongated by the resonance and have lower energy transitions than would be expected otherwise. Electromagnetic energy may also be absorbed by a molecule from the microwave and radiowave regions (see Table 4–2). Low-energy transitions involve the spin of electrons in the microwave region and the spin of nuclei in the radiowave region. The study of these transitions constitutes the fields of electron spin resonance (ESR) and nuclear magnetic resonance (NMR) spectroscopy. These various forms of molecular spectroscopy are discussed in the following sections.

ULTRAVIOLET AND VISIBLE SPECTROPHOTOMETRY

When organic molecules in solution, or as a liquid, are exposed to light in the visible and ultraviolet regions of the spectrum (see Table 4–2), they absorb light of particular wavelengths depending on the type of electronic transition that is associated with the absorption. Such electronic transitions depend on the electron bonding within the molecule.[1] For example, paraffins that contain σ-type bonds can undergo only $\sigma \rightarrow \sigma^*$ electronic transitions from their lowest energy, or ground, state. The * indicates the excited state of the electron after absorption of a quantized amount of energy. These σ electronic transitions occur exclusively from the relatively high energy available from short-wavelength radiation in the vacuum ultraviolet region (wavelengths typically between 100 and 150 nm). If a carbonyl group is present in a molecule, however, the oxygen atom of this functional group possesses a pair of nonbonding (n) electrons that can undergo $n \rightarrow \pi^*$ or $n \rightarrow \sigma^*$ electronic orbital transitions. These transitions require a lower energy than do $\sigma \rightarrow \sigma^*$ transitions and therefore occur from the absorption of longer wavelengths of radiation. For acetone, these $n \rightarrow \pi^*$ or $n \rightarrow \sigma^*$ transitions occur at 280 and 190 nm, respectively. For aldehydes and ketones, the region of the ultraviolet spectrum between 270 and 290 nm is associated with their carbonyl $n \rightarrow \pi^*$ electronic transitions, and this fact can be used for identification. Thus, the types of electronic orbitals present in the ground state of the molecule dictate the region of the spectrum in which absorption can take place. Those parts of a molecule that can be directly associated with an absorption of ultraviolet or visible light, such as the carbonyl group, are called *chromophores*.

The magnitude of the absorption of light at a fixed wavelength can be calculated by using *Beer's law*. This equation relates the amount of light absorbed (A) to the concentration of absorbing substance (c in g/liter) and the length of the path of radiation passing through the sample (b in cm) as

$$A = abc \qquad (4\text{–}9)$$

in which a is a constant known as *absorptivity* for a particular absorbing species (in units of liter g^{-1} cm^{-1}). If the units of c are moles/liter, then the constant is termed ϵ, the *molar absorptivity* (in units of liter $mole^{-1}$ cm^{-1}). The absorptivity depends not only on the molecule whose absorbance is being determined, but also on the type of solvent being used, as well as on the temperature and the wavelength of light used for the analysis. The quantity A is termed the *absorbance* and is related to the *transmittance* of light (T) by

$$A = \log \frac{I_0}{I} = -\log T \qquad (4\text{–}10)$$

in which I_0 is the intensity of the incident light beam and I is in the intensity of light after it emerges from the sample.

Example 4–2. (*a*) A solution of $c = 2 \times 10^{-5}$ moles/liter of chlordiazepoxide dissolved in 0.1 N sodium hydroxide was placed in a fused silica cell having an optical path of 1 cm. The absorbance A was found to be 0.648 at a wavelength of 260 nm. What is the molar absorptivity? (*b*) If a solution of chlordiazepoxide had an absorbance of 0.298 in a 1-cm cell at 260 nm, what is its concentration?

(*a*)

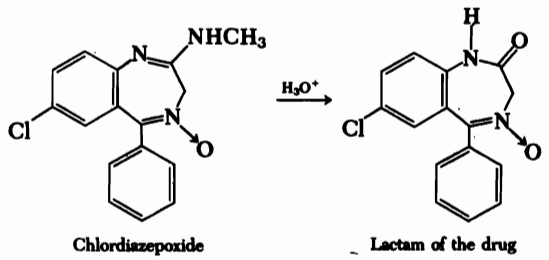
$$\epsilon = \frac{A}{bc} = \frac{6.48 \times 10^{-1}}{1 \times (2 \times 10^{-5})}$$

$$= 3.24 \times 10^4 \text{ liter mole}^{-1} \text{ cm}^{-1}$$

(*b*)

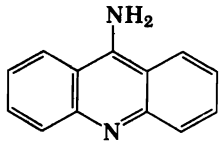
$$c = \frac{A}{b\epsilon} = \frac{2.98 \times 10^{-1}}{1 \times (3.24 \times 10^4)} = 9.20 \times 10^{-6} \text{ moles/liter}$$

The large value of ϵ indicates that chlordiazepoxide absorbs strongly at this wavelength. This molar absorptivity is characteristic of the drug dissolved in 0.1 N NaOH at this wavelength, and is not the same in 0.1 N HCl. A lactam is formed from the drug under an acid condition that has an absorbance maximum at 245 rather than 260 nm, and a correspondingly different ϵ value.



Chlordiazepoxide — Lactam of the drug

Example 4–3. Aminacrine is an antiinfective agent with the following molecular structure:



Aminacrine

Its highly conjugated acridine ring produces a complex ultraviolet spectrum in dilute sulfuric acid that includes absorption maxima at 260, 313, 326, 381, 400, and 422 nm. The molar absorptivities of the absorbances at 260 and 313 nm are 63,900 and 1130 liter $mole^{-1}$ cm^{-1} respectively. What is the minimum amount of aminacrine that can be detected at each of these two wavelengths?

If we assume an absorbance level of $A = 0.002$ corresponding to a minimum detectable concentration of the drug, then, at 260 nm,

$$c = \frac{A}{b \times \epsilon} = \frac{0.002}{1 \times 63900} = 3.13 \times 10^{-8} \text{ mole/liter}$$

while at 381 nm

$$c = \frac{0.002}{1 \times 1130} = 1.77 \times 10^{-6} \text{ mole/liter}$$

Nearly 100 times greater sensitivity in detecting the drug is possible with the 260-nm absorption band. The absorbance level of 0.002 was chosen by judging this value to be a significant signal above instrumental noise (i.e., interference generated by the spectrophotometer in the absence of the drug). For a particular analysis, this minimum absorbance level for detection of a compound will depend on both the instrumental conditions and the sample state, for example, the solvent chosen for dissolution of the sample.

Characteristic ϵ values for selected drugs together with their wavelength of maximum absorbance (at which the ϵ values were calculated) are given in Table 4–4. Sometimes the absorbance found in the literature is expressed as $E^{1\%}_{1cm}$. This is the absorbance through a 1-cm path length of a solution containing 1 g of solute per 100 mL of solution. The $E^{1\%}_{1cm}$ term is being discontinued and replaced by the ϵ value for molar absorptivity.

A molecule may have more than one characteristic absorption wavelength band, and the complete spectrum in the ultraviolet and visible wavelength regions can provide information for the positive identification of a compound. A recording spectrophotometer is usually used to obtain such a spectrum. A schematic diagram of a typical double-beam spectrophotometer is shown in Figure 4–2. The beam of light from the source, usually a deuterium lamp, passes through a prism or grating monochromator to sort the light according to wavelength and spread the wavelengths over a wide range. This permits a particular wavelength region to be easily selected by passing it through the appropriate slits. The selected light is then split into two separate beams by a rotating mirror, or "chopper"—one beam for the reference, which is typically the blank solvent used to dissolve the sample, and the other for the sample cell. After each beam passes through its respective cell, it is reflected onto a second mirror in another chopper assembly, which alternatively selects either the reference or the combined beams to focus onto the photomultiplier detector. The rapidly changing current signal from the detector is proportional to the intensity of the particular beam, and this is fed into an amplifier, which electronically separates the signals of the reference beam from those of the sample beam. The final difference in beam signals is automatically recorded on a strip-chart recorder. The recording obtained is a plot of the intensity, usually as absorbance, against the wavelength, as shown in Figure 4–3. Standard solutions of known but varying concentration are used in quantitative analysis as the samples in the spectrophotometer. The absorbance of each solution is determined at one selected wavelength (typically an absorption maximum). The absorbance is plotted against the concentration, as shown in Figure 4–4, to obtain what is known as a Beer's law plot. The concentration of an "unknown" sample can then be determined by interpolation from such a graph.

Spectrophotometry is a useful tool for studying chemical equilibria or determining the rate of chemical reactions. The chemical species participating in the equilibria must have different absorption spectra, and one simply observes the variation in absorption at a representative wavelength for each species while the pH or other equilibrium variable is changed. If one determines the concentrations of the species from Beer's law and knows the pH of the solution, one can

TABLE 4–4. *Molar Absorptivity of Some Drugs Measured in a 1-cm Cell*

Drug	ϵ (liter $mole^{-1}$ cm^{-1})	Wavelength (nm)	Solvent
Codeine phosphate	1,570	284	Water
Colchicine	29,200	243	Ethanol
(+)-3-Hydroxy-*N*-methylmorphinan (Dextrorphan)	2,360	279	0.1 *N* Sulfuric acid
Reserpine	14,500	267	Chloroform
Riboflavine	35,500	222	Water
Tetracycline hydrochloride	16,200	380	Water
Tolazoline	24	257	Ethanol
Prednisolone	17,500	263	Ethanol

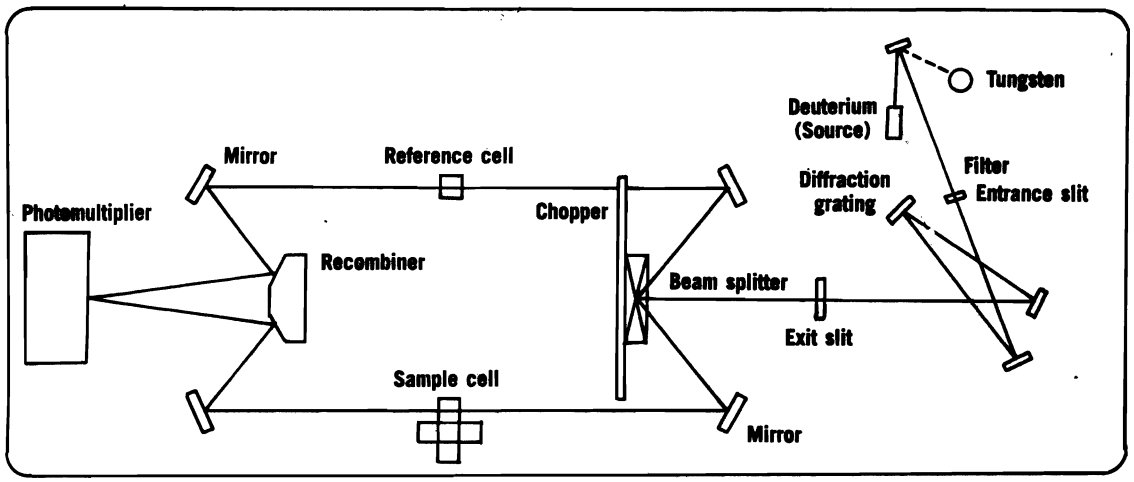


Fig. 4–2. Schematic diagram of a double-beam spectrophotometer, Bausch and Lomb Spectronic 2000. (From J. P. Malone, L. E. DeLong, and J. C. Defendorf, Am. Lab. 12, 78, 1980. Copyright 1980 by International Scientific Communications, Inc.)

calculate an approximate pK_a for a drug. For example, if the drug is a free acid (HA) in equilibrium with its base (A^-), then

$$pK_a = pH + \log [HA]/[A^-] \qquad (4-11)$$

When $[HA] = [A^-]$, as determined by their respective absorbances in the spectrophotometric determination, $pK_a \cong pH$.

Example 4–4. Phenobarbital shows a maximum absorption of 240 nm as the monosodium salt (A^-), while the free acid (HA) shows no absorption maxima in the wavelength region from 230 to 290 nm. If the free acid in water is slowly titrated with known volumes of dilute NaOH, measuring the pH of the solution and the absorbance at 240 nm after each titration, one reaches a maximum absorbance value at pH 10 after the addition of 10 mL of titrant. How can pK_a be determined from this titration?

By plotting the absorbance against the pH over the titration range to pH = 10, one may obtain the midpoint in absorbance, where half the free acid has been titrated, and $[HA] = [A^-]$ (Fig. 4–5). The pH corresponding to this absorbance midpoint is approximately equal to the pK_a, namely, pK_{a_1} for the first ionization stage of phenobarbital. This midpoint occurs at a pH of 7.3; therefore, the $pK_a \cong 7.3$. For more accurate pK_a determinations, refer to the discussion beginning on page 204.

Reaction rates can be measured easily if a particular reaction species has an absorption spectrum that is noticeably different from the spectra of other reactants or products. One can follow the rate of appearance or disappearance of the selected species by recording its absorbance at specific times during the reaction process. If no other reaction species absorbs at the

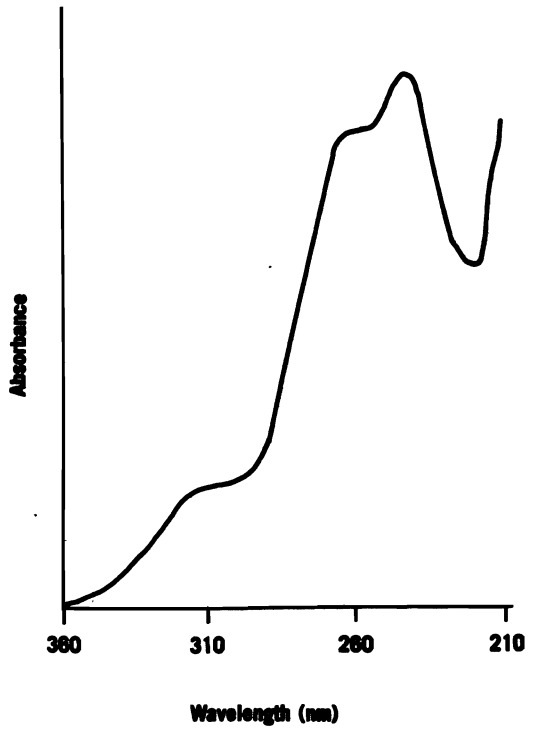


Fig. 4–3. The absorbance of light by a solution of chlordiazepoxide as a function of the wavelength in nanometers.

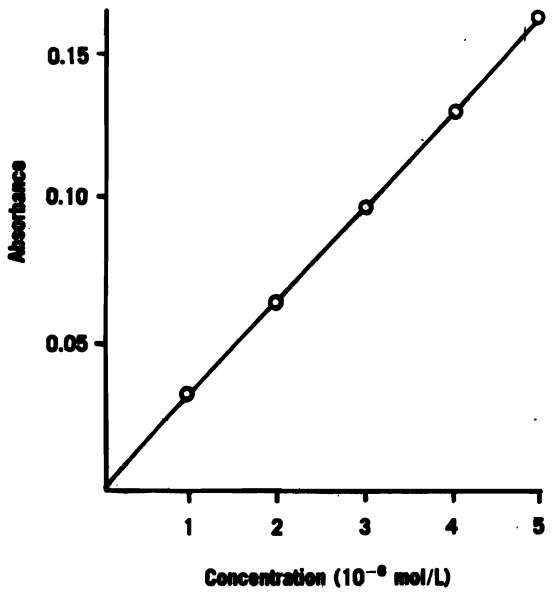


Fig. 4–4. A Beer's law plot of absorbance against the concentration of chlordiazepoxide.

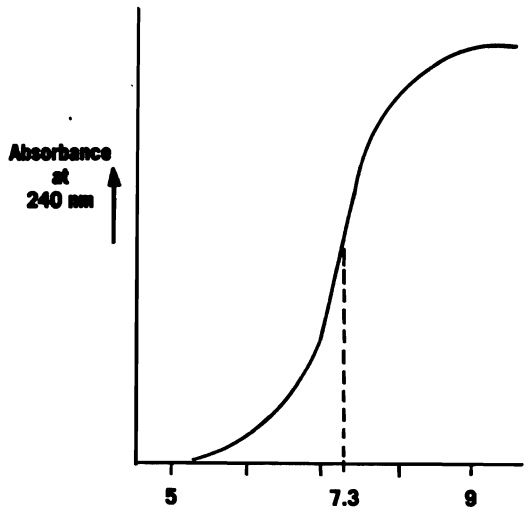


Fig. 4–5. Spectrophotometric titration curve for phenobarbital. The absorbance of the monosodium salt at 240 nm is plotted against the pH of the solution.

particular wavelength chosen for this determination, the reaction rate will simply be proportional to the rate of change of absorbance with reaction time.

An example of the use of spectrophotometry for the determination of reaction rates in pharmaceutics is given in the work of Jivani and Stella,[2] where the disappearance of *para*-aminosalicylic acid from solution was used to determine its rate of decarboxylation.

Spectrophotometry can be used to study enzyme reactions and to evaluate the effects of drugs on enzymes. For example, the analysis of clavulanic acid can be accomplished by measuring the ultraviolet absorption of penicillin G at 240 nm, as described by Gutman et al.[3] Clavulanic acid inhibits the activity of β-lactamase enzymes, which are capable of degrading penicillin G to penicilloic acid: where R is $C_6H_5CH_2$—. The method first requires that the rate of absorbance change at 240 nm be measured with a solution containing penicillin G and a β-lactamase enzyme. Duplicate experiments are then performed with increasing standard concentrations of clavulanic acid. These show a decrease in absorbance change, equivalent to the enzyme inhibition from the drug, as the concentration of the drug increases. The concentration of an unknown amount of clavulanic acid is measured by comparing its rate of enzyme inhibition with that of the standards.

Although these applications are often helpful in pharmaceutical calculations, the major use of spectrophotometry today is in the field of quantitative analysis, in which the absorbance of chromophors is determined. Various applications of spectrophotometry are discussed by Schulman and Vogt.[4]

FLUORESCENCE AND PHOSPHORESCENCE

A molecule that initially absorbs ultraviolet light to reach an excited state and then emits ultraviolet or visible light in returning to the ground state is said to undergo *photoluminescence*. This emission of light may be described as either fluorescence or phosphorescence, depending on the mechanism by which the electron finally returns to the ground state.

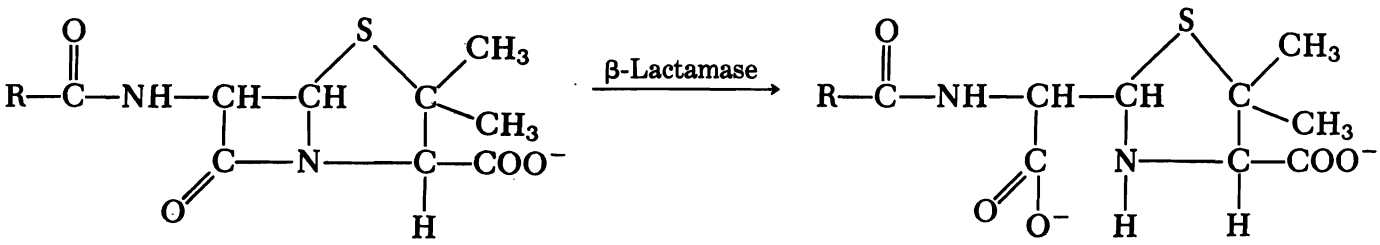
The overall mechanism can be described as

$$\underset{\text{(Ground state)}}{S_0} + UV \rightarrow \underset{\text{(Singlet)}}{S^*} \rightarrow S_0 + \text{Fluorescence}$$

$$S^* \rightarrow \underset{\text{(Triplet)}}{T^*} \rightarrow S_0 + \text{Phosphorescence}$$

in which, in addition to the singlet excited state (S*) discussed previously, we have a triplet (T*) state, associated with the production of phosphorescence. The triplet state of the excited electron occurs when the excited singlet electron changes spin so that it is now of the same spin as its originally paired electron in the ground-state orbital. The triplet state usually cannot be achieved by excitation from the ground state, this being termed a "forbidden" transition according to the quantum theory. It is usually reached through the process of *intersystem crossing*, in which the excited singlet (S*) converts spontaneously to a triplet by a change in electron spin, usually with some energy loss. These changes, together with the energies involved, are represented schematically in Figure 4–6.

The triplet state (T*) is usually considered more stable (i.e., having a longer lifetime) than the excited singlet state (S*). The length of time during which light will be emitted after the molecule has become excited depends on the lifetime of the electronic transition. Therefore, we can expect phosphorescence to occur for a longer period after excitation than fluorescence. Ordinarily, fluorescence occurs between 10^{-6} to 10^{-9} second after excitation. Because of this short lifetime, fluorescence is usually measured while the molecule is being excited. A typical filter fluorometer is shown in Figure 4–7. Fluorescence intensity is measured in this



Penicillin G R = $C_6H_5CH_2$– Penicilloic acid

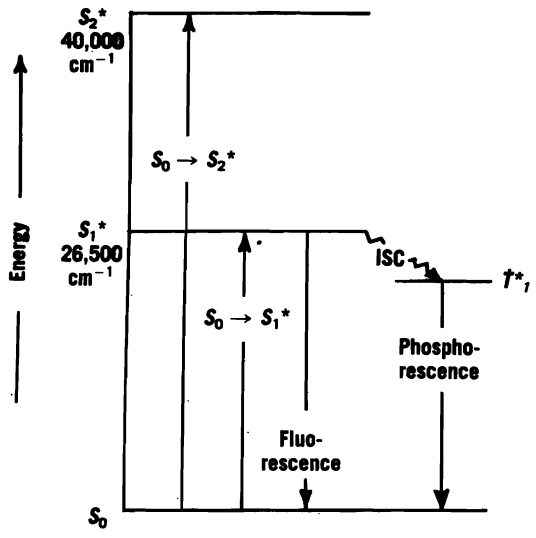


Fig. 4–6. Schematic energy-level diagram for a molecule that fluoresces or phosphoresces. ISC stands for intersystem crossing; S_0, S*, and T are described in the text.

system by placing the photomultiplier detector at right angles to the light beam that is producing the excitation. The signal intensity is recorded as relative fluorescence against a standard solution. Since photoluminescence can occur in any direction from the sample, the detector will sense a part of the total emission at a characteristic wavelength and will not be capable of detecting radiation from the light beam used for excitation. Fluorescence normally has a longer wavelength than the radiation used for the excitation, principally because of internal energy losses within the excited molecule before the fluorescent emission occurs. Phosphorescence typically has still longer wavelengths than fluorescence, owing to the energy difference that occurs in intersystem crossing as well as the loss of energy due to internal conversion over a longer lifetime.

Photoluminescence occurs only in those molecules that can undergo the specified photon emissions after excitation with consequent return to the ground state. Many molecules do not possess any photoluminescence, although they can absorb ultraviolet light. In these cases, the return to the ground state from the singlet excited state occurs through the internal conversion of excitation energy into vibrational energy or through collisions with other molecules resulting in energy transfer. These energy conversions result finally in the production of heat rather than photoluminescence. Most often, a molecule that fluoresces or phosphoresces contains at least one aromatic ring. Examples of drugs that fluoresce are given in Table 4–5 along with their characteristic excitation and emission wavelengths, which can be used for qualitative or quantitative analyses. Photoluminescent analysis is normally more sensitive and selective than absorption spectrophotometry.

A thorough review of the applications of photoluminescence to the analysis of pharmaceuticals is given by Schulman and Sturgeon.[5]

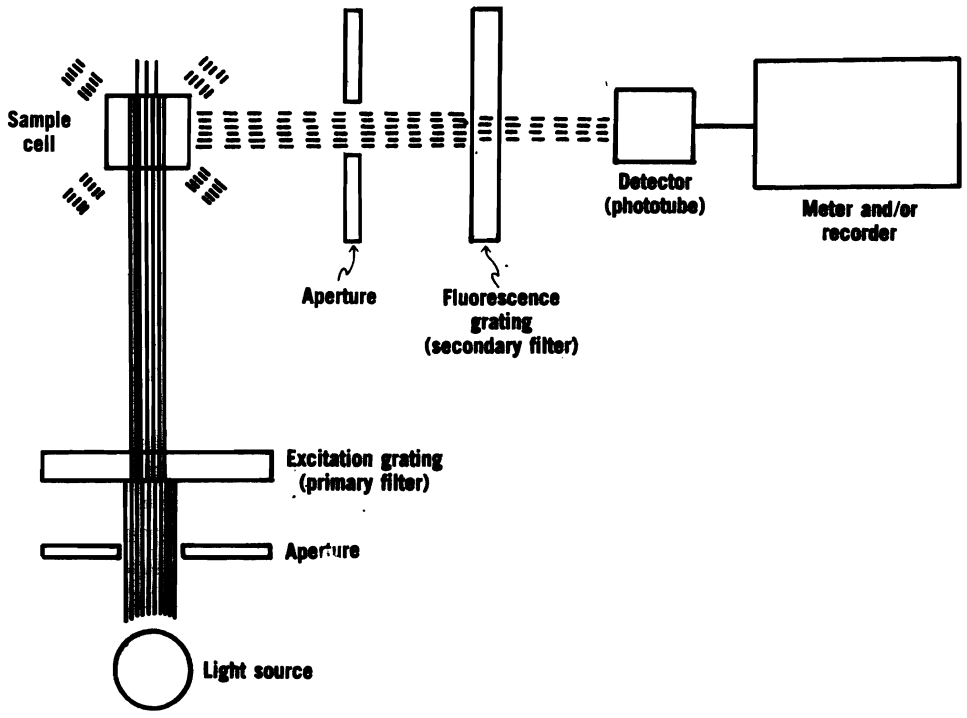


Fig. 4–7. Schematic diagram of a filter fluorometer. (From G. H. Schenk, *Absorption of Light and Ultraviolet Radiation*, Allyn and Bacon,

TABLE 4–5. *Fluorescence of Some Drugs*

Drug	Excitation Wavelength (nm)	Emission Wavelength (nm)	Solvent
Phenobarbital	255	410–420	0.1 *N* NaOH
Hydroflumethiazide	333	393	1 *N* HCl
Quinine	350	~450	0.1 *N* H_2SO_4
Thiamine	365	~440	Isobutanol, after oxidation with ferricyanide
Aspirin	280	335	1% Acetic acid in chloroform
Tetracycline hydrochloride	330	450	0.05 *N* NaOH(aq)
Fluorescein	493.5	514	pH-2 (aq.)
Riboflavine	455	520	Ethanol
Hydralazine	320	353	Conc. H_2SO_4

DIELECTRIC CONSTANT AND INDUCED POLARIZATION

A molecule can maintain a separation of electric charge either through induction by an external electric field or by a permanent charge separation within a polar molecule. To fully understand the concepts of charge separation, it is necessary to understand the concept of the dielectric constant.

Consider two parallel conducting plates, such as the plates of an electric condenser, which are separated by some medium across a distance r, as shown in Figure 4–8, and apply a potential across the plates. Electricity will flow from the left plate to the right plate through the battery until the potential difference of the plates equals that of the battery supplying the initial potential difference. The *capacitance*, C (in farads), is equal to the quantity of electric charge, q (in coulombs), stored on the plates, divided by the potential difference, V (in volts), between the plates:

$$C = q/V \qquad (4-12)$$

The capacitance of the condenser in Figure 4–8 depends on the type of medium separating the plates as well as on the thickness r. When a vacuum fills the space between the plates, the capacitance is C_0. This value is used as a reference to compare capacitances when other substances fill the space. If water fills the space, the capacitance is increased, since the water molecule can orientate itself so that its negative end lies nearest the positive condenser plate and its positive end lies nearest the negative plate. This alignment provides additional movement of charge because of the increased ease with which electrons can flow between the plates. Thus, additional charge can be placed on the plates per unit of applied voltage.

The capacitance of the condenser filled with some material, C_x, divided by the reference standard C_0, is referred to as the *dielectric constant*, ϵ:

$$\epsilon = C_x/C_0 \qquad (4-13)$$

The dielectric constant ordinarily has no dimensions, since it is the ratio of two capacitances. Dielectric constants of some liquids are listed in Table 4–6. By

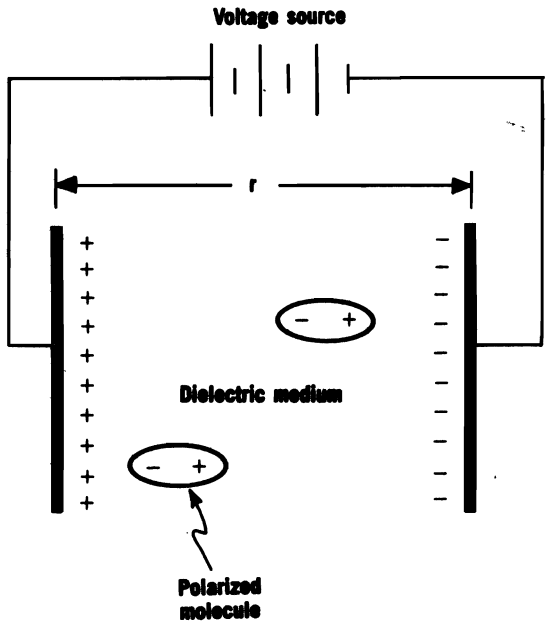


Fig. 4–8. Parallel plate condenser.

definition, the dielectric constant of a vacuum is unity. Dielectric constants can be determined by oscillometry, in which the frequency of a signal is kept constant by electrically changing the capacitance between the two parallel plates. The liquid whose dielectric constant is being measured is placed in a glass container between the two plates during the experiment. The dielectric constants of solvent mixtures can be related to drug solubility as described by Gorman and Hall[6] and ϵ for drug vehicles can be related to drug plasma concentration as reported by Pagay et al.[7]

If nonpolar molecules in a suitable solvent are placed between the plates of a charged capacitor, an *induced polarization* of the molecules can occur. This *induced dipole* occurs because of the separation of electric charge within the molecule when it is placed in the electric field between the plates. The electrons and nuclei are displaced from their original positions in this induction process. This temporary induced dipole moment is proportional to the field strength of the

TABLE 4–6. *Dielectric Constants of Some Liquids at 25° C*

Substance	Dielectric Constant, ϵ	
N-Methylformamide	182	
Hydrogen cyanide	114	
Formamide	110	
Water	78.5	
Glycerol	42.5	
Methanol	32.6	
Tetramethylurea	23.1	
Acetone	20.7	
n-Propanol	20.1	
Isopropanol	18.3	
Isopentanol	14.7	
1-Pentanol	13.9	
Benzyl alcohol	13.1	
Phenol	9.8	(60° C)
Ethyl acetate	6.02	
Chloroform	4.80	
Hydrochloric acid	4.60	
Diethyl ether	4.34	(20° C)
Acetonitrile	3.92	
Carbon disulfide	2.64	
Triethylamine	2.42	
Toulene	2.38	
Beeswax (solid)	2.8	
Benzene	2.27	
Carbon tetrachloride	2.23	
1,4-Dioxane	2.21	
Pentane	1.84	(20° C)
Furfural	41	(20° C)
Pyridine	12.3	
Methyl salicylate	9.41	(30° C)

capacitor and the *induced polarizability*, α_p, which is a characteristic property of the particular molecule. Polarizability is defined as the ease with which an ion or molecule can be polarized by any external force, whether it be an electric field, light energy, or another molecule. Large-size anions have large polarizabilities because of their loosely held outer electrons. Polarizabilities for molecules are found in Table 4–7. The units on α_p are $Å^3$ or 10^{-24} cm^3.

From electromagnetic theory, it is possible to obtain the relationship

$$\frac{\epsilon - 1}{\epsilon + 2} = \frac{4}{3}\pi n\alpha_p \qquad (4-14)$$

in which n is the number of molecules per unit volume. Equation (4–14) is known as the *Clausius–Mossotti equation*. Multiplying both sides by the molecular weight of the substance, M, and dividing both sides by the density, ρ, we obtain

TABLE 4–7. *Polarizabilities*

Molecule	$\alpha_p \times 10^{24}$ cm^3
H_2O	1.68
N_2	1.79
HCl	3.01
HBr	3.5
HI	5.6
HCN	5.9

$$\left(\frac{\epsilon - 1}{\epsilon + 2}\right)\frac{M}{\rho} = \frac{4}{3}\frac{\pi nM\alpha_p}{\rho} = \frac{4}{3}\pi N\alpha_p = P_i \qquad (4-15)$$

in which N is Avogadro's number, 6.023×10^{23} $mole^{-1}$, and P_i is known as the *induced molar polarization*. P_i represents the induced dipole moment per mole of nonpolar substance when the electric field strength of the condenser, V/m in volts per meter, is unity.

Example 4–5. Chloroform has a molecular weight of 119 g/mol and a density of 1.43 g/cm^3 at 25° C. What is the induced molar polarizability of chloroform?

$$P_i = \frac{(\epsilon - 1)}{(\epsilon + 2)} \times \frac{M}{\rho} = \frac{(4.8 - 1)}{(4.8 + 2)} \times \frac{119}{1.43} = 46.5 \text{ cm}^3\text{/mole}$$

The concept of induced dipole moments can be extended from the condenser model just discussed to the model of a nonpolar molecule in solution surrounded by ions. In this case, an anion would repel molecular electrons while a cation would attract them. This would cause an interaction of the molecule in relation to the ions in solution and produce an induced dipole. The distribution and ease of attraction or repulsion of electrons in the nonpolar molecule will affect the magnitude of this induced dipole, as would the applied external electric field strength.

PERMANENT DIPOLE MOMENT OF POLAR MOLECULES

In a polar molecule, the separation of positively and negatively charged regions can be permanent, and the molecule will possess a *permanent dipole moment*, μ. This is a nonionic phenomenon, and although regions of the molecule may possess charges, these charges should balance each other so the molecule as a whole will have no net charge. The water molecule, for example, possesses a permanent dipole. The magnitude of the permanent dipole, μ, is independent of any induced dipole from an electric field. It is defined as the vector sum of the individual charge moments within the molecule, including those from bonds and lone-pair electrons. The vectors depend on the distance of separation between the charges. The unit of μ is the *debye*, with 1 debye equal to 10^{-18} esu cm.* This is derived from the charge on the electron (about 10^{-10} esu) multiplied by the average distance between charged centers on a molecule (about 10^{-8} cm).

In an electric field, molecules with permanent dipole moments can also have induced dipoles. The polar molecule, however, tends to orient itself with its negatively charged centers closest to positively charged centers on other molecules *before* the electric field is applied, so that when the applied field is present, the orientation is in the direction of the field. Maximum

*The esu (electrostatic unit) is the measure of electrostatic charge, defined as a charge in a vacuum that repels a like charge 1 centimeter away with a force of 1 dyne. In SI units, 1 debye = 3.34×10^{-30} coulomb-meter.

dipole moment occurs when the molecules are oriented most perfectly. Absolutely perfect orientation can never occur owing to the thermal energy of the molecules, which contributes to agitation against the molecular alignment. The *total* molar polarization, P, is the sum of induction and permanent dipole effects:

$$P = P_i + P_0 = \left(\frac{\epsilon - 1}{\epsilon + 2}\right)\frac{M}{\rho} \qquad (4-16)$$

in which P_0 is the orientation polarization of the permanent dipoles. P_0 is equal to $4\pi N\mu^2/9\,kT$, in which k is the Boltzmann constant, 1.38×10^{-23} J° K^{-1}. Since P_0 depends on the temperature, T, equation (4–16) can be rewritten in a linear form as

$$P = P_i + A\frac{1}{T} \qquad (4-17)$$

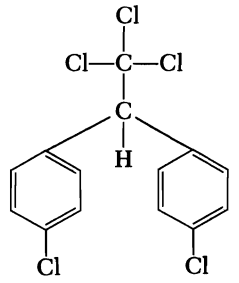
in which the slope A is $4\pi N\mu^2/9k$, and P_i is the y intercept. If P is obtained at several temperatures and plotted against $1/T$, the slope of the graph can be used to calculate μ, and the intercept can be applied to compute α_p. The values of P can be obtained from equation (4–16) by measuring the dielectric constant and the density of the polar compound at various temperatures. The dipole moments of several compounds are listed in Table 4–8.

TABLE 4–8. *Dipole Moments of Some Compounds*

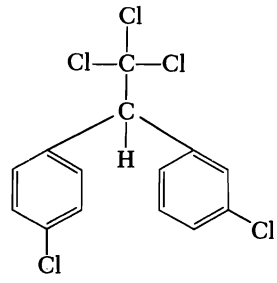
Compound	Dipole Moment (Debye units)
p-Dichlorobenzene	0
H_2	0
Carbon dioxide	0
Benzene	0
1,4-Dioxane	0
Carbon monoxide	0.12
Hydrogen iodide	0.38
Hydrogen bromide	0.78
Hydrogen chloride	1.03
Dimethylamine	1.03
Barbital	1.10
Phenobarbital	1.16
Ethylamine	1.22
Formic acid	1.4
Acetic acid	1.4
Phenol	1.45
Ammonia	1.46
m-Dichlorobenzene	1.5
Tetrahydrofuran	1.63
n-Propanol	1.68
Chlorobenzene	1.69
Ethanol	1.69
Methanol	1.70
Dehydrocholesterol	1.81
Water	1.84
Chloroform	1.86
Cholesterol	1.99
Ethylenediamine	1.99
Acetylsalicylic acid	2.07
o-Dichlorobenzene	2.3
Acetone	2.88
Hydrogen cyanide	2.93
Nitromethane	3.46
Acetanilide	3.55
Androsterone	3.70
Acetonitrile	3.92
Methyltestosterone	4.17
Testosterone	4.32
Urea	4.56
Sulfanilamide	5.37

In solution, the permanent dipole of a solvent such as water can strongly interact with the solute molecules. This interaction contributes to solvent effect and is associated, in the case of water, with the hydration of ions and molecules. The symmetry of the molecule can also be associated with its dipole moment. For example, benzene and *p*-dichlorobenzene are symmetric planar molecules and have dipole moments of zero. Meta and ortho derivatives of benzene, however, are not symmetric and have significant dipole moments, as listed in Table 4–8.

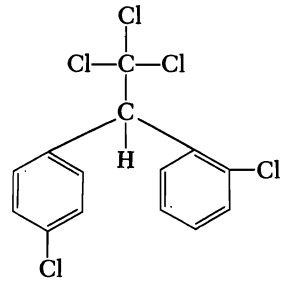
Permanent dipole moments can be correlated with biologic activities of certain molecules to obtain valuable information about the relationship of physical properties and charge separation in a class of compounds. For example, the insecticidal activity of the three isomers of DDT, shown in the following structures, can be associated with their permanent dipole moments. The para isomer, *p,p'*-DDT, has the smallest dipole moment and the greatest activity. This may be due to the fact that greater solubility in a nonpolar solvent may be related to a small dipole moment for a solute. The more soluble molecule most readily penetrates the lipoidal membranes of the insect and attacks the enzymes of the insect's nervous system. Hence, the lower the dipole moment of the isomer, the greater its insecticidal action.



DDT
Para isomer
μ = 1.10 (toxic)



DDT
Meta isomer
μ = 1.55 (intermediate toxicity)



DDT
Ortho isomer
μ = 1.90 (least toxic)

The importance of dipole interactions should not be underestimated. For ionic solutes and nonpolar solvents, ion-induced dipole interactions have an essential role in solubility phenomena. For drug-receptor bonding, dipole forces are believed to contribute to this essentially noncovalent interaction, as described by Kollman.[8] For solids composed of molecules with permanent dipole moments, the dipole force contributes to the crystalline arrangement and overall structural nature of the solid. Ice crystals are organized through their dipole forces. Additional interpretations of the significance of dipole moments are given by Minkin et al.[9]

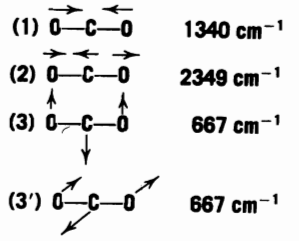


Fig. 4–9. The normal vibrational modes of CO_2 and their respective wavenumbers, showing the directions of motion in reaching the extreme of the harmonic cycle. (Modified from W. S. Brey, *Physical Chemistry and Its Biological Applications*, Academic Press, New York, 1978, p. 316.)

INFRARED SPECTROSCOPY

The study of the interaction of electromagnetic radiation with vibrational or rotational resonances within a molecular structure is termed *infrared spectroscopy*. Normally, infrared radiation in the region from about 2.5 to 50 μm, equivalent to 4000 to 200 cm^{-1} in wavenumber, is used in commercial spectrometers to determine most of the important vibration or vibration–rotation transitions. The individual masses of the vibrating or rotating atoms or functional groups, as well as the bond strength and molecular symmetry, determine the frequency (and, therefore, also the wavelength) of the infrared absorption. The absorption of infrared radiation occurs only if the permanent dipole moment of the molecule changes with a vibrational or rotational resonance. The molecular symmetry relates directly to the permanent dipole moment, as already discussed. Bond stretching or bending *resonances* (i.e., the harmonic oscillations associated with the stretching or bending of the bond) may affect this symmetry, thereby shifting the dipole moment as found for (2), (3), and (3′) in Figure 4–9. Other resonances, such as (1) for CO_2 in Figure 4–9, do not affect the dipole moment and do not produce infrared absorption. Resonances that *shift* the dipole moments can give rise to infrared absorption by molecules, even those considered to have no permanent dipole moment, such as benzene or CO_2. The frequencies of infrared absorption bands correspond closely to vibrations from particular parts of the molecule. The bending and stretching vibrations for acetaldehyde, together with the associated infrared frequencies of absorption, are shown in Figure 4–10. In addition to the fundamental absorption bands, shown in this figure, each of which corresponds to a vibration or vibration–rotation resonance and a change in the dipole moment, weaker overtone bands may be observed for multiples of each of these frequencies (in wavenumbers). For example, an overtone band may appear for acetaldehyde at 3460 cm^{-1}, which corresponds to twice the frequency (2 × 1730 cm^{-1}) for the carbonyl stretching band. Since the frequencies are simply associated with harmonic motion of the radiant energy, the overtones may be thought of as simple multiples that are exactly in phase with the fundamental frequency and can therefore "fit" into the same resonant vibration within the molecule.

Since the vibrational resonances of a complex molecule often can be attributed to particular bonds or groups, they behave as though they resulted from vibrations in a diatomic molecule. This means that vibrations produced by similar bonds and atoms are associated with infrared bands over a small frequency range, even though these vibrations may occur in completely different molecules. Some characteristic infrared stretching vibrations are listed according to bond and atomic group in Table 4–9. The infrared spectrum of a molecule can be used for structural identification by applying tables such as Table 4–9. This qualitative use is the principal application of infrared spectroscopy in pharmacy. A typical infrared spectrum of theophylline is shown in Figure 4–11. The spectrum "fingerprints" the drug and provides one method of verifying compounds. The individual bands can be associated with particular groups. For example, the band at 1660 cm^{-1}, (*a*) in Figure 4–11, is due to a carbonyl stretching vibration for theophylline.

Infrared spectra can be complex, and characteristic frequencies vary depending on the physical state of the molecule being examined. For example, hydrogen bonding between sample molecules may change the spectra. For alcohols in dilute carbon tetrachloride solution, there is little intermolecular hydrogen bonding, and the hydroxyl stretching vibration occurs at about 3600 cm^{-1}. The precise position and shape of the infrared band associated with the hydroxyl group depends on the concentration of the alcohol and the degree of hydrogen bonding. Steric effects, the size and relative charge of neighboring groups, and phase changes can effect similar frequency shifts.

The use of infrared spectroscopy in pharmacy has centered on its applications for drug identification, as described by Chapman and Moss.[10] The development of Fourier Transform-Infrared (FT-IR) spectrometry, as described by Durig,[11] has enhanced infrared applica-

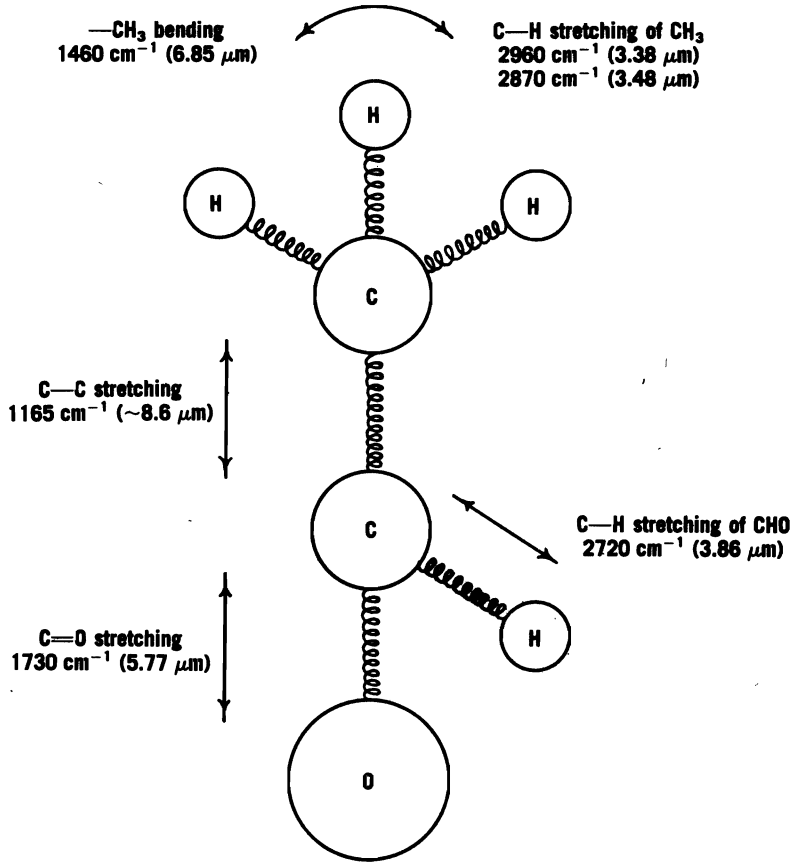


Fig. 4–10. Bending and stretching frequencies for acetaldehyde. (From H. H. Willard et al., *Instrumental Methods of Analysis*, 4th Edition, Van Nostrand, New York, 1965.)

TABLE 4–9. *Characteristic Infrared Stretching Vibrations of Some Functional or Atomic Groups*

Group	Characteristic Wavenumber Range (cm^{-1})
C—F (monofluoro)	1110–1000
C—Cl (monochloro)	730– 650
C—Br (monobromo)	680– 515
C—I (monoiodo)	600– 500
C=N	1620–1690
C≡N	2100–2300
N=N	1500–1560
N—H	3200–3600
C—C	600–1500
C=C (olefin)	1620–1700
C=C (aromatic)	1430–1650
C≡C	2100–2300
C—O (alcohol)	1075–1400
C=O	1600–1900
C—H (alkane)	2850–3000
C—H (alkene)	3000–3100
C—H (aromatic)	3000–3100
O—H	3200–3700

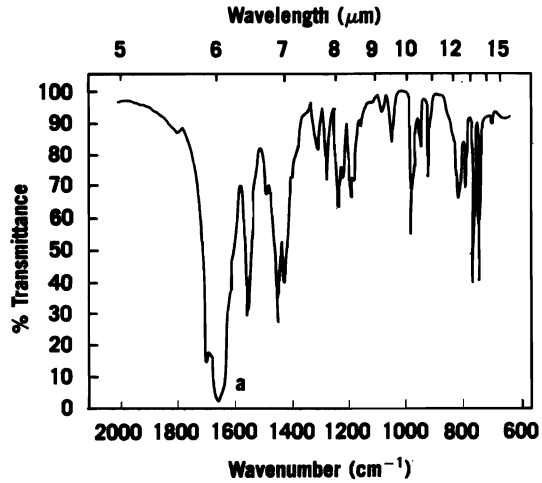


Fig. 4–11. Infrared spectrum of theophylline. (From E. G. C. Clarke, Ed., *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1969.)

tions for both qualitative and quantitative analysis of drugs owing to the greater sensitivity and the enhanced ability to analyze aqueous samples with FT-IR instrumentation. A thorough survey of the techniques and applications of infrared spectroscopy is provided by Smith[12] and by Willard et al.[13]

ELECTRON SPIN AND NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Electromagnetic radiation is characterized as waves that have both an electric vector and a magnetic vector at 90° to each other, as shown in Figure 4–12. Certain forms of spectroscopy measure differences in magnetic

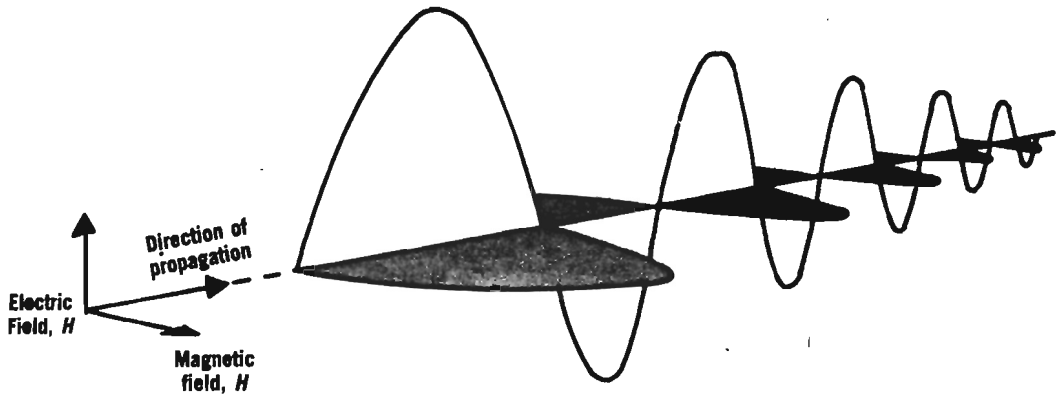


Fig. 4–12. Wave model of electromagnetic radiation showing electrical and magnetic vectors.

energy levels of electrons or nuclei through their relationship to certain frequencies of electromagnetic radiation. A species with an odd number of (or unpaired) electrons placed in an external magnetic field can produce resonance between energy levels of the unpaired electron's magnetic moment at a frequency in the microwave region of the electromagnetic spectrum (see Table 4–2). This resonance is associated with the spin of the unpaired electron, and the study of this effect is termed electron spin resonance (ESR) or, alternatively, electron paramagnetic resonance (EPR) spectroscopy. The microwave frequency at which resonance occurs depends upon the external magnetic field. For example, a frequency at 10 Hz* typically will be found for resonances produced with a magnetic field H of 0.35 T.† Resonance of the unpaired electron is specifically associated with the energy difference, ΔE, between the two spin states that an electron can have according to the quantum theory. When the electron is an external magnetic field,

$$\Delta E = h\nu = g\beta_e H \qquad (4\text{–}18)$$

in which ν is the resonance frequency in the microwave region, β_e is a constant known as the *Bohr magneton* with a value of 9.27×10^{-24} joule/tesla, H is the applied magnetic field, and g is termed the spectroscopic *splitting factor*. The *g factor* is characteristic for certain metal complexes with unpaired electrons. For organic free radicals, g is nearly equal to its value for a free electron, 2.0023, which is the ratio of the electron's *spin* magnetic moment to its *orbital* magnetic moment.

Electron spin resonance spectroscopy has been applied to the study of free radical processes, including pathways of photosynthesis, and to the structure of metal complexes. It is useful only for species that possess unpaired electrons. The addition of substances with unpaired electrons to systems such as lipids or enzymes that do not contain odd electrons, however, affords the lipids or enzymes a *spin label*. This permits studies of the structural environment near the spin label through changes in the pattern of the ESR spectrum. Applications of ESR are described by Swartz et al.[14]

The interaction of electromagnetic radiation from the radiowave region of the spectrum (see Table 4–2) with the spin of *nuclei* in a magnetic field is studied by nuclear magnetic resonance (NMR) spectroscopy. All atomic nuclei have a charge attributed to their protons and, in addition, may have a spin about their nuclear axis. Spinning charges, whether they be nuclei or electric currents in closed circuits, generate magnetic fields, that is, they have magnetic moments. The total angular momentum of the spinning charges from the particles of a particular nucleus is characterized by the spin quantum number, I. A nucleus in the ground state, which is the only state we shall discuss here since we are dealing with low-energy transitions, can have a value of I from 0, which denotes no nuclear spin, increasing to $\frac{1}{2}$, 1, $\frac{3}{2}$, etc. This value of I is directly related to the atomic number and mass number of the nucleus so that nuclei with even atomic and mass numbers have $I = 0$ and no spin and no magnetic moment. Such nuclei include ^{12}C and ^{16}O. Nuclei with odd mass numbers have I of half-integral value, while nuclei with odd atomic numbers but even mass numbers have I of integral value. Both of these last cases give rise to nuclei with magnetic moments and, consequently, NMR signals. Examples include ^{1}H, ^{13}C, ^{15}N, and ^{19}F, which have $I = \frac{1}{2}$, and ^{2}H and ^{14}N, which have $I = 1$.

The concept of NMR is based on the fact that nuclei with magnetic moments, μ, *precess*, that is, rotate like a gyroscope, about the axis of an applied magnetic field. This precession occurs only through certain orientations, or nuclear *spin states*, with their own I values, as shown in Figure 4–13. The nuclear spin states are separated by the energy difference ΔE, so that

$$\Delta E = h\nu = \mu H(1 - \sigma)/I \qquad (4\text{–}19)$$

in which σ is the shielding constant for a particular atom (i.e., a measure of its susceptibility to induction from a

*Hertz, Hz., is frequency in waves per second.

†The tesla, T, is the unit of magnetic flux density: One T induces a voltage of 1 volt in a 1-m long conductor that is moving at 1 m/sec. The tesla is equivalent to 10^4 gauss, the unit that the tesla replaced.

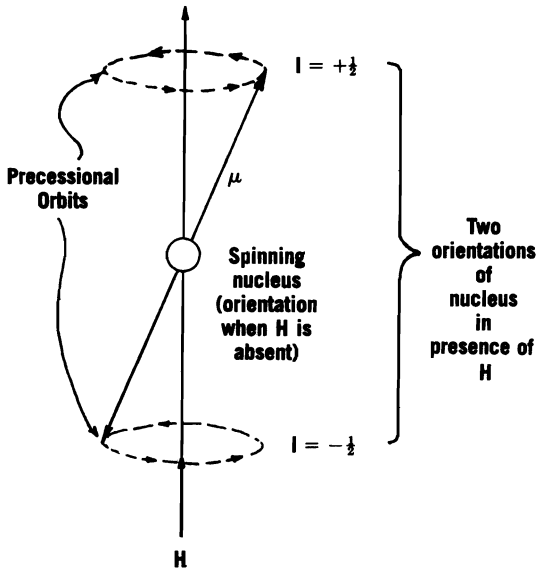


Fig. 4–13. Precession of a nucleus with magnetic moment, μ, about the axis of an applied magnetic field, H. (Modified from D. Betteridge and H. E. Hallam, *Modern Analytical Methods*, The Chemical Society, London, 1972, p. 201.)

magnetic field), H is the external magnetic field strength, μ is the nuclear magnetic moment, and ν is some radio frequency. In actual practice, equation (4–19) can be applied to spectroscopy by gradually varying the magnetic field strength, H, while keeping the radio frequency, ν, constant. At some particular H value, a nuclear spin transition will take place that flips the nuclei from one spin state to another (for example, from $I = -\frac{1}{2}$ to $+\frac{1}{2}$, as in Fig. 4–13). In some spectrometers, the experiment can be done in the reverse fashion: keeping H constant and varying ν. In either case, equation (4–19) applies, and the particular value of ν depends on H.

Example 4–6. (*a*) What is the energy change associated with the ^{1}H nuclear spin of chloroform, which has a shielding constant, σ, of -7.25×10^{-6} and a nuclear magnetic moment, μ, of 1.410620×10^{-26} J/T in a magnetic field H of 1 tesla? The spin quantum number I for ^{1}H is 0.5.

From equation (4–19):

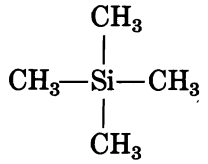
$$\Delta E = \mu H(1 - \sigma)/I = (1.410620 \times 10^{-26}) \times 1 \times (1.00000725)/(0.5)$$
$$= 2.821260 \times 10^{-26} \text{ J}^*$$

(*b*) What is the radio frequency at which resonance will occur for this nuclear spin transition under the stated conditions? From equation (4–19):

$$\nu = \frac{\Delta E}{h} = \frac{2.821260 \times 10^{-26}}{6.626196 \times 10^{-34}} = 4.257738 \times 10^7 \text{ Hz}$$

Tetramethylsilane (TMS) is often used as a reference compound in proton NMR because the resonance frequency of its one proton signal, from its four identical methyl groups, is below that for most other compounds. In addition, TMS is relatively stable and inert.

*We have assumed an exaggerated level of significance for this value, i.e., six significant digits after the decimal point, to show a subsequent relationship.



Tetramethylsilane

Example 4–7. What is the radio frequency at which resonance occurs for TMS in a magnetic field of 1 tesla?

The shielding constant is 0.000, and $\Delta E = 2.821240 \times 10^{-26}$ J for TMS, so

$$\nu = \frac{\Delta E}{h} = \frac{2.821240 \times 10^{-26}}{6.626196 \times 10^{-34}} = 4.257707 \times 10^7 \text{ Hz}$$

From the examples just given, it is important to note that the difference in the resonance frequency between chloroform and TMS at a constant magnetic field strength is only $(4.257738 \times 10^7) - (4.257707 \times 10^7) = 310$ Hz or waves/sec. This small radio frequency difference is equivalent to more than half the total range within which ^{1}H NMR signals are detected. Consequently, the instrument needs to scan only a relatively narrow range of radio frequencies with a constant magnetic field strength for what is termed *frequency-swept NMR*. Alternatively, the instrument may sweep a narrow region of magnetic field strength while the radio frequency is constant, for *field-swept NMR*.

The value of the shielding constant, σ, for a particular nucleus will depend on local magnetic fields, including those produced by nearby electrons within the molecule. This effect is promoted by placing the molecule within a large external magnetic field, H. Greater shielding will occur with higher electron density near a particular nucleus, and this reduces the frequency (assuming frequency-swept NMR) at which resonance takes place. Thus, for TMS, the high electron density from the Si atom produces enhanced shielding and, therefore, a lower resonance frequency. The relative difference between a particular NMR signal and a reference signal (usually from TMS for proton NMR) is termed the *chemical shift*, δ, given in parts per million (ppm). It is defined as

$$\delta = (\sigma_r - \sigma_s) \times 10^6 \qquad (4\text{–}20)$$

in which σ_r and σ_s are the shielding constants for the reference and sample nucleus, respectively.

If the separation between the sample and reference resonance is ΔH or $\Delta\nu$, then

$$\delta = \frac{\Delta H}{H_R} \times 10^6 = \frac{\Delta \nu}{\nu_R} \times 10^6 \qquad (4\text{–}21)$$

in which H_R or ν_R are the magnetic field strength or radio frequency for the nuclei* depending on whether field-swept or frequency-swept NMR is used.

*In our example, the proton ^{1}H resonates at 42.57×10^6 Hz at a field strength of 1 tesla.

Example 4–8. What is the chemical shift of the chloroform proton using TMS as a reference?

Substituting the frequencies obtained from *Examples 4–6b* and *4–7* into equation (4–21), we obtain the chemical shift:

$$\delta = \frac{\Delta\nu}{\nu_R} \times 10^6$$

$$= \frac{(4.257738 \times 10^7) - (4.257707 \times 10^7)}{42.57 \times 10^6} \times 10^6$$

$$= \frac{310 \times 10^6}{42.57 \times 10^6} = 7.28 \text{ ppm}$$

This is an approximate value owing to the relative accuracy used to determine each frequency in the example. The accepted experimental value for this chemical shift is 7.25 ppm. Identical chemical shifts are obtained using either frequency or field sweeping as the experimental measurement, since the relative changes (i.e., ΔH or $\Delta\nu$) to the reference value are directly proportional by either NMR method. The chemical shift of a nucleus provides information about its local magnetic environment and therefore can "type" a nuclear species. Table 4–10 lists some representative proton chemical shifts. Figure 4–14 shows a proton NMR spectrum for benzyl acetate, $CH_3COOCH_2C_6H_5$, using TMS as a reference. Notice that each signal band represents a particular type of proton, that is, the proton at $\delta = 2.0$ is from the CH_3 group, while that at 5.0 is due to CH_2, and the protons at about 7.3 are from the aromatic protons. The *integral* curve above the spectrum is the sum of the respective band areas, and its stepwise height is proportional to the number of protons represented by each band.

An example of the application of NMR to the direct analysis of a pharmaceutical is given by Hanna and Lau-Cam[15] for succinylcholine chloride injections. This NMR assay involves the addition of a known amount of acetamide, as an internal standard, to a freeze-dried sample of the succinylcholine injection. The mixture is dissolved in deuterium oxide (D_2O) and the NMR spectrum obtained. The integral of the band at 3.27 ppm, from the 18 protons in CH_3 groups attached to N atoms in succinylcholine chloride, that is,

TABLE 4–10. *Proton Chemical Shifts for Representative Chemical Groups or Compounds*

Compound or Group	ppm
TMS $(CH_3)_4Si$	0.00
CH_4	0.23
Cyclohexane	1.44
Acetone	2.08
CH_3Cl	3.06
$CHCl_3$	7.25
Benzene	7.27
Ethylene	5.28
Acetylene	1.80
R—OH (hydrogen-bonded)	0.5–5.0
R_2—NH	1.2–2.1
Carboxylic acids (R—COOH)	10–13
H_2O	~4.7

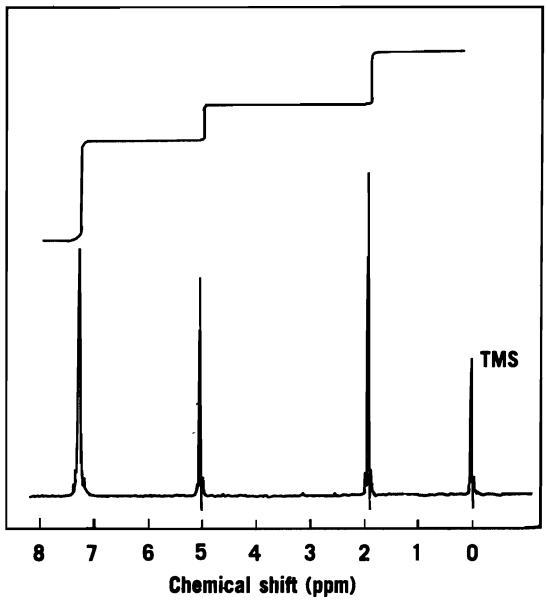
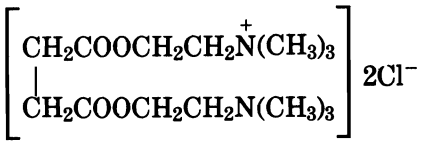


Fig. 4–14. Proton NMR spectrum of benzyl acetate with TMS as a reference. The TMS band appears at the far right. The upper curve is an integration of the spectral bands, the height of the step at each being proportional to the area under that band. (From W. S. Brey, *Physical Chemistry and Its Biological Applications*, Academic Press, New York, 1978, p. 498.)



Succinylcholine Chloride

and the integral from the band at 2.01 ppm, due to the three methyl protons of acetamide (CH_3CONH_2), are determined. The amount of succinylcholine chloride in an injection sample is then calculated from

$$C = W/V \times Iu/Is \times EWu/EWs \qquad (4\text{–}22)$$

in which C is the concentration, in mg/mL, of the succinylcholine chloride, W is the weight, in mg, of acetamide taken as the internal standard, V is the volume, in mL, of succinylcholine chloride injection being tested, Iu and Is are the average integrals of the 3.27-ppm band from succinylcholine chloride and the 2.01-ppm band from acetamide, respectively, EWu is the formula weight (molecular weight) of succinylcholine chloride divided by the number of protons producing the signal band (i.e., 361.31/18), and EWs is the formula weight of the internal standard divided by its signal protons (i.e., 59.07/3).

Example 4–9. What is the concentration (in mg/mL) of succinylcholine chloride in a 1-mL injection sample to which 93 mg of acetamide internal standard is added, and which produces average integral signals at 3.27 ppm and 2.01 ppm of 2158 and 2045 units, respectively?

$$C = W/V \times Iu/Is \times EWu/EWs = 93/1 \times 2158/2045 \times \frac{361.31/18}{59.07/3}$$

$$= 93 \times 1.06 \times 20.07/19.69$$

$$= 100 \text{ mg/mL}$$

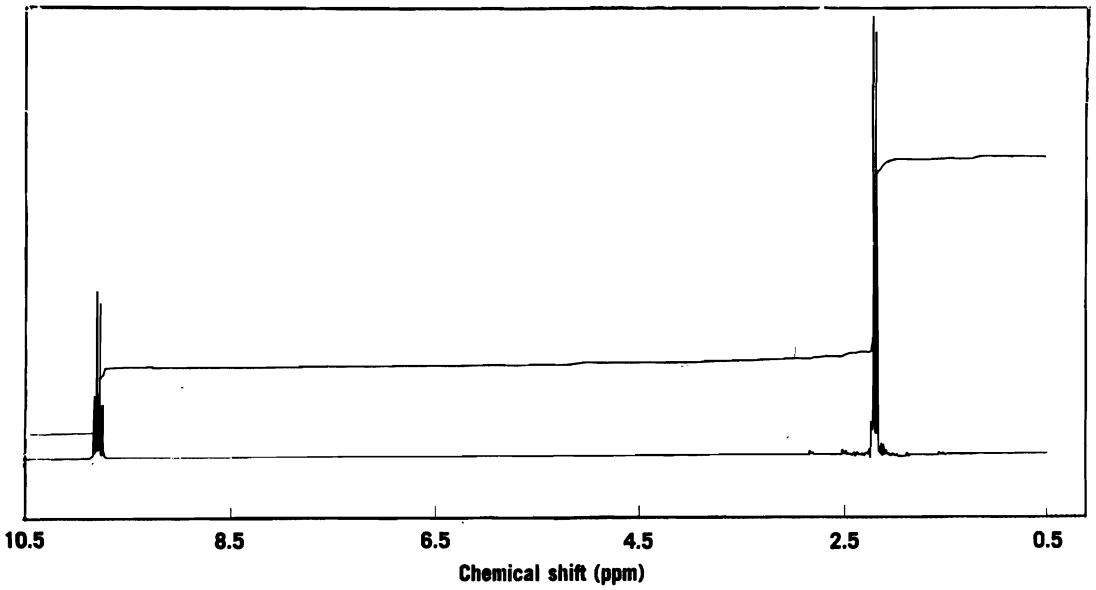


Fig. 4–15. Proton NMR spectrum of acetaldehyde. (From A. S. V. Burgen and J. C. Metcalfe, J. Pharm. Pharmacol. 22, 156, 1970.)

In Figure 4–14, the signal bands are of simple shape, with little apparent complexity Such sharp single bands are known as *singlets* in NMR terminology. In most NMR spectra, the bands are not as simple, since each particular nucleus can be coupled by spin interactions to neighboring nuclei. If these neighboring nuclei are in different local magnetic environments, owing primarily to differences in electron densities, splitting of the bands can occur. This leads to *multiplet* patterns, with several lines for a single resonant nucleus. The pattern of splitting in the multiplet can provide valuable information concerning the nature of the neighboring nuclei.

Figure 4–15 shows the proton NMR spectrum of acetaldehyde, CH_3CHO, in which the doublet of the CH_3 group (right side of the figure) is produced from coupling to the neighboring single proton, and the quartet of the lone proton in the CHO group (left side of the figure) is produced from coupling to the three methyl protons. In any molecule, if the representative coupled nuclei are in proportion as A_X:A_Y on neighboring groups, then the resonance band for A_X will be split into $(2_Y \times I + 1)$ lines, in which I is the spin quantum number for the nuclei, while that for A_Y will be split into $(2_X \times I + 1)$ lines, assuming the nuclei are in different local environments. For example, with acetaldehyde, A_X:A_Y is CH_3:CH, and the resulting proton splitting pattern is A_X (CH_3) as two lines and A_Y (CH) as four lines. This splitting produces *first-order* spectra when the difference in ppm between all the lines of a multiplet (known as the *coupling constant, J*) is small compared with the difference in the chemical shift, δ, between the coupled nuclei. First-order spectra produce simple multiplets with intensities determined by the coefficients of the binomial expansion: a doublet of intensity 1:1; a triplet, 1:2:1; a quartet, 1:3:3:1. Further details of multiplicity and the interpretation of NMR spectra can be found in the book by Bauer et al.[16]

The typical range for NMR chemical shifts depends on the nucleus being observed: for protons, it is about 15 ppm with organic compounds, while it is about 400 ppm for ^{13}C or ^{19}F spectra. Table 4–11 gives the basic NMR resonance for certain pure isotopes, together with their natural abundances. As the natural abundance of the isotope decreases, the relative sensitivity of NMR gets proportionally smaller.

TABLE 4–11. *Basic NMR Resonances and Natural Abundance of Selected Isotopes**

Isotope	NMR Frequency (MHz) at Field Strength (T)		Natural Abundance (%)
	1.0000 T	2.3487 T	
$^{1}_{1}H$	42.57	100.00	99.985
$^{13}_{6}C$	10.71	25.14	1.108
$^{15}_{7}N$	4.31	10.13	0.365
$^{19}_{9}F$	40.05	94.08	100

*From A. J. Gordon and R. A. Ford, *The Chemist's Companion*, Wiley, New York, 1972, p. 314.

The development of ^{13}C NMR spectroscopy in recent years has been influenced by the application of *spin decoupling* to intensify and simplify the otherwise complex ^{13}C NMR spectra. Decoupling of the proton spins is produced by continuously irradiating the entire proton spectral range with broad-band radiofrequency radiation. This decoupling produces the collapse of multiplet signals into simpler and more intense signals. It also produces an effect known as the *nuclear Overhauser effect* (NOE), in which decoupling of the protons produces a dipole–dipole interaction and energy transfer to the carbon nuclei, resulting in greater relaxation (i.e., rate of loss of energy from nuclei in the higher spin state to the lower spin state), and, consequently, a greater population of carbon nuclei in the lower spin state (see Fig. 4–13). Since this greater population in the lower spin state permits a greater absorption signal in NMR, the NOE can increase the carbon nuclei signal by as much as a factor of three. These factors—proton spin decoupling and the NOE—have enhanced the sensitivity of ^{13}C NMR and, therefore, compensate for the low natural abundance of ^{13}C as well as the smaller magnetic moment, μ, of ^{13}C compared with that for hydrogen.

A powerful extension of this enhancement of ^{13}C spectra involves systematically decoupling only specific protons by irradiating at particular radiofrequencies rather than by broad-band irradiation. Such systematic decoupling permits individual carbon atoms to show multiplet collapse and signal intensification, as mentioned above, when protons coupled to the particular carbon atom are irradiated. These signal changes allow particular carbon nuclei in a molecular structure to be associated with particular protons, and produce what is termed *two-dimensional NMR spectrometry*. The techniques involved in these experiments are described by Farrar.[17]

Nuclear magnetic resonance is a versatile tool in pharmaceutical research. Spectra can provide powerful evidence for a particular molecular conformation of a drug, including the distinction between closely related isomeric structures. This identification is normally based on the relative position of chemical shifts as well a peak multiplicity and other parameters associated with spin coupling. Drug–receptor interactions can be distinguished and characterized through specific changes in the NMR spectrum of the unbound drug after the addition of a suitable protein binder. These changes are due to restrictions in drug orientation. Burgen and Metcalfe[18] describe applications of NMR to problems involving drug-membrane and drug–protein interactions. An illustration of these interactions analyzed by both ESR and NMR spectroscopy is given by Lawrence and Gill[19] using ESR, and by Tamir and Lichtenberg[20] using proton-NMR techniques. The ESR and proton-NMR results show that the psychotropic tetrahydrocannabinols reduce the molecular ordering in the bilayer of liposomes used as simple models of biologic membranes. These results suggest that the cannabinoids exert their psychotropic effects by way of a nonspecific interaction of the cannabinoid with lipid constituents, principally cholesterol, of nerve cell membranes. The use of NMR in pharmaceutical research, with particular reference to analytical problems, has been reviewed by Rackham.[21]

REFRACTIVE INDEX AND MOLAR REFRACTION

Light passes more slowly through a substance than through a vacuum. As light enters a denser substance, the advancing waves at the interface are modified by being closer together owing to their slower speed and shorter wavelength, as shown in Figure 4–16. If the light enters the denser substance at an angle, as shown, one part of the wave slows down more quickly as it passes the interface, and this produces a bending of the wave toward the interface. This phenomenon is called *refraction*. If light enters a less dense substance, it is refracted away from the interface rather than toward it. The relative value of this effect between two substances is given by the *refractive index*, n:

$$n = \frac{\sin i}{\sin r}$$

$$= \frac{\text{velocity of light in first substance}}{\text{velocity of light in second substance}} \qquad (4\text{–}23)$$

in which sin i is the sine of the angle of the incident ray of light and sin r is the sine of the angle of the refracted ray. Normally, the numerator is taken as the velocity of light in air, and the denominator is the material being investigated. The refractive index, by this convention, is greater than 1 for substances denser than air. Theoretically, the reference state where $n = 1$ should be for light passing through a vacuum; however, the use of air as a reference produces a difference in n of only

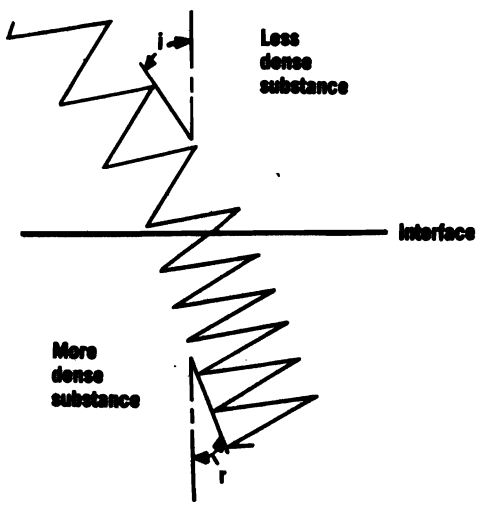


Fig. 4–16. Waves of light passing an interface between two substances of different density.

0.03% from that in a vacuum and is more commonly used.

Refractive index varies with the wavelength of light and the temperature. Normally, these values are identified when a refractive index is listed; for example, n_D^{20} signifies the refractive index using the *D*-line emission of sodium, at 589 nm, at a temperature of 20° C. Pressure must also be held constant in measuring the refractive index of gases. Refractive index can be used to identify a substance, to measure its purity, and to determine the concentration of one substance dissolved in another. Typically, a refractometer is used to determine refractive index.

The *molar refraction*, R_m, is related to both the refractive index and the molecular properties of a compound being tested. It is expressed as

$$R_m = \frac{n^2 - 1}{n^2 + 2}\left(\frac{M}{\rho}\right) \qquad (4-24)$$

in which M is the molecular weight and ρ is the density of the compound. The R_m value of a compound can often be predicted from the structural features of the molecule. Each constituent atom or group contributes a portion to the final R_m value, as discussed earlier in connection with additive-constitutive properties (see Table 4–1). For example, acetone has an R_m produced from three carbons (R_m = 7.254), six hydrogens (6.6), and a carbonyl oxygen (2.21) to give a total R_m of 16.1 cm^3/mol. Because R_m is independent of the physical state of the molecule, this value can often be used to distinguish between structurally different compounds, such as keto and enol tautomers.

Light incident upon a molecule induces vibrating dipoles, and the greater the refractive index at a particular wavelength, the greater is the dipolar induction. The interaction of light photons with the polarizable electrons of a dielectric causes a reduction in the velocity of light. The dielectric constant, being a measure of polarizability, is greatest when dipolar interactions with light are proportionally large. The refractive index for light of long wavelengths, n_∞, is related to the dielectric constant for a nonpolar molecule, ϵ, by the expression:

$$\epsilon = n_\infty^2 \qquad (4-25)$$

Molar polarization, P_i, equation (4–15), can be considered roughly equivalent to molar refraction, R_n, and can be written as

$$P_i = \left(\frac{n_\infty^2 - 1}{n_\infty^2 + 2}\right)\frac{M}{\rho} = \frac{4}{3}\pi N\alpha_p \qquad (4-26)$$

From this equation, the polarizability α_p of a nonpolar molecule may be obtained from a measurement of refractive index. For practical purposes, the refractive index at a finite wavelength is used. This introduces only a relatively small error, approximately 5%, in the calculation.

OPTICAL ROTATION

By passing light through a polarizing prism, such as a Nicol prism, the randomly distributed vibrations of radiation are sorted so that only those vibrations occurring in a single plane are emitted. The velocity of this *plane-polarized* light can become slower or faster as it passes through a substance, in a manner similar to that discussed for refraction. This change in velocity results in refraction of the polarized light in a particular direction for an *optically active* substance. A clockwise rotation, looking into the beam of polarized light, defines a substance that is *dextrorotatory*, whereas a counterclockwise rotation defines a *levorotatory* substance. The dextrorotatory substance, which may be thought of as rotating the beam to the right, produces an *angle of rotation*, α, that is defined as positive (+); while the levorotatory substance, which would rotate the beam to the left, has an α that is defined as negative (−). Molecules that have an asymmetric center and therefore lack symmetry about a single plane are optically active, whereas symmetric molecules are optically inactive and consequently do not rotate the plane of polarized light. Optical activity can be considered as the interaction of a plane-polarized radiation with electrons in a molecule to produce electronic polarization. This interaction rotates the direction of vibration of the radiation by altering the electric field. A *polarimeter* is used to measure optical activity.

Optical rotation, α, depends on the density of an optically active substance, since each molecule provides an equal but small contribution to the rotation. The *specific rotation*, $\{\alpha\}_\lambda^t$, at a specified temperature t and wavelength λ (usually the *D*-line of sodium), is characteristic for a pure, optically active substance. It is expressed by the equation

$$\{\alpha\}_\lambda^t = \frac{\alpha v}{lg} \qquad (4-27)$$

in which l is the length in decimeters (dm*) of the light path through the sample, and g is the number of grams of optically active substance in v mL of volume. If the substance is dissolved in a solution, the solvent as well as the concentration should be reported with the specific rotation. The specific rotations of some drugs are found in Table 4–12. The subscript D on [α] indicates that the measurement of specific rotation is made at a wavelength (λ) of 589 nm for sodium light. When the concentration is not specified, as in Table 4–12, the concentration is assumed to be 1 gram per milliliter of solvent. The specific rotation of steroids, carbohydrates, aminoacids, and other compounds of biologic importance are found in the *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, Fl.

*Decimeters was the unit chosen because of the long sample cells normally used in polarimeters. The decimeter = 10 cm = 1/10 m.

TABLE 4–12. *Specific Rotations*

Drug	$[\alpha]_D$	Temperature (°C)	Solvent
Ampicillin	+283°	20	Water
Aureomycin	+296°	23	Water
Benzylpenicillin	+305°	25	Water
Camphor	+41° to +43°	25	Ethanol
Colchicine	−121°	17	Chloroform
Cyanocobalamin	−60°	23	Water
Ergonovine	−16°	20	Pyridine
Nicotine	−162°	20	Pure liquid
Propoxyphene	+67°	25	Chloroform
Quinidine	+230°	15	Chloroform
Reserpine	−120°	25	Chloroform
Tetracycline hydrochloride	−253	24	Methanol
d-Tubocurarine chloride	+190°	22	Water
Yohimbine	+51° to +62°	20	Ethanol

Optical Rotatory Dispersion. Optical rotation changes as a function of the wavelength of light, and *optical rotatory dispersion* (ORD) is the measurement of the angle of rotation as a function of the wavelength. By varying the wavelength of light, the specific rotation for an optically active substance will change. A graph of specific rotation versus wavelength shows an inflection and then passes through zero at the wavelength of maximum absorption of polarized light as shown in Figure 4–17. This change in specific rotation is known as the *Cotton effect.* By convention, compounds whose specific rotations show a maximum *before* passing through zero as the wavelength of polarized light becomes smaller are said to show a *positive Cotton effect,* whereas if {α} shows a maximum *after* passing through zero (under the same conditions of approaching shorter wavelengths), the compound shows a *negative Cotton effect.* Enantiomers can be characterized by the Cotton effect, as shown in Figure 4–18. In addition, ORD is often useful for the structural examination of organic compounds. For example, one can readily distinguish between two steroids with keto groups at positions 3 and 17 by examination of rotatory dispersion curves.

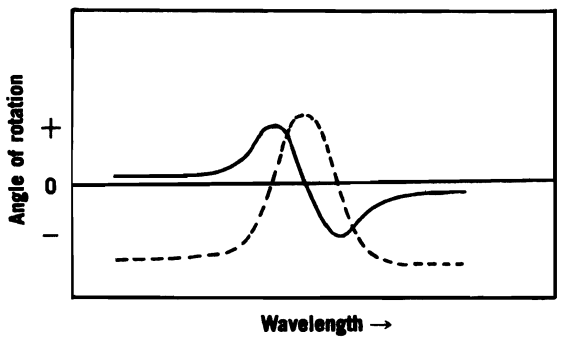


Fig. 4–17. The Cotton effect. Variation of the angle of rotation (solid line) in the vicinity of an absorption band of polarized light (dashed line). (Modified from W. S. Brey, *Physical Chemistry and Its Biological Applications,* Academic Press, New York, 1978, p. 330.)

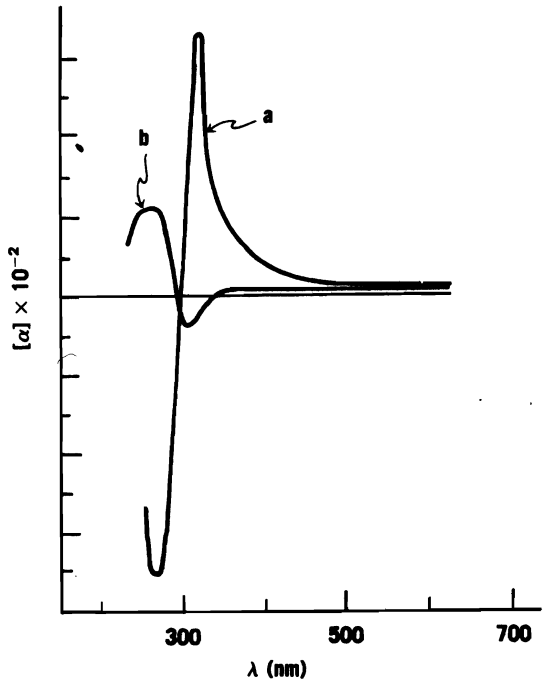
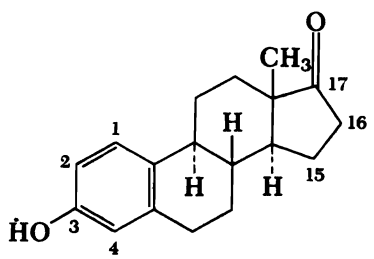


Fig. 4–18. ORD curves for (*a*) *cis*-10-methyl-2-decalone and (*b*) trans-10-methyl-2-decalone. Note that (*a*) has a positive cotton effect, while (*b*) has a negative cotton effect. (From *Optical Rotatory Dispersion,* by C. Djerassi. Copyright © 1960, McGraw-Hill Book Company, New York. Used with the permission of McGraw-Hill Book Company.)



Estrone (a 17-keto steroid)

Detailed discussion of ORD is given by Crabbe.[22]

CIRCULAR DICHROISM

Plane-polarized light is described as the vector sum of two circularly polarized components. Circularly polarized light has an electric vector that spirals around the direction of propagation. In plane-polarized light, there can be two such vectors, each spiraling in the opposite direction. For an optically active substance, the values of the index of refraction n of the two vectors cannot be the same. This difference changes the relative rate at which the polarized light spirals about its direction of propagation.

Likewise, the speeds of the two components of polarized light become unequal as they pass through an optically active substance that is capable of absorbing light over a selected wavelength range. This is the same as saying that the two components of polarized light have different absorptivities at a particular wavelength of light. This effect causes circularly polarized light to become elliptically polarized, and this is termed *circular dichroism* (CD). The Cotton effect is the unequal absorption of light by the two components of circularly polarized light in the wavelength region near an absorption band. Circular dichroism spectra are plots of molar ellipticity, ([θ]), which is proportional to the difference in absorptivities between the two components of circularly polarized light, against the wavelength of light. Molar ellipticity is given by

$$[\theta] = \frac{[\psi]M}{100} = 3300\,(\epsilon_L - \epsilon_R)\ \text{deg liter mole}^{-1}\ \text{dm}^{-1} \qquad (4\text{–}28)$$

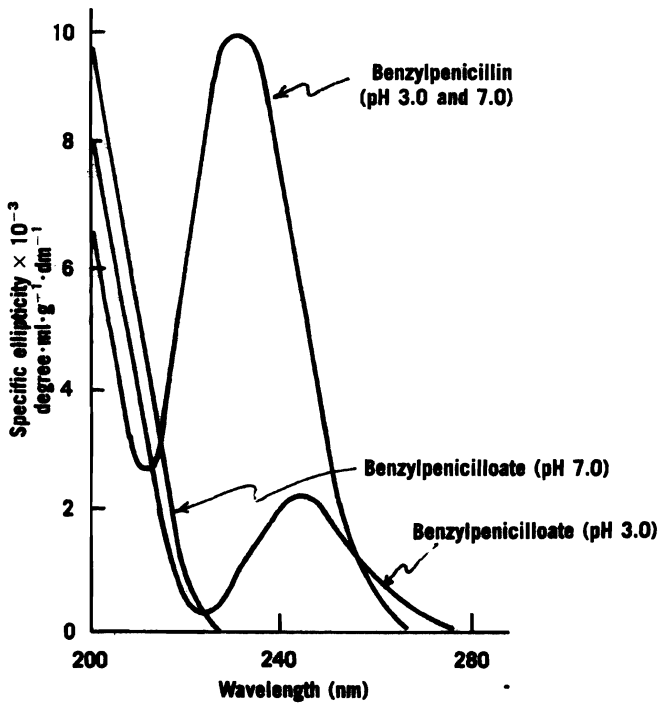


Fig. 4–19. CD spectra of benzylpenicillin and its penicilloic acid derivative at pH 7.0. (From C. E. Rassmussen and T. Higuchi, J. Pharm. Sci. **60**, 1616, 1971, reproduced with permission of the copyright owner.)

where 1 dm is written for 10 cm, and [ψ] is the specific ellipticity analogous to specific rotation, M is the molecular weight, and ϵ_L *and* ϵ_R are the molar absorptivities for the left and right components of circularly polarized light at a selected wavelength. The applications of CD to interactions between small molecules and macromolecules are reviewed by Perrin and Hart.[23] Its application to the determination of the activity of penicillin was described by Rasmussen and Higuchi.[24] The activity is measured as the change in the CD spectra of penicillin after addition of penicillinase, which enzymatically cleaves the β-lactam ring to form the penicilloate ion, as shown for penicillin G on p. 84. Typical CD spectra for benzylpenicillin and its hydrolysis product are shown in Figure 4–19. The direct determination of penicillins by CD and the distinction of penicillins from cephalosporins by their CD spectra has been described by Purdie and Swallows.[25] The penicillins all have single positive CD bands with maxima at 230 nm, while the cephalosporins have two CD bands, a positive one with a maximum at 260 nm and a negative one with maximum at 230 nm (wavelengths for maxima are given to within ±2 nm). This permits easy differentiation between penicillins and cephalosporins by CD spectropolarimetry.

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Problems

4–1. The wavelength for the detection of lithium by its atomic emission spectra is 670.8 nm. What is the energy of the photon of radiation that corresponds to this emission line for lithium?

Answer: 2.93×10^{-19} J

4–2. Describe at least one limitation to the usefulness of atomic emission methods for the detection of trace metals (for example, selenium) dissolved in blood. (J. A. Dean, Editor, *Lange's Handbook of Chemistry*, McGraw-Hill, New York, 1979, contains tables of emission lines.)

4–3. A urine sample is being analyzed for trace levels of copper by atomic emission. The following flame emission intensities (EI) were obtained at a wavelength of 324.8 nm for the 24-hour urine sample, which had a total volume of 980 mL. A set of copper samples (CS) yielded EI values as shown in the table.

Data for *Problem 4–3*

Copper samples (CS)	Emission intensity (EI)
Cu standard, 0.5 μg/mL	20
Cu standard, 1.0 μg/mL	38
Cu standard, 1.5 μg/mL	61
Cu standard, 2.0 μg/mL	80
Urine sample	32

What is the concentration of copper in the 24-hour urine sample? If the normal copper level in urine is approximately 20 μg per 24-hour sample, does this calculated copper concentration indicate an unusually high pathologic condition?

Hint: Regress CS in μg/mL against EI: CS = $a + b$ EI. From the equation, if the relationship is linear, obtain μg/mL for copper in the urine sample having an EI of 32. This is the result per milliliter; but the volume of the 24-hour urine sample is 980 mL. Calculate the micrograms of copper in the urine over a 24-hour period.

Answer: 797 μg per 24-hour sample. This is an unusually high copper level in urine and indicates a pathologic condition.

4–4. A single tablet of colchicine is dissolved in 100 mL of ethanol and its absorbance maximum at 243 nm is measured as 0.438 in a 1-cm path-length cell. Using the molar absorptivity for colchicine given in Table 4–4, what is the amount, in mg, of colchicine in the tablet? The molecular weight of colchicine is 399.4 g/mole.

Answer: 0.60 mg per tablet.

4–5. The ultraviolet spectrum of saccharin has absorption maxima in methanol at 285, 276, and 226 nm and corresponding molar absorptivities (ε) of 775, 951, and 8570 liter mole^{-1} cm^{-1}, respectively. Assuming a minimum absorbance level of $A = 0.002$, what is the minimum detectable concentration of saccharin at each of its absorption maxima wavelengths? Which of these wavelengths would be suitable for the analysis of the amount of saccharin in a tablet with a label claim of 1/4 grain sodium saccharin when the tablet is dissolved in 50 mL of methanol? The molecular weight of sodium saccharin is 205.16 g/mole. Assume that the pathlength of the cell is 1 cm.

Answer: At 285 nm, $c = 2.6 \times 10^{-6}$ mole/liter; at 276 nm, $c = 2.1 \times 10^{-6}$ mole/liter; and at 226 nm, $c = 2.3 \times 10^{-7}$ mole/liter. The 1/4 grain sodium saccharin tablet in a 50-mL solution provides a concentration of 1.58×10^{-3} mole/liter, which is larger than the minimum detectable concentrations at 285, 276, and 226 nm. Any of these three wavelengths is suitable for the analysis.

4–6. A sample of 20 acetaminophen tablets is selected to determine the average amount of active ingredient. The weight of a tablet is 400 mg. Acetaminophen tablets are finely powdered and a portion of the powder (400 mg) is transferred to a 250-mL volumetric flask, diluted with ethanol to volume, mixed, and filtered. One milliliter of the filtrate is transferred to a second 250-mL volumetric flask and diluted with ethanol to volume. The absorbance of this solution, A, is 0.340 at 250 nm, in a 1-cm cell. The molar absorptivity, ε, of acetaminophen is 13,500, and its molecular weight is 151.2 g/mole. Calculate the deviation from the labeled amount (250 mg per tablet) in the sample of tablets assayed. The quantity of acetaminophen must be within the limits of 90 and 110 per cent.

Answer: The amount of acetaminophen per tablet is 237.5 mg. The deviation from the labeled claim is 5 per cent, which is within the limits established.

4–7. The Beer's law plot, as shown in Figure 4–4, is a straight line relating absorbance to concentration. Describe an experimental condition in which the Beer's law plot might be a curved line, with the slope of the curve decreasing at higher concentrations. From a molecular point of view, what is the cause of deviations from ideal solution behavior at high concentrations?

4–8. The molecular weight of diethyl ether is 74.12 g/mole and its density is 0.7134 g/cm^3 at 20° C. What is the induced molar polarization, P_i, of diethyl ether? See Table 4–6 for the dielectric constant of diethyl ether. What is the calculated induced polarizability, α_p, for diethyl ether at 20° C?

Answer: $P_i = 54.73$ cm^3/mole; $\alpha_p = 2.17 \times 10^{-23}$ cm^3

4–9. The following table of concentrations and absorbance values, A, was produced for solutions of nitrazepam in 0.1-N sulfuric acid. The absorbance A was measured at 277.5 nm. What is the average molar absorptivity, ε, of nitrazepam in 0.1-N sulfuric acid calculated from the three sets of data in the table? A 1-cm path-length cell was used for the experiment. Draw the Beer's law plot associated with the data given in the table. The molecular weight of nitrazepam is 281.3 g/mole.

Data for *Problem 4–9*

Concentration (C) (mg/L)	Absorbance (A)
0.394	0.06
0.844	0.13
1.160	0.25

Answer: Average molar absorptivity, $\epsilon = 4.29 \times 10^4$ liter mole^{-1} cm^{-1}.

4–10. The $E_{1cm}^{1\%}$ value for the ultraviolet absorbance of indomethacin at 318 nm is 182 per 100 mL g^{-1} cm^{-1}. (See page 82 for $E_{1\,cm}^{1\%}$.) What is the molar absorptivity, ε, corresponding to this $E_{1\,cm}^{1\%}$ value? The molecular weight of indomethacin is 357.81 g/mole.

Answer: $\epsilon = 6512$ liter mole^{-1} cm^{-1}

4–11.* The methyl group protons of nicotine have a shielding constant σ of -2.2×10^{-6} and the nuclear magnetic moment, μ_H, is 1.41062×10^{-26} J/T for the proton in NMR spectroscopy. The spin quantum number, I, is equal to 1/2. (a) What is the frequency ν_R at which resonance will occur for the methyl group of nicotine at a magnetic field strength H of 1 tesla? (b) What is the chemical shift, δ, in ppm of the methyl protons of nicotine under the same conditions

*Both an H and a lower case h appear in this problem and they must not be confused. H is the magnetic field given in the units of *teslas* and h is the Planck's constant, 6.6262×10^{-27} erg sec.

using TMS as a reference? TMS has a resonance frequency ν_R of 4.257707×10^7 Hz under the stated conditions. See *Examples 4–6, 4–7,* and *4–8.*

Answer: (a) $\nu_R = 4.257716 \times 10^7$ Hz. (b) Chemical shift, $\delta = 2.11$ ppm. (*Note:* δ may equal 2.16 if ν is carried out to several more decimal places.)

4–12. A blood serum sample is being analyzed for isoniazid by fluorescence induced with salicylaldehyde. The following relative fluorescence emission intensities are obtained for a blank sample with no drug, a standard of 0.80 μg/mL, and the serum sample: 1.2, 60.5, and 38.4, respectively. Assuming that the emission intensity is proportional to the isoniazid concentration, determine the isoniazid concentration in μg/mL in the serum.

Answer: The standard isoniazid sample of 0.80 μg/mL yields a fluorescence emission intensity of 60.5–1.2, or 59.3 where 1.2 is a correction for the blank. The isoniazid in the serum sample produces a fluorescence intensity of 38.4–1.2. Thus, by the method of proportions, one directly obtains the isoniazid concentration, 0.50 μg/mL.

4–13. An aqueous solution of maltose containing 15.3 g per 100 mL was observed in a polarimeter to have a rotation of 20° at 25° C using the sodium D-line. The polarimeter cell was 10 cm long. What is the specific rotation, $\{\alpha\}_D^{25^\circ}$, of maltose? *Note:* cell length must be expressed in decimeters.

Answer: $\{\alpha\}_D^{25^\circ} = 131^\circ$

4–14. Calculate the total molar polarization for water, using the dielectric constant at 25° C given in Table 4–6 and equation (4–16).

Answer: $P = 17.4$ cm^3/mole

4–15. A forensic scientist is attempting to identify a sample as either a codeine or cocaine salt by infrared spectroscopy. The infrared spectrum shows no strong bands between 1600 and 2000 cm^{-1}, some strong bands in the region of 1400 to 1500 cm^{-1}, and some broad bands in the region 3200 to 3700 cm^{-1}. Based on this data, which compound is associated with the spectrum? Use Table 4–9 as a guide.

4–16. The diphenhydramine hydrochloride content of a capsule formulation can be determined by proton NMR using t-butyl alcohol as an internal standard.[26] The integral I_u of the 6 N-methyl protons in the diphenhydramine band at 2.85 ppm is divided by the integral I_s of the nine methyl protons of t-butyl alcohol in the band at 1.27 ppm using equation (4–22). If a single capsule's contents v is assayed, using $W = 25$ mg of t-butyl alcohol as the internal standard, and the average integrals of the bands at 2.85 and 1.27 ppm are 1200 and 7059 units, respectively, what is the amount C (in mg per capsule) of diphenhydramine hydrochloride in the capsule? The formula weights (molecular weights) for diphenhydramine hydrochloride EW_u and t-butyl alcohol EW_s are 291.9 g/mole and 74.1 g/mole, respectively.

Answer: 25 mg of diphenhydramine hydrochloride per capsule

4–17. Explain why deuterium oxide rather than water is used to dissolve the sample of succinylcholine chloride for NMR analysis, as described in *Example 4–9.* Give examples of other solvents that are suitable for proton NMR analysis. (*Hint:* See references to books at the end of this chapter for examples of suitable solvents for NMR spectroscopy.)

4–18. (a) Calculate the molar refraction, R_m, of methanol using Table 4–1 for the molar refraction of contributing atoms and groups. Compare the result with that obtained by using equation (4–24). The refractive index n of methanol is 1.326, its molecular weight is 32.04 g/mole, and its density is 0.7866 g/cm^3 at 25° C.

(b) What are the units on R_m?

Answer: The values in Table 4–1 yield R_m for methanol as 8.343 cm^3/mole; and with the density of methanol as 0.7866 g/cm^3 at 25° C, equation (4–24) yields the value $R_m = 8.218$. The student should readily be able to attach the proper units to R_m.

4–19. What is the molar ellipticity $[\Theta]$ for a penicillin V solution with a specific ellipticity, $[\Psi]$, of 1.04×10^5 deg mL/g dm at 230 nm? Penicillin has a molecular weight of 350 g/mole.

Answer: From equation (4–28), the molar ellipticity $[\Theta]$ is 364 deg liter mole^{-1} dm^{-1}.

4–20. The refractive index n for quinoline, an antimalarial drug, is 1.627 at 20° C using light from the D-line emission of sodium. If the incident light, passing through air, is at an angle of 45° from the perpendicular to the surface of the quinoline liquid, what is the angle of its direction inside the quinoline?

Answer: 25°45′

4–21. The specific rotation of digoxin at 20° C using light from the D-line of sodium, $\{\alpha\}_D^{20}$, is +30.4°. If an optical rotation, α, of +15.2° is obtained at the same temperature and wavelength of light for a 10-mL solution in a 1-dm length cell, wavelength of light for a 10-mL solution in a 1-dm length cell, what is the concentration, g, of digoxin (in g/mL) in the solution?

Answer: $g = 5$ grams in 10 mL or a concentration of 0.5 g/mL.