4 Determination of the Physical Properties of Molecules

Chapter Objectives

At the conclusion of this chapter the student should be able to:

1. Understand the nature of intra- and intermolecular forces that are involved in stabilizing molecular and physical structures.
2. Understand the differences in the energetics of these forces and their relevance to different molecules.
3. Understand the differences in energies between the vibrational, translational, and rotational levels and define their meaning.
4. Understand the differences between atomic and molecular spectroscopic techniques and the information they provide.
5. Appreciate the differences in the strengths of selected spectroscopic techniques used in the identification and detection of pharmaceutical agents.
6. Define the electromagnetic radiation spectrum in terms of wavelength, wave number, frequency, and the energy associated with each range.
7. Define and understand the relationships between atomic and molecular forces and their response to electromagnetic energy sources.
8. Define and understand ultraviolet and visible light spectroscopy in terms of electronic structure.
9. Define and understand fluorescence and phosphorescence in terms of electronic structure.
10. Understand electron and nuclear precession in atoms subjected to electromagnetic radiation and its role in the determination of atomic structure in a molecule.
11. Understand polarization of light beams and the ability to use polarized light to study chiral molecules.
12. Understand fundamental principles of refraction of electron and neutron beams and how these beams are used to determine molecular properties.

Molecular Structure, Energy, and Resulting Physical Properties

An atom consists of a nucleus, made up of neutrons (neutral in charge) and protons (positively charged), with each particle carrying a weight of approximately 1 g/mole. In addition, electrons (negatively charged) exist in atomic orbits surrounding the nucleus and have a significantly lower weight. Charged atoms arise from an imbalance in the number of electrons and protons and can lead to ionic interactions (discussed in Chapter 2). The atomic mass is derived from counting the number of protons and neutrons in a nucleus. Isotopes may also exist for a given type of atom. For example, carbon has an atomic number of 6, which describes the number of protons, and there are several carbon isotopes with different numbers of neutrons in the nucleus: ^{11}C (with five neutrons), ^{12}C (with six neutrons), ^{13}C (with seven neutrons), ^{14}C (with eight neutrons), and ^{15}C (with nine neutrons).1 Carbon-13, ^{13}C, is a common isotope used in nuclear magnetic resonance (NMR) and kinetic isotope effect studies on rates of reaction, and ^{14}C is radioactive and used as a tracer for studies that require high sensitivity and for carbon dating. Both ^{11}C and ^{15}C are very short-lived, having half-lives of 20.3 min and 2.5 sec, respectively, and are not used in practical applications.

Molecules arise when interatomic bonding occurs. The molecular structure is reflected by the array of atoms within a molecule and is held together by bonding energy, which relies heavily on electron orbital orientation and overlap. This is illustrated in the Atomic Structure and Bonding Key Concept Box. Each bond in a complex molecule has an intrinsic energy and will have different properties, such as reactivity. The properties within a molecule depend on intramolecular interactions, and each molecule will possess a net energy of bonding that is defined by its unique composition of atoms. It is also important to note

that for macromolecules like proteins or synthetic polymers, the presence of neighboring charges or dipole interactions, steric hindrance and repulsion, Debye forces, and so on, within the backbone or functional groups of the molecule can also give rise to distortions in the primary intramolecular bonding energies and can even give rise to additional covalent bonding, for example, disulfide bridges in a protein like insulin.

The more bonding that an atom participates in, the lower is the density of the electron cloud around the nucleus and the greater is the shared distribution of atomic electrons in the interatomic bonded space. For example, because of differences in atomic properties such as electronegativity, the nature of each interatomic bond can vary greatly. Here, the “nature” refers to the energy of the bond, the rotation around the bond, the vibration and motion of atoms in the bond, the rotation of a nucleus of an atom in a bond, and so on.

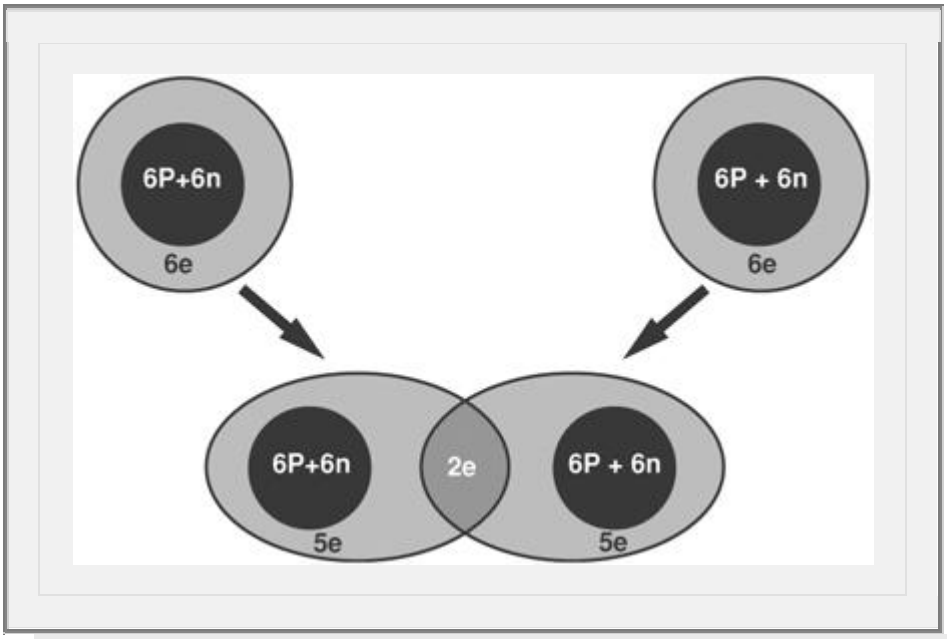
As an example of the nature of a bond, consider the carbonyl–amide bond between two amino acids in a protein

P.78

(Fig. 4-1), as described by Mathews and van Holde.3 The oxygen in the carbonyl is very electronegative and pulls electrons toward itself. The lone pair of electrons on the nitrogen remains unbound, but nitrogen has a considerably lower electronegativity than oxygen. Recall that the covalent bond occurs between the carbon (which also has a low electronegativity) in the carbonyl group and the amine of the next amino acid. However, the electrons in the carbon–oxygen double bond in the carbonyl are pulled much closer to the oxygen due to its greater electronegativity, causing a partially negatively charged oxygen. The carbon then carries a partial positive charge and pulls the lone pair of electrons from the nitrogen. This forms a partial double bond, which causes the nitrogen to carry a partial positive charge, yielding a net dipole (see Permanent Dipole Moment of Polar Molecules section). Interestingly, in extended secondary structures, in particular α-helices, the effective dipole of the peptide bond can have a strongly stabilizing effect on secondary structure.4 This reduces rotation around the carbon–nitrogen bond, making only*cis/trans* isomers allowable. Although this is not discussed here, the student should recall discussions about energetically favored bond rotations in organic chemistry. The *trans*peptide bond orientation is energetically preferred, with the *cis* conformer appearing in only specialized cases, in particular, around the conformationally constrained proline. As discussed in general biochemistry courses, the secondary structure of a peptide is then determined by rotation around the*φ* and Ψ angles only. Finally, amino acids naturally occur in the L-conformer, with the exception of glycine. This chirality is followed in almost all peptides and proteins, resulting in a profound effect on function. An example of chirality with an L- and a D-amino acid is presented in the Key Concept Box Chirality of Amino Acids as a refresher.

Key Concept

Atomic Structure and Bonding

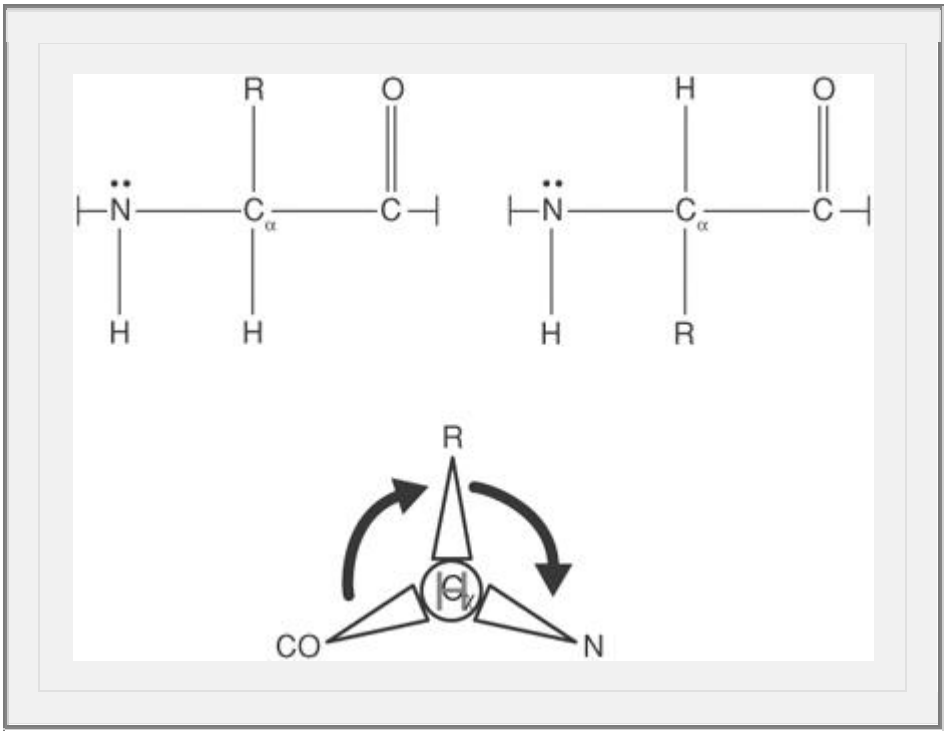


Consider two carbon atoms that are not bound. Each nucleus, carrying six protons (6P) and six neutrons (6n) and a net positive charge, is surrounded by a fairly uniform, six-electron (6e) cloud. Consider what happens when the two carbons form a covalent bond. Two electrons, one from each carbon, fill the hybrid orbital space between the atoms. The nucleus no longer is surrounded by a uniform cloud of equal charge. The nucleus is now surrounded closely by five electrons and has an electron that is shared in the bond. The charge distribution around each nucleus is similar to that of the atom alone, yet its shape is different. As we will see in this chapter, the difference in this distribution relates to the physical property of the molecule. When the atoms alone or in the molecule are exposed to external energy fields, they will behave differently. Therefore, changes in atomic order when molecular bonding occurs can be measured by different techniques using different levels of external energy.

Key Concept

Chirality of Amino Acids

All α-amino acids, with the exception of glycine, have a chiral α-carbon. The L-conformer is preferred, yet the D-conformer is also found. To determine the conformer, Richardson2 and others proposed the “corn crib” structure. In connection with the figure shown here, if one holds the α-helix in front of one's eyes and looks down the bond with the α-carbon, reading clockwise around the α-carbon, one sees the word “CORN” pointing into the page for the L-amino acid conformer.



Additive and Constitutive Properties

A study of the physical properties of drug molecules is a prerequisite for product formulation and leads to a better understanding of the relationship between a drug's molecular

and physicochemical properties and its structure and action. These properties come from the molecular bonding order of the atoms in the molecule and 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). For example, mass is an additive property (the molecular weight is the sum of the masses of each atom in the molecule), whereas optical rotation may be thought of as a constitutive property because it depends on the chirality of the molecule, which is determined by atomic bonding order.

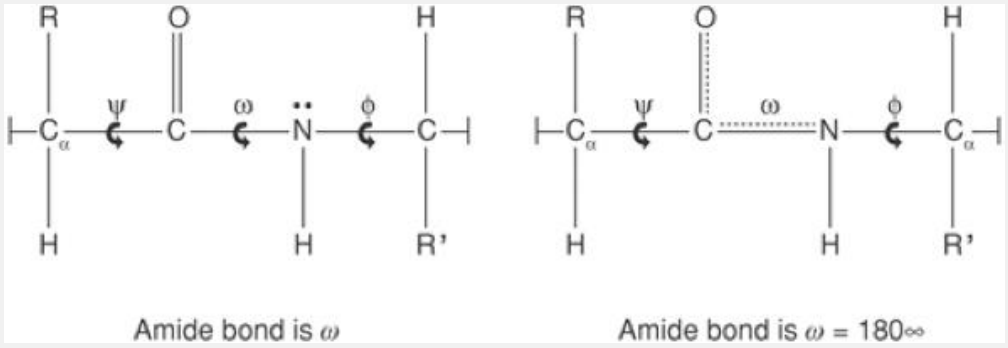
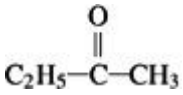


Fig. 4-1. The nature of an amide bond within a peptide/protein and its effect on

structure. As mentioned in the text, the*trans*isomer of a peptide is energetically favored due to steric hinderance of the amino acids. Proline (tertiary amine group) and glycine (two hydrogens, little steric hinderence from the R group) can more readily adapt a *trans*isomer conformation in a peptide or protein than can the other amino acids.

Many physical properties are constitutive and yet have some measure of additivity. The molar refraction of a compound, for example, is the sum of the refraction of the atoms and the array of functional groups making up the compound. Because the arrangements of atoms in each group are different, the refractive index of two molecules will also be different. That is, the individual groups in two different molecules contribute different amounts to the overall refraction of the molecules.

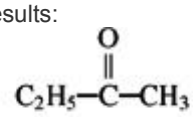
A sample calculation will clarify the principle of additivity and constitutivity. The molar refractions of the two compounds



and

$$CH_3{-}CH{=}CH{-}CH_2{-}OH,$$

which have exactly the same number of carbon, hydrogen, and oxygen atoms, are calculated using Table 4-1. We obtain the following results:



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 and type of atoms, their molar refractions are different. The molar refractions of the atoms are additive, but the carbon and oxygen atoms are constitutive in refraction. Refraction of a single-bonded carbon does not add equally to that of 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. These will be more comprehensively discussed in this chapter and in other sections of the book.

Molecular perturbations result from external sources that exert energy on molecules. Actually, every molecule that exists does so under atmospheric conditions provided that ideality is excluded. As such, it exists in a perturbed natural state. Hence, physical properties encompass the specific relations between the atoms in 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, whereas that of optical rotation uses plane-polarized light to describe the optical rotation of molecules organized in a particular bonding pattern. Therefore, a physical property should be easily

measured or calculated, and such measurements should be reproducible for an individual molecule under optimal circumstances.

Table 4-1 Atomic and Group Contributions to Molar Refraction*

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

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

When one carefully associates specific physical properties with the chemical nature of closely related molecules, one can (*a*) describe the spatial arrangement of atoms in drug molecules, (*b*) provide evidence for the relative chemical or physical behavior of a molecule, and (*c*) suggest methods for the qualitative and quantitative analysis of a particular pharmaceutical agent. The first and second of these often lead to implications about the 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. The level of understanding of the physical properties of molecules has

expanded greatly and, with increasing advances in technology, the development of computer-based computational tools to develop molecules with ideal physical properties has become commonplace. Nowhere has the impact of computational modeling been of broader importance than in drug discovery and design, where computational tools offer great promise for enhancing the speed of drug design and selection. For example, computer modeling of therapeutic targets (e.g., HIV protease) is widely utilized. This approach is based on the screening of known or predicted physical properties (e.g., the protein conformation of the HIV protease) of a therapeutic target in a computer to elucidate area(s) where a molecule can be synthesized to optimally bind and either block (antagonist) or enhance (agonist) the actions of the target. This type of modeling usually reveals a limited number of atomic spatial arrangements and types, such as a functional group, that can be utilized in the design of a molecule to elicit the desired pharmacologic response. Other computational programs can then be utilized to compute the number of molecules that might make reasonable drugs based on these molecular restrictions.

It is important to note that these models are relatively new but offer great potential for the future of drug discovery and design. It is also important to note that all of these computational approaches are based solely on the physical properties of molecule(s) that have been determined through years of research. The ability to design and test these molecules using a computer and to rapidly synthesize chemical libraries has also dramatically increased the need for technologically superior equipment to measure the physical properties of these molecules to confirm that the actual properties match those that are predicted. Toward that end, chemical libraries can reach well over 10,000 individual molecules and can be tested for numerous molecular properties in modern *high-throughput systems*. In fact, “virtual” libraries routinely contain millions of compounds. This aids in the ability of scientists to select one lead compound whose potential to make it into clinical use is greater than all of the other molecules screened. Finally, the ability to measure the physical properties of a lead compound is critical to assuring that during the transition from computer to large-scale manufacturing, the initially identified molecule remains physically the same. This is a guideline that the Food and Drug Administration requires to ensure human safety and drug efficacy.

The remainder of this chapter describes some of the well-defined interactions that are important for determining the physical properties of molecules. All of the quantities presented are expressed in Système International (SI) units for all practical cases.

Dielectric Constant and Induced Polarization

Electricity is related to the nature of charges in a dynamic state (e.g., electron flow). When a charged molecule is at rest, its properties are defined by electrostatics. For two charges separated by a distance *r*, their potential energy is defined by Coulomb's law,

$$U(r) = \frac{q_1 q_2}{4\pi\varepsilon_0 r} \qquad \textbf{(4–1)}$$

where the charges q_1 and q_2 are in coulombs (C), *r* is in meters, ε_0 (the permittivity constant) = 8.854×10^{-12} C2 N^{-1} m^{-2}, and the potential energy is in joules. Coulomb's law can be used to describe both attractive and repulsive interactions. From equation **(**4-1**)**, it is clear that electrostatic interactions also rely on the permittivity of the medium in which the charges exist. Different solvents have differing permittivities due to their chemical nature, polar or nonpolar. For a more comprehensive discussion, see Bergethon and Simons.5

When no permanent charges exist in molecules, a measure of their polarity (i.e., the electronic distribution within the molecule) is given by the property we called the dipole moment (μ). In the simplest description, a dipole is a separation of two opposing charges over a distance *r* (Fig. 4-2) and is generally described by a vector whose magnitude is given by $\mu = qr$. Dipoles do not have a net charge, but this charge separation can often create chargelike interactions and influence several physical and chemical properties. In complex molecules, the electron distribution can be approximated so that overall dipole moments can be estimated from partial atomic charges. A key feature of this calculation is the symmetry of the molecule. A symmetric molecule will display no dipole moment either because there is no charge

separation (e.g., H_2, O_2, N_2) or as a consequence of cancellation of the dipole vectors (e.g., benzene, methane).

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. The

dipolar nature of a peptide bond is an example of a fixed or permanent dipole, and its effects on the bonding structure were discussed earlier in this chapter. In proteins, the permanent dipolar nature of the peptide bond and side chains can stabilize secondary structures like α-helices and can also influence the higher-order conformation of a protein. Induced dipoles in a protein can also influence hydrophobic core protein structures, so both types of dipole play a prominent role in the stabilization of protein-derived therapeutics. Permanent dipoles in a molecule are discussed in the next section.

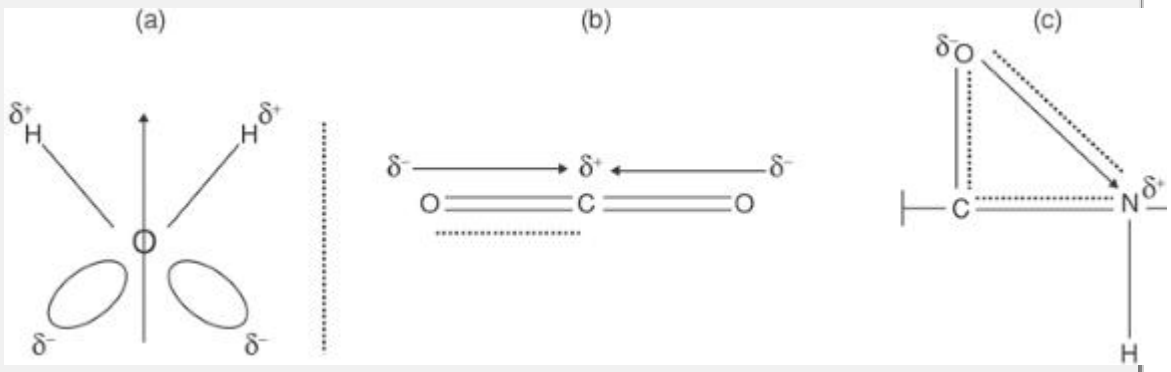


Fig. 4-2. Vectorial nature of permanent dipole moments for (*a*) water, (*b*) carbon dioxide, and (*c*) a peptide bond. The distance *r* is given by the dashed line for each molecule. The arrow represents the conventional direction that a dipole moment vector is drawn, from negative to positive.

To properly discuss dipoles and the effects of solvation, one must understand the concepts of polarity and dielectric constant. Placing a molecule in an electric field is one way to induce a dipole. Consider two parallel conducting plates, such as the plates of an electric condenser, which are separated by some medium across a distance *r*, and apply a potential across the plates (Fig. 4-3). 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 [F]) is equal to the quantity of electric charge (*q*, in coulombs) stored on the plates divided by the potential difference (*V*, in volts [V]) between the plates:

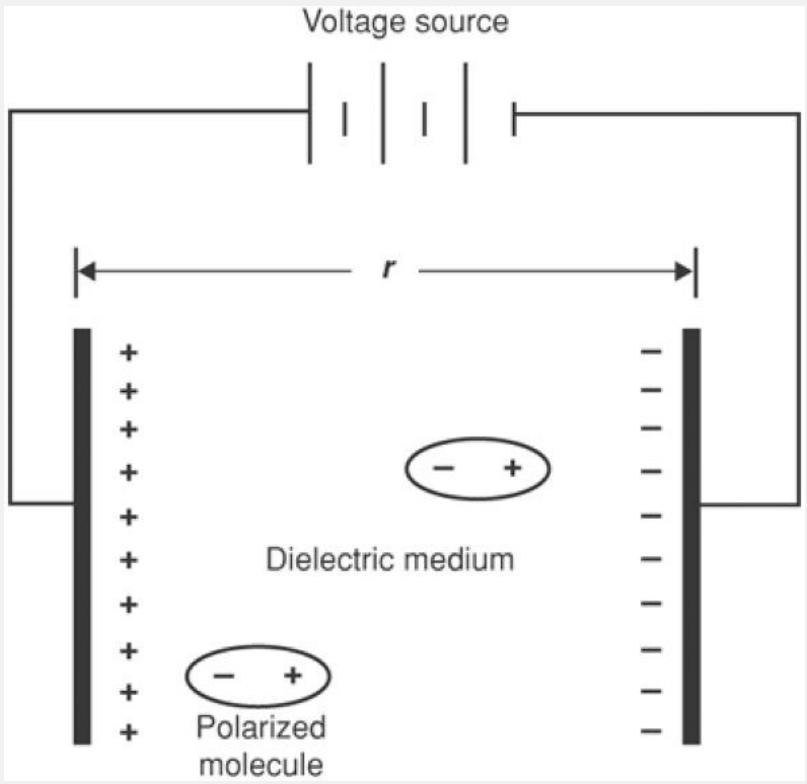


Fig. 4-3. Parallel-plate condenser.

$$C = q/V \qquad (4\text{–}2)$$

The capacitance of the condenser in Figure 4-3 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 because 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 (see Fig. 4-3). 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:

$$\varepsilon = C_x/C_0 \qquad (4\text{–}3)$$

The dielectric constant ordinarily has no dimensions because it is the ratio of two capacitances. By definition, the dielectric constant of a vacuum is unity. Dielectric constants of some liquids are listed in Table 4-2. The dielectric constants of solvent mixtures can be related to drug solubility as described by Gorman and Hall,6and ε for drug vehicles can be related to drug plasma concentration as reported by Pagay et al.7

The dielectric constant is a measure of the ability of molecule to resist charge separation. If the ratios of the capacitances are close, then there is greater resistance to a charge separation. As the ratios increase, so too does the molecule's ability to separate charges. Note that field strength and temperature play a role in these measurements. If a molecule has a permanent dipole, it will have minimal resistance to charge separation and will migrate in the field. However, the converse is also true. Consider a water molecule at the atomic level with its positively charged nuclei drawn toward the negative plate and its negatively charged electron cloud pulled and oriented toward the positive plate. Oxygen is strongly

electronegative and will have a stronger attraction to the positive pole than will a less electronegative atom. Likewise, hydrogen atoms are more electropositive and will move further toward the positive plate than an atom that is more electronegative. Conceptually, this will reflect less resistance to the field.

Table 4-2 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	
l-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	
Toluene	2.38	
Beeswax (solid)	2.8	
Benzene	2.27	
Carbon tetrachloride	2.23	
l,4-Dioxane	2.21	
Pentane	1.84	(20°C)
Furfural	41	(20°C)
Pyridine	12.3	
Methyl salicylate	9.41	(30°C)

A molecule placed in that field will align itself in the same orientation as the water molecules even though the extent of the alignment and induced charge separation will dramatically differ due to atomic structure. For this discussion, consider a molecule like pentane. Pentane is an aliphatic hydrocarbon that is not charged and relies on van der Waals interactions for its primary attractive energies. Pentane is not a polar molecule, and carbon is not strongly electronegative. Carbon–hydrogen bonds are much stronger (less acidic) than oxygen–hydrogen bonds; therefore, the electrons in the σ bonds are more shared. The electronic structure would not favor a large charge separation in the molecule and should

have a lower dielectric constant. If a polar functional group such as an alcohol moiety is added to generate 1-pentanol, an enhanced ability to undergo charge separation and an increased dielectric constant is the result. This is readily observed in Table 4-2, where the dielectric constant for pentane (at 20°C) is 1.84, that for 1-pentanol (at 25°C) is 13.9, and that for isopentanol is 14.7 (at 25°C).

Key Concept

Polarizability

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 or light energy or through interaction with another molecule. Large anions have large polarizabilities because of their loosely held outer electrons. Polarizabilities for molecules are given in the table. The units for α_p are Å3 or 10^{-24} cm^3.

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

When nonpolar molecules like pentane are placed in a suitable solvent between the plates of a charged capacitor, an *induced polarization* of the molecules can occur. Again, this *induced dipole* occurs because of the separation of electric charge within the molecule due to the electric field generated between the plates. The displacement of electrons and nuclei from their original positions is the main result of the induction process. This temporary induced dipole moment is proportional to the field strength of the capacitor and the *induced polarizability*, α_p, which is a characteristic property of the particular molecule.

From electromagnetic theory, it is possible to obtain the relationship

$$\frac{\varepsilon - 1}{\varepsilon + 2} = \frac{4}{3}\pi n\alpha_p \qquad \textbf{(4–4)}$$

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

$$\left(\frac{\varepsilon - 1}{\varepsilon + 2}\right)\frac{M}{\rho} = \frac{4}{3}\frac{\pi n M\alpha_p}{\rho} = \frac{4}{3}\pi N\alpha_p = P_i \qquad \textbf{(4–5)}$$

where 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 4-1
Polarizability of Chloroform
Chloroform has a molecular weight of 119 g/mole and a density of 1.43 g/cm^3 at 25°C. What is its induced molar polarizability? We have

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

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, whereas 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 partial charges, these charges balance each other so that the molecule does not have a net charge. Again, we can relate this to atomic structure by considering the electronegativity of the atoms in a bond. Water molecules possess a permanent dipole due to the differences in the oxygen and the hydrogen. The magnitude of the permanent dipole moment, 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 itself, including those from bonds and lone-pair electrons. The vectors depend on the distance of separation between the charges. Figure 4-2 provides an illustration of dipole moment vectors for water, carbon dioxide, and a peptide bond. The unit of μ is the *debye*, with 1 debye equal to 10^{-18} electrostatic unit (esu) cm. The esu is the measure of electrostatic charge, defined as a charge in a vacuum that repels a like charge 1 cm away with a force of 1 dyne. In SI units, 1 debye = 3.34×10^{-30} C-m. 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 or 1 Å).

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. When the applied field is present, the orientation is in the direction of the field. Maximum dipole moments occur when the molecules are almost perfectly oriented with respect to the fields. Absolutely perfect orientation can never occur owing to thermal energy of the molecules, which contributes to molecular motion that opposes the molecular alignment. The *total* molar polarization,P, is the sum of induction and permanent dipole effects:

$$P = P_i + P_0 = \left(\frac{\varepsilon - 1}{\varepsilon + 2}\right)\frac{M}{\rho} \qquad (4\text{–}6)$$

where 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} joule/K. Because P_0depends on the temperature, T, equation **(**4-6**)** can be rewritten in a linear form as

$$P = P_i + A\frac{1}{T} \qquad (4\text{–}7)$$

where 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 **(**4-6**)** 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 4-3.

In solution, the permanent dipole of a solvent such as water can strongly interact with solute molecules. This interaction contributes to the 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, which is observed with carbon dioxide (no net dipole) in Figure 4-2. Likewise, 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 4-3.

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 (Chapter 9). For drug–receptor binding, dipole–dipole interactions are essential noncovalent forces that contribute to enhance the pharmacologic effect, as described by Kollman.8 For solids composed of molecules with permanent dipole moments, the dipole interactions contribute to the crystalline arrangement and overall structural nature of the solid. For instance, water molecules in ice crystals are organized through their dipole forces. Additional interpretations of the significance of dipole moments are given by Minkin et al.9

Electromagnetic Radiation

Electromagnetic radiation is a form of energy that propagates through space as oscillating electric and magnetic fields at right angles to each other and to the direction of the propagation, shown in Figure 4-4a. Both electric and magnetic fields can be described by sinusoidal waves with characteristic amplitude, *A*, and frequency, *v*. The common representation of the electric field in two dimensions is shown in

Figure 4-4b. This frequency, *v*, is the number of waves passing a fixed point in 1 sec. 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 the velocity of propagation, *v*:

Table 4-3 Dipole Moments of Some Compounds

Compound	Dipole Moment (Debye Units)
p-Dichlorobenzene	0
H_2	0
Carbon dioxide	0
Benzene	0
l,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

$$\nu = \nu\lambda \qquad (4\text{–}8)$$

The frequency of the radiation depends on the source and remains constant; however, the velocity depends on the composition of the medium through which it passes. In a vacuum, the wave of radiation travels at its maximum, the speed of light, $c = 2.99792 \times 10^8$ m/sec. Thus, the frequency can be defined as

$$\nu = c/\lambda \qquad (4\text{–}9)$$

The wave number, *[v with bar above]*, defined as the reciprocal of the wavelength, is another way of describing the electromagnetic radiation:

$$\overline{\nu} = \frac{1}{\lambda} = \frac{\nu}{\nu} \qquad \textbf{(4–10)}$$

The wave number (in cm^{-1}) represents the number of wavelengths found in 1 cm of radiation in a vacuum. It is widely used because it is directly proportional to the frequency, and thus to the energy of radiation, *E*, given by the famous and fundamental relationship of Planck and Einstein for the light quantum or energy of a photon:

$$E = h\nu = hc\overline{\nu} \qquad \textbf{(4–11)}$$

where *h* is Planck's constant, which is equal to 6.6261×10^{-34} joules.

The electromagnetic spectrum is classified according to its wavelength or its corresponding wave number, as illustrated in Table 4-4. The wavelength becomes shorter as the corresponding radiant energy increases. Most of our knowledge about atomic and molecular structure and properties comes from the interaction of electromagnetic radiation with matter. The electric field component is mostly responsible for phenomena such as transmission, reflection, refraction, absorption, and emission of electromagnetic radiation, which give rise to many of the spectroscopic techniques discussed in this chapter. The magnetic component is responsible for the absorption of energy in electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR), techniques described at the end of the chapter.

Atomic and Molecular Spectra

All atoms and molecules absorb electromagnetic radiation at certain characteristic frequencies. According to elementary quantum theory, ions and molecules can exist only in certain discrete states characterized by finite amounts of energy (i.e., energy levels or electronic states) because electrons are in motion around the positively charged nucleus in atoms. Thus, the radiant energy absorbed by a chemical species has only certain discrete values corresponding to the individual transitions from one energy state (E_1) to a second, upper energy state (E_2) that can occur in an atom or molecule (Fig. 4-5). The pattern of absorption frequencies is called an *absorption spectrum* and is produced if radiation of a particular wavelength is passed through a sample and measured. If the energy of the radiation is decreased upon exit, the change in the intensity of the radiation is due to electronic excitation.

In some instances, electromagnetic radiation can also be produced (*emission*) when the excited species (atoms, ions, or molecules excited by means of the heat of a flame, an electric

current spark, absorption of light, or some other energy source) relax to lower energy levels by releasing energy as photons (*hv*). The emitted radiation as a function of the wavelength or frequency is called the emission spectrum.

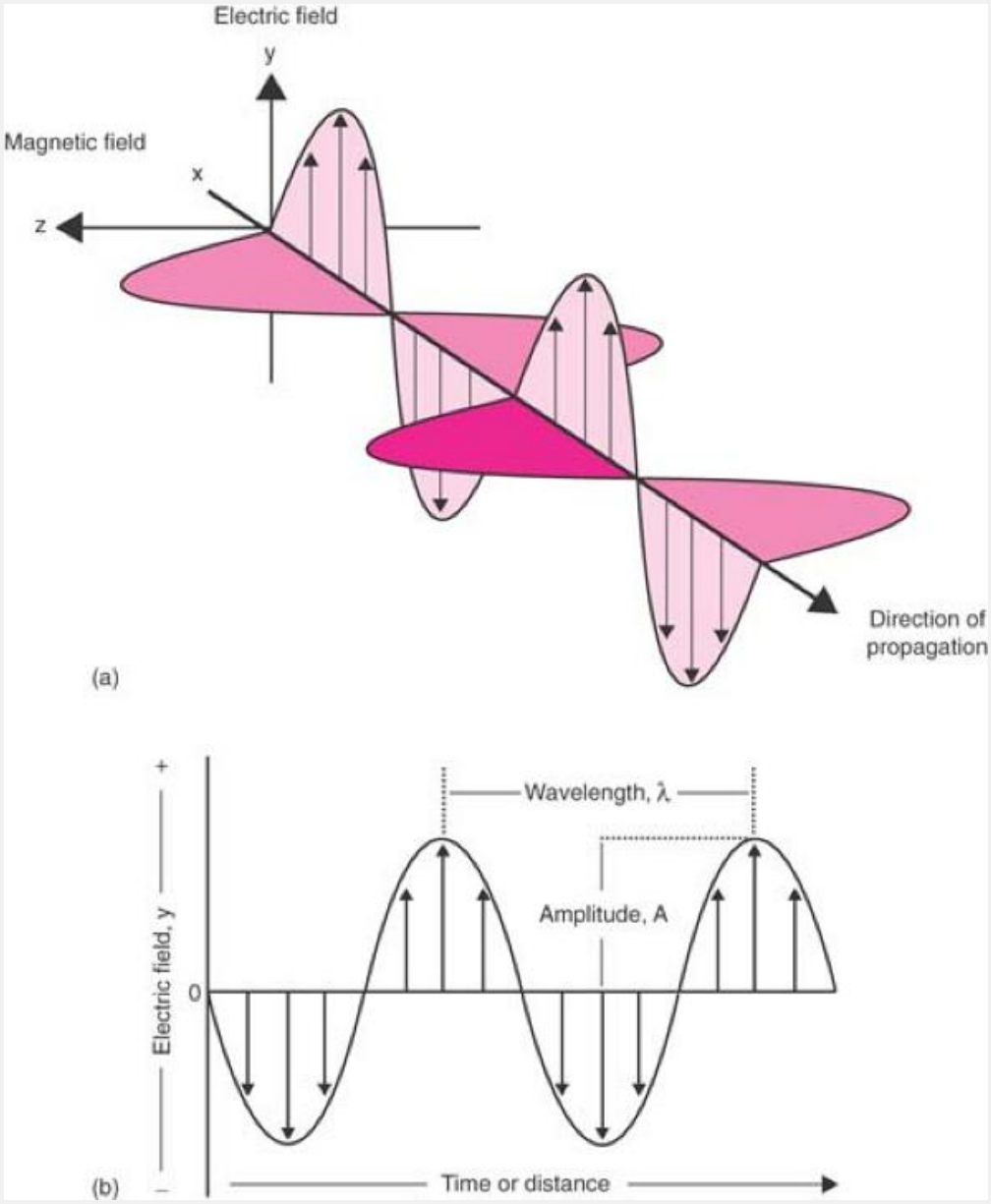


Fig. 4-4. The oscillating electric and magnetic fields associated with electromagnetic radiation.

Atomic spectra are the simplest to describe and usually present a series of lines corresponding to the frequencies of specific electronic transition states. An exact description of these transitions is possible only for the hydrogen atom, for which a complete quantum mechanical solution exists.10 Even so, the absorption and emission spectra for the hydrogen atom also can be explained using a less sophisticated theory such as the Bohr's model. In this approach, the energy of an electron moving in a definite orbit around a positively charged nucleus in an atom is given by

$$E = -\frac{2\pi^2 Z^2 m e^4}{n^2 h^2} \qquad (4\text{–}12)$$

where Z is the atomic number, m is the mass of the electron (9.1×10^{-31} kg), n is an integer number corresponding to the principal quantum number of the orbit, e is the charge on the electron (1.602×10^{-19} coulomb or 1.519×10^{-14} $m^{3/2}$ $kg^{1/2}$ sec^{-1}), and h is Planck's constant.

Bohr's model also assumes that electrons moving in these orbits are stable (i.e., angular momentum is quantized), and therefore absorption or emission occurs only when electrons change orbit. Thus, the characteristic frequency of the photon absorbed or emitted corresponds to the absolute value of the difference of the energy states involved in an electronic transition in an atom. Thus, the difference between electron energy levels, E_2 - E_1, having respective quantum numbers $n_2$and n_1, is given by the expression

$$E_2 - E_1 = \frac{2\pi^2 Z^2 me^4}{h^2}\left(\frac{1}{n_1^2} - \frac{1}{n_2^2}\right) \qquad \textbf{(4–13)}$$

The energy of a photon of electromagnetic radiation E is related to the frequency of the radiation by equation (4-12). Substituting the energy difference by the corresponding

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characteristic wave number according to equation (4-11) in equation (4-13), we obtain

$$\bar{\nu} = \frac{2\pi^2 Z^2 me^4}{h^3 c}\left(\frac{1}{n_1^2} - \frac{1}{n_2^2}\right) \qquad \textbf{(4–14)}$$

Table 4-4 Electromagnetic Radiation Ranges According to Wavelength, Wave number, Frequency, and Energy, and Techniques That Utilize These Forms of radiation*

Electomagnetic Radiation	Wavelength λ (m)	Wave Number [v with bar above] (cm^{-1})	Frequency (Hz)	Energy (joule)	Representative Techniques (Not Inclusive)
Gamma rays	$<1 \times 10^{-11}$	$>1 \times 10^{9}$	$>3 \times 10^{19}$	$>2 \times 10^{-14}$	Microscopy, photography, radioactivity tracer
X-rays	1×10^{-11}–1×10^{-8}	1×10^{9} –1×10^{6}	3×10^{19}–3×10^{16}	2×10^{-14} –2×10^{-17}	Microscopy, photography, radioactivity tracer, diffraction, ionization
Vacuum ultraviolet	1×10^{-8}–2×10^{-7}	1×10^{6} –5	3×10^{16}–1.5 ×	2×10	Spectrophotometry

		$\times 10^4$	10^{15}	$-17 – 9.9 \times 10^{-19}$	
Near-ultraviolet	2×10^{-7}–4×10^{-7}	$5 \times 10^4 – 2.5 \times 10^4$	1.5×10^{15}–7.5×10^{14}	$9.9 \times 10^{-19} – 5 \times 10^{-19}$	Spectrophotometry, fluorescence, circular dichroism
Visible light	4×10^{-7}–7×10^{-7}	$2.5 \times 10^4 – 1.4 \times 10^4$	7.5×10^{14}–4×10^{14}	$5 \times 10^{-19} – 3 \times 10^{-19}$	Spectrophotometry, fluorescence, light scattering, optical rotation
Near-infrared	7×10^{-7}–5×10^{-6}	$1.4 \times 10^4 – 2 \times 10^3$	4×10^{14}–6×10^{13}	$3 \times 10^{-19} – 4 \times 10^{-20}$	Vibrational and rotational spectroscopy, solid–state characterization
Mid (intermediate) infrared	5×10^{-6}–3×10^{-5}	$2 \times 10^3 –$	6×10^{13}–$1 \times$	4×10	Vibrational and rotational spectroscopy

		3.33 × 10^2	10^{13}	-20 – 6.6 × 10^{-21}	
Far infrared	3×10^{-5}–1×10^{-3}	3.33 × 10^2 –10	1×10^{13}–3×10^{11}	6.6 × 10^{-21} –2 × 10^{-22}	Vibrational and rotational spectroscopy
Microwave	1×10^{-3}–0.1	10–0.1	3×10^{11}–3×10^9	2 × 10^{-22} –2 × 10^{-24}	Electron paramagnetic resonance
Radio	>0.1	<0.1	$<3 \times 10^9$	<2 × 10^{-24}	Nuclear magnetic resonance

*Information on the values was derived in part from the NASA Web site http://imagine.gsfc.nasa.gov/docs/science/know_11/spectrum_chart.html and Campbell and Dweb.[12]

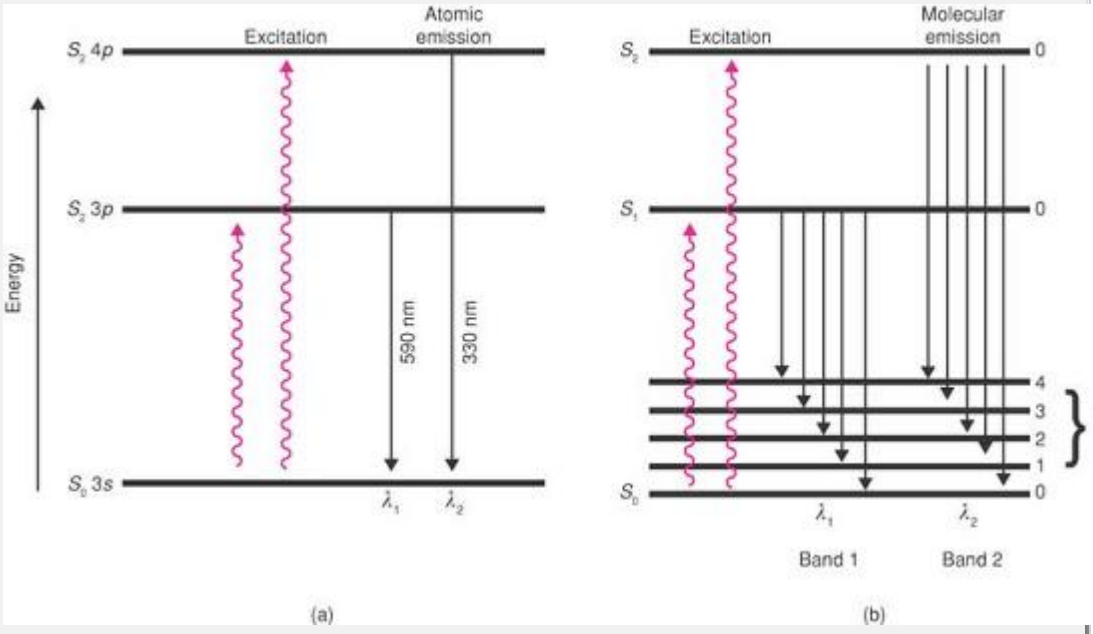

Fig. 4-5. Comparison of (*a*) atomic and (*b*) molecular energy levels.

where n_1 and n_2 are the principal quantum numbers for the orbital states involved in an electronic transition of the atom from level E_1 to E_2.

The first term on the right in equation **(**4-14**)** is composed of fundamental constants and the atomic number *Z*. For the hydrogen atom $Z = 1$, and substitution of the appropriate values affords a single constant R_8 of value 109,737.3 cm^{-1}. This value is calculated from the Bohr's theory and assumes that the nucleus of infinite mass was stationary with respect to the electron orbit. A more accurate analysis involves the replacement of the mass of the electron by the reduced mass of the electron in the hydrogen atom, $\mu_H = 0.9994558m$. The corrected theoretical value of R_8, now called R_H, is (109737.3) (0.9994558) = 109,677.6 cm^{-1}. The experimental value for this constant, also known as the *Rydberg constant*, is R_H = 109,678.76 cm^{-1}. The remarkable agreement between the theoretical and experimental values of R_H gave initial support to Bohr's theory, although it was later abandoned for the more complete quantum mechanical treatment.10Nevertheless, the absorption and emission spectra for the hydrogen atom are fully explained by Bohr's theory. Thus, introducing R_H in equation **(**4-14**)**, we find the characteristic wave number only from the values of *n*, the principal quantum number of the electron's orbit for the transition, given by the simple relation

$$\bar{\nu} = R_H \left(\frac{1}{n_1^2} - \frac{1}{n_2^2} \right) \qquad \textbf{(4–15)}$$

Example 4-2
Energy of Excitation

(*a*) What is the energy (in joules and cm^{-1}) 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, $n_2 = 2$?

We can solve this problem by using equation (4-13) and replacing the values of all physical constants involved and using $Z = 1$; we find

$$E_2 - E_1 = \frac{2 \times (3.14)^2 \times (1)^2 \times (9.1 \times 10^{-31}) \times (1.519 \times 10^{-14})^4}{(6.629 \times 10^{-34})^2} \times \left(\frac{1}{(1)^2} - \frac{1}{(2)^2} \right)$$
$$= 1.63 \times 10^{-18} \text{ joule}$$

Note that joule = kg × m^2 × sec^{-2}.

To obtain the answer in cm^{-1}, we can use equation (4-11) to convert joules into wave number, or directly use equation (4-15):

$$\bar{\nu} = R_H\left(\frac{1}{n_1^2} - \frac{1}{n_2^2}\right)$$

Using this equation we obtain,

$$\bar{\nu} = (109{,}677.6\ \text{cm}^{-1})\frac{1}{(1)^2} - \frac{1}{(2)^2} = 82{,}258.2\ \text{cm}^{-1}$$

and therefore, from equation (4-10), the wavelength of the spectral line when an electron passes from the n = 1 orbital to the n = 2 orbital is

$$\lambda = \frac{1}{\bar{\nu}} = \frac{1}{82{,}258.2} = 1.22 \times 10^{-5} \quad \text{or} \quad 122\ \text{nm}$$

This is the first line of the Lyman ultraviolet series of the atomic spectra for hydrogen. Note that, from equation **(**4-12**)**, the electron of the hydrogen atom in the ground state, n = 1, has a lower energy (-2.18×10^{-18} joule) than that in the next highest electron state, n = 2 (-0.55×10^{-18} joule).

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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 atom according to

$$H - le^- \rightarrow H^+$$

is also 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

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

Because $1/\infty$ [congruent] 0,

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

This is equivalent to $-E$ for n = 1, according to equation **(**4-13**)**. Thus, the ionization potential exactly equals the negative energy of the electron in the ground state with a value of 10,9677.6 cm^{-1} (equals 13.6 eV).

Virtually all atoms but hydrogen have more than one electron. Therefore, approximate methods are used to evaluate electronic transition states for most atoms. In these atoms, spin pairing energy and electron–electron repulsion arise, so that description of the electronic states is not as straightforward as in the hydrogen atom. For practical purposes, most elements of the periodic table display a characteristic atomic absorption and emission spectrum associated with the electronic transition states that can be used to identify and quantify specific elements. Some of the more sensitive spectral wavelengths associated with particular atoms are given in Table 4-5. For instance, the simplified energy-level diagram for the sodium atom in Figure 4-5a illustrates the source of the lines in a typical emission spectrum of gas-phase sodium. The single-outer-electron ground state for sodium is located in the 3*s* orbital. After excitation (i.e., absorption of energy as shown by wavy lines), two emission lines (about 330 and 590 nm) result from the transitions $4p \rightarrow 3s$ and $3p \rightarrow 3s$, respectively. Many types of atomic spectrometers are available depending on the methods used for atomization and introduction of the sample. Further detailed information can be found in the references cited in this chapter.

Table 4-5 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

Atomic spectroscopy has pharmaceutical applications in analysis of 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 depressive disorder), can be analyzed by atomic spectroscopy to check for overdosing of lithium salts.

In addition to having electronic states, molecules have quantized *vibrational states*, which are associated with energies due to interatomic vibrations (e.g., stretching and bending), and rotational states, which are related to the rotation of molecules around their center of gravity. These additional energy states available for electron transitions make the spectra of molecules more complex than those of atoms. In the case of vibration, the interatomic bonds may be thought of as springs between atoms (see Fig. 4-14) that can vibrate in various stretching or bending configurations depending on their energy levels. 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 is typically about 10^{-18} joule (electronic transitions absorb in the ultraviolet and visible light region between 180 and 780 nm; see Example 4-2), where the energy involved in vibrational changes is about 10^{-19} to 10^{-20} joule (infrared region) 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. The rotational energy levels are lower than the vibrational levels and are drawn in a similar manner to the quantized vibrational levels in the electronic states, as shown

in Figure 4-5b. The translational states are so numerous and the energy levels between translational states are so small that they are normally considered 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 4-5, the absorption of radiation (wavy lines) leads to two different energy transitions, ΔE, which result in the electronic transition from the lowest level of the ground state (S_0) to an excited electronic state (S_1 or S_2). Electronic transitions of molecules involve energies corresponding to ultraviolet or visible radiation.

Purely vibrational transitions may occur within the same electronic state (e.g., a change from level 1 to 2 in S_0) and involve near-infrared (IR) radiation. Rotational transitions (not shown in Fig. 4-5) are associated with low-energy radiation over the entire infrared wavelength region. The relatively large energy associated with an electronic transition usually leads to a variety of concurrent, 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 radio wave regions (see Table 4-4). Low-energy transitions involve the spin of electrons in the microwave region and the spin of nuclei in the radio wave region. The study of these transitions constitutes the fields of EPR and NMR spectroscopy. These various forms of molecular spectroscopy are discussed in the following sections.

Ultraviolet and Visible Spectrophotometry

Electromagnetic radiation in the ultraviolet (UV) and visible (Vis) regions of the spectrum fits the energy of electronic transitions of a wide variety of organic and inorganic molecules and ions. Absorbing species are usually classified according to the type of molecular energy levels involved in the electronic transition, which depends on the electronic bonding within the molecule.[11,12] Commonly, covalent bonding occurs as a result of a pair of electrons moving around the nuclei in a way that minimizes both internuclear and interelectronic Coulombic repulsions. Combinations of atomic orbitals (i.e., overlap) give rise to *molecular orbitals*, which are locations in space with an associated energy in which bonding electrons within a molecule can be found.

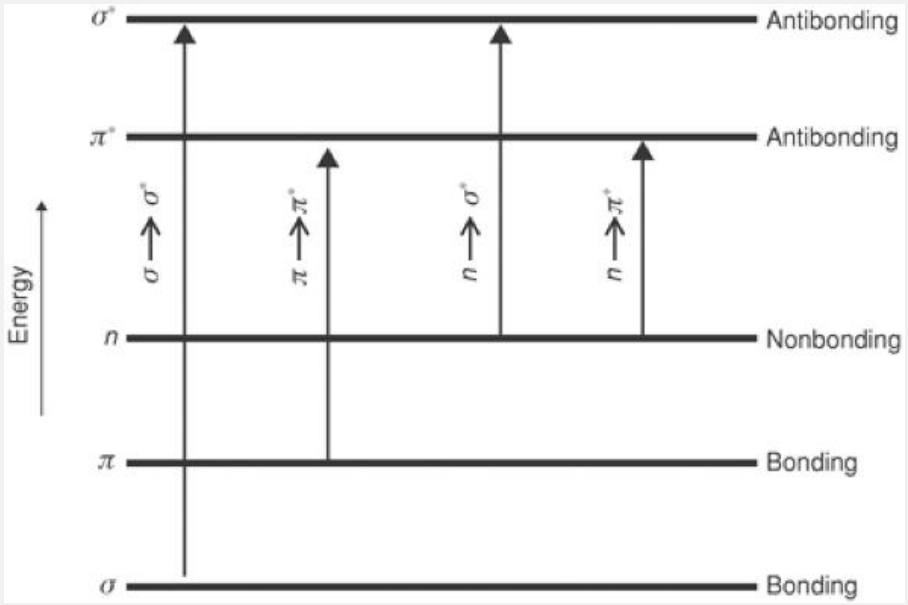

Fig. 4-6. Molecular orbital electronic transitions commonly found in ultraviolet–visible spectroscopy.

For instance, the molecular orbitals commonly associated with single covalent bonds in organic species are called sigma orbitals (σ), whereas a double bond is usually described as containing two types of molecular orbitals: one sigma and one pi (π). Molecular orbital energy levels usually follow the order shown in Figure 4-6.

Thus, when organic molecules are exposed to light in the UV–Vis regions of the spectrum (see Table 4-4), they absorb light of particular wavelengths depending on the type of electronic transition that is associated with the absorption. For example, hydrocarbons that contain σ-type bonds can undergo only $\sigma \rightarrow \sigma^*$ electronic transitions from their ground state. The asterisk (*) indicates the antibonding molecular orbital occupied by the electron in the excited state after absorption of a quantized amount of energy. These electronic transitions occur at 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 (see Fig. 4-6). For acetone, $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ 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 their 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*.

Most applications of absorption spectroscopy to organic molecules rely on transitions from n or π electrons to π* because the energies associated with these electronic transitions fall in an experimentally convenient region from 200 to 700 nm.

The amount of light absorbed by a sample is based on the measurement of the transmittance, T, or the absorbance, A, in transparent cuvettes or cells having a path length b (in cm) according to equation (4-16):

$$A = -\log T = \log(I_0/I) = \varepsilon bC \qquad (4\text{–}16)$$

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

Equation **(**4-16**)** is also known as *Beer's law*, and relates the amount of light absorbed (*A*) to the concentration of absorbing substance (*C* in mole/liter), the length of the path of radiation passing through the sample (*b* in cm), and the constant ε, known as the *molar absorptivity* for a particular absorbing species (in units of liter/mole cm). The molar absorptivity depends not only on the molecule whose absorbance is being determined but also on the type of solvent being used, the temperature, and the wavelength of light used for the analysis.

Example 4-3
Absorbance Maxima Changes Due to Chemical Instability

a. A solution of $c = 2 \times 10^{-5}$ mole/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?
c. $$\varepsilon = \frac{A}{bc} = \frac{6.48 \times 10^{-1}}{1 \times (2 \times 10^{-5})} = 3.24 \times 10^{4} \text{ liter/mole cm}$$
d. $$c = \frac{A}{b\varepsilon} = \frac{2.98 \times 10^{-1}}{1 \times (3.24 \times 10^{4})} = 9.20 \times 10^{-6} \text{ mole/liter}$$

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 as it would be in 0.1 N HCl. A lactam is formed from the drug under acid conditions that has an absorbance maximum at 245 rather than 260 nm and a correspondingly different ε value. Large values for molar absorptivities are usually found in molecules having a high degree of conjugation of chromophores, such as the conjugated double bonds in 1,3-butadiene, or the combination of carbonyl or carboxylic acids with double bonds as in α, β unsaturated ketones. In particular, highly conjugated molecules such as aromatic hydrocarbons and heterocycles typically display absorption bands characteristic of $\pi \rightarrow \pi^*$ transitions and have significantly higher ε values.

Example 4-4
Absorption Maxima Calculation

Aminacrine is an anti-infective agent with the following molecular structure:

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 cm, 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,

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

and 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, which is reflective of a higher number of electronic transitions that can occur at the higher energy. 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, the minimum absorbance level for detection of a compound depends on both the instrumental conditions and the sample state, for example, the solvent chosen for dissolution of the sample. Instrumental conditions vary across spectrophotometers, and will be discussed later. With regard to the sample conditions, solvents may have dramatically different polarities, which are addressed in the Dielectric Constant and Induced Polarization section. The polarity of a solvent may influence the bonding state of a molecule by changing the attractive and repulsive forces in the molecule through solvation. For example, a polar compound dissolved in water will have different capacities to undergo electronic transitions than if it were dissolved in a nonpolar, aprotic solution.
As illustrated in Example 4-4, a molecule may have more than one characteristic absorption wavelength band, and the complete UV–Vis wavelength absorption spectrum can provide information for the positive identification of a compound. Each functional group has different associated molecular bonding and electronic structure, which will have different wavelengths that maximally absorb energy. Therefore, the resulting spectrum for a molecule can be used as a "molecular fingerprint" to determine the molecular structure. Changes in the order of bonding or in electronic structure will

alter the UV–Vis spectrum, as illustrated with chlordiazepoxide and its lactam. As a result, many different molecular properties can be monitored by using a UV–Vis spectrophotometer including chemical reactions, complexation, and degradation, making it a powerful tool for pharmaceutical scientists.

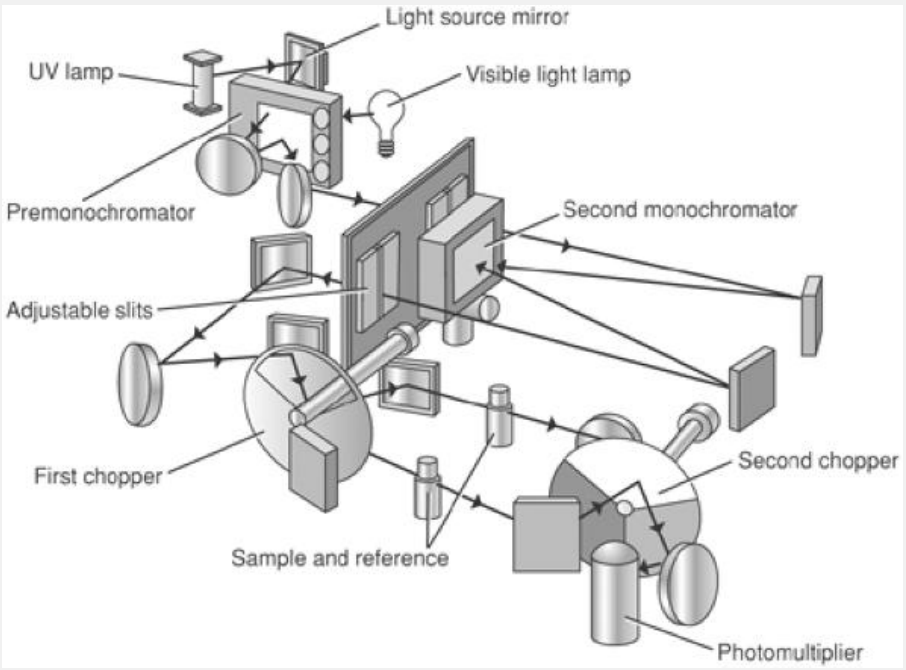

Fig. 4-7. A double-beam ultraviolet–visible spectrophotometer. (Courtesy of Varian, Inc., Palo Alto, CA.)

Absorption spectroscopy is one of the most widely used methods for quantitative analysis due to its wide applicability to both organic and inorganic systems, moderate to high sensitivity and selectivity, and good accuracy and convenience. Modern spectroscopic instrumentation for measuring the complete molecular UV–Vis absorption spectra is commonplace in both industrial and academic settings. Two main types of spectrophotometers, usually coupled to personal computers for data analysis, are commercially available: double-beam and diode-array instruments.

A schematic diagram of a traditional double-beam UV–Vis spectrophotometer is shown in Figure 4-7. 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," with one beam passed through the reference, which is typically the blank solvent used to dissolve the sample, and the other through the sample cell containing the test molecule. 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. In addition, it is common practice to first put the solvent in the sample cell and set the spectrophotometer's absorbance to zero to serve as the baseline reference. The samples can then be placed in the sample cell and measured, with the difference from the baseline being reported. The sample data are reported as a plot of the intensity, usually as absorbance, against the wavelength, as shown for the chlordiazepam lactam in Figure 4-8.

Figure 4-9 is an illustration of a typical diode-array spectrophotometer. Note that these instruments have simpler optical components, and, as a result, the radiation throughput is much higher than in traditional double-beam instruments. After the light beam passes the sample, the radiation is focused on an entrance slit and directed to a grating. The transducer is a diode array from which resolutions of 0.5 nm can be reached. A single scan from 200 to 1100 nm takes only 0.1 sec, which leads to a significant improvement in the signal-to-noise ratio by accumulating multiple scans in a short time.

Typically, a calibration curve from a series of standard solutions of known but varying concentration is used to generate standard curves for quantitative analysis. An absorbance spectrum can be used to determine one wavelength, typically an absorption maximum, where the absorbance of each sample can be efficiently measured. The absorbance for standards is measured at this point and plotted against the concentration, as shown in Figure 4-10, 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 due to changes in the electronic structure in the associated or dissociated states (Chapter 8), which influence the bonding structure. This change results in 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 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 the pK_a is defined by

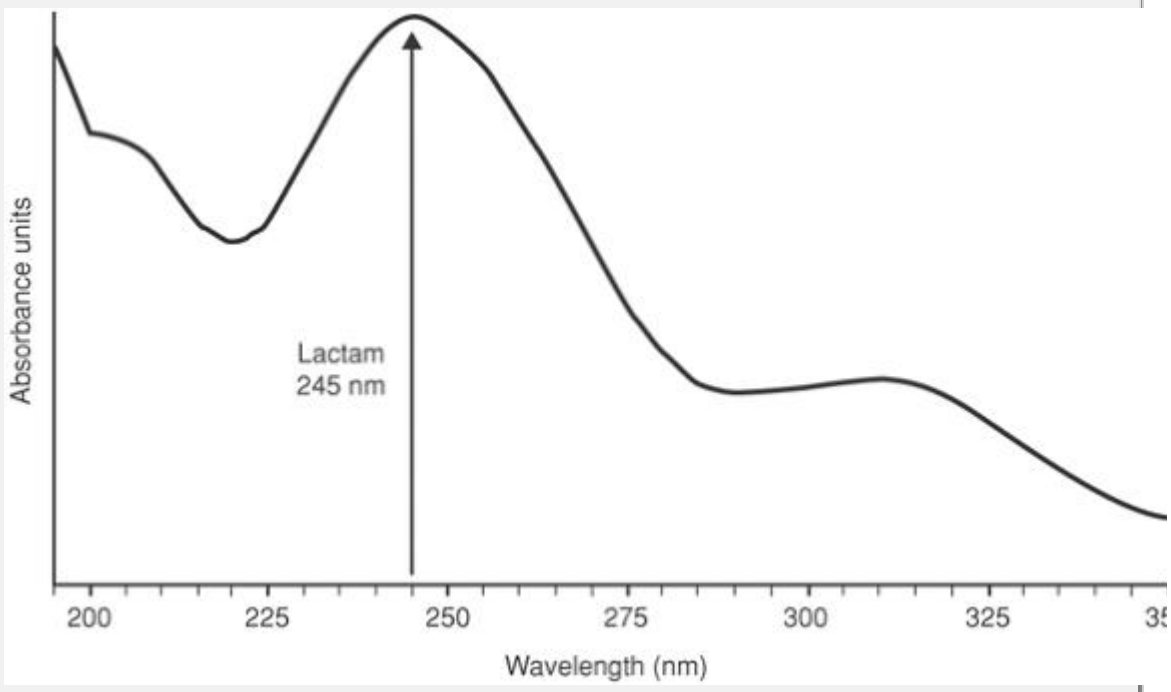

Fig. 4-8. The absorbance maxima of chlordiazepoxide lactam as a function of the wavelength. (Modified from http://chrom.tutms.tut.ac.jp/JINNO/DRUGDATA/17chlordia-zepoxide.html)

$$pK_a = pH + \log\frac{[HA]}{[A^-]} \qquad (4\text{–}17)$$

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

Example 4-5

Ionization State and Absorption

Phenobarbital shows a maximum absorption of 240 nm as the monosodium salt (A^-), whereas 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 and the pH of the solution and the absorbance at 240 nm are measured 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?

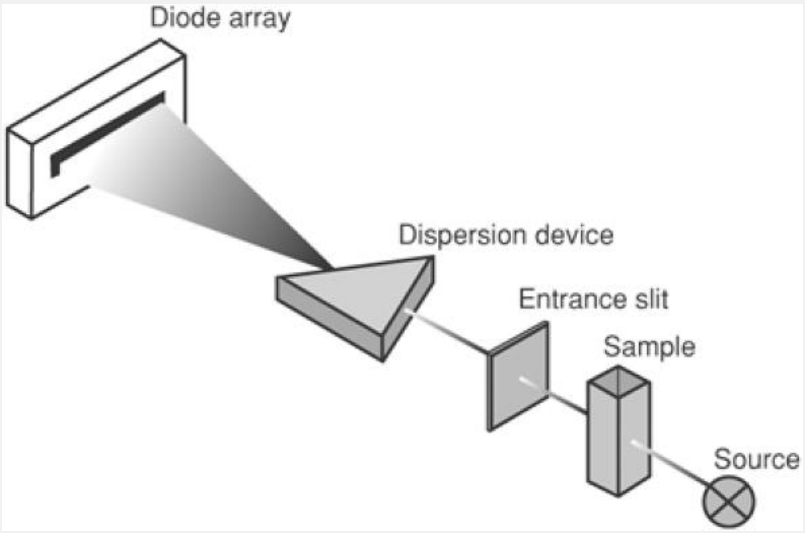

Fig. 4-9. Schematic of an HP-8453 diode-array ultraviolet–visible spectrophotometer. (Courtesy of Hewlett-Packard, Palo Alto, CA.)

By plotting the absorbance against the pH over the titration range to pH = 10, one can obtain the midpoint in absorbance, where half the free acid has been titrated, and [HA] = [A^-] (Fig. 4-8). The pH corresponding to this absorbance midpoint is approximately equal to the pK_a, namely, pK_{a1} for the first ionization stage of phenobarbital. This midpoint occurs at a pH of 7.3; therefore, pK_a [congruent] 7.3. For more accurate pK_a determinations, refer to the discussion in Chapter 8.

Reaction rates can also be measured when 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 particular wavelength chosen for this determination, the reaction rate will simply be proportional to the rate of change of absorbance with reaction time. Standard curves can be generated if the selected reactant is available in a pure form. It must be noted that the assumption that the other species or reaction intermediates do not interfere with the selected reactant can also be flawed, as discussed later. An example of the use of spectrophotometry for the determination of reaction rates in pharmaceutics is given by Jivani and Stella,13 who used the disappearance of *para*-aminosalicylic acid from solution to determine its rate of decarboxylation.

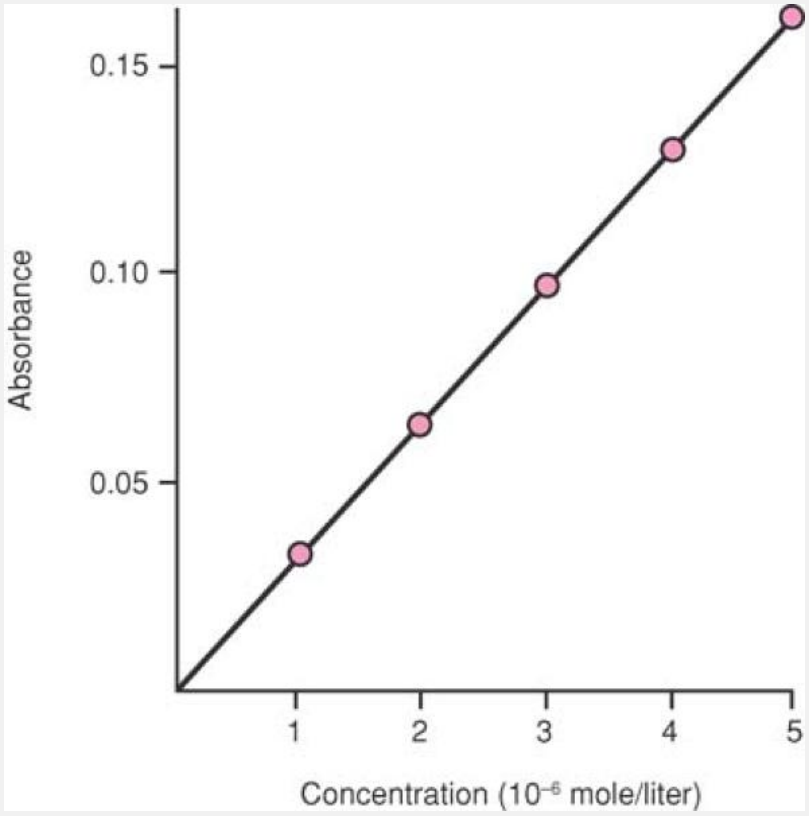

Fig. 4-10. A Beer's-law plot of absorbance against the concentration of chlordiazepoxide.

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.14 Clavulanic acid inhibits the activity of β-lactamase enzymes, which convert 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.

The primary weakness with a spectrophotometer used in such a manner is that it measures all of the species in a sample even though a single wavelength might be selected for its molecular detection specificity. UV–Vis spectra generated at a given wavelength(s) cannot properly detect changes in the species that arise during a reaction. For example, a reaction intermediate(s) may be generated that has stronger absorption at the wavelength selected than the reactant or the product being measured. Finally, there are many different reaction pathways that include multiple species with overlapping absorption, so the selection of a single wavelength for detection does not provide good selectivity. One way to correct this is to generate a UV–Vis absorption scan across a wide wavelength range and measure changes in several specific absorption maxima. However, the same problems can obfuscate the data in this approach as well. Therefore, in many cases, the complex reaction milieu is not readily adaptable to a single spectroscopic method. Thus, an ability to separate the individual species and quantitate their levels would provide enhanced sensitivity. UV–Vis spectrophotometers are also used in conjunction with many other methods for detecting molecules, for example, high-performance liquid chromatography

(HPLC), which eliminates species interference by separating the compounds before detection occurs. The major use of spectrophotometry is in the field of quantitative analysis, in which the absorbance of chromophores is determined. Various applications of spectrophotometry are discussed by Schulman and Vogt.15

Fluorescence and Phosphorescence

Luminescence is an emission of radiation in the ultraviolet, visible, or near-IR regions from electronically excited species. An electron in an atom or a molecule can be excited by means of absorbing energy, for instance a photon of light, to reach an electronic excited state (see prior discussion of orbital transitions). The excited state is relatively short-lived, and the electron can return to its ground state via radiative and nonradiative energy emission. If the preferred path to return to the ground state involves releasing energy through internal conversions by changes in vibrational states or through collisions with the environment (e.g., solvent molecules), then the molecule will not display luminescence. Many chemical species, however, emit radiation when returning to the ground state either as fluorescence or as phosphorescence, depending on the mechanism by which the electron finally returns to the ground state.

Figure 4-11 shows a simplified energy diagram (also known as a Perrin–Jablonsky diagram) of the typical mechanisms that a chemical species undergoes after being electronically excited. The absorption of a photon usually excites an electron from the ground state toward its excited states without changing its spin (i.e., a singlet ground state will absorb into a singlet excited state, called a spin-allowed transition).

The triplet state usually cannot be achieved by excitation from the ground state, this being termed a "forbidden" transition according to quantum theory. It is usually reached through the process of *intersystem crossing* (ISC), which is a nonradiative transition between two isoenergetic vibrational levels belonging to electronic states of different multiplicities. For example, the excited singlet (S_1) in the 0 vibrational mode can move to the isoenergetic vibrational level of T_n triplet state, then vibrational relaxation can bring it to the lowest vibrational level of T_1, with the concomitant energy loss (see Fig. 4-11). From T_1, radiative emission to S_0 can occur, which is called phosphorescence.

The excited triplet state (T_1) is usually considered more stable (i.e., having a longer lifetime) than the excited singlet state (S_1). 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 after fluorescence. Ordinarily, fluorescence occurs between 10^{-10} and 10^{-7} sec after excitation, whereas the lifetime for phosphorescence is between 10^{-7} and 1 sec. Because of its short lifetime, fluorescence is usually measured while the molecule is being excited. Phosphorescence uses a pulsed excitation source to allow enough time to detect the emission. It should be noted that measurements of

fluorescence lifetimes on the order of femtoseconds have been demonstrated to be valuable in studying transition states, as described by Zewail.16

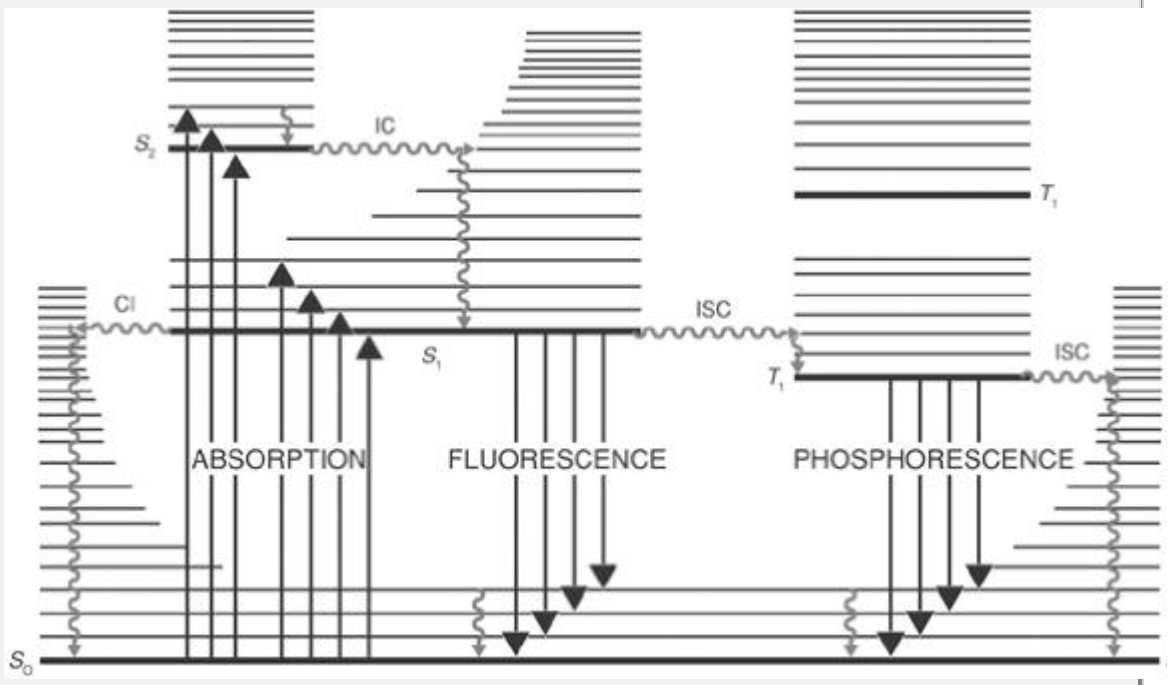

Fig. 4-11. A Perrin–Jablonski diagram illustrating the multiple pathways by which absorbed molecular energy can be released. Relaxation can occur through a nonradiative pathway or radiative emission of fluorescence or phosphorescence. The singlet (*S*) and triplet (*T*) states pertain to the electronic structure in the molecule after excitation.

Fluorescence normally has a longer wavelength than the radiation used for the excitation phase, principally because of internal energy losses within the excited molecule before the fluorescent emission $S_1 \rightarrow S_0$ occurs (Fig. 4-11). The gap between the first absorption band and the maximum fluorescence is called the Stokes shift. Phosphorescence typically has even longer wavelengths than fluorescence owing to the energy difference that occurs in ISC as well as the loss of energy due to internal conversion over a longer lifetime.

A representative schematic of a typical fluorometer is shown in Figure 4-12. Generally, fluorescence intensity is measured in these instruments by placing the photomultiplier detector at right angles to the incident light beam that produces the excitation. The signal intensity is recorded as relative fluorescence against a standard solution. Because 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.

Fluorometry is a very sensitive technique, up to 1000 times more sensitive than spectrophotometry. This is because the fluorescence intensity is measured above a low background level, whereas in measuring low absorbances, two large signals that are slightly different are compared. Recently, advances in instrumentation have made it possible to detect fluorescence at a single-molecule level.17

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 largely absorb light. Most often, molecules that display fluorescence or phosphorescence contain a rigid conjugated structure such as aromatic hydrocarbons, rhodamines, coumarins, oxines, polyenes, and so on, or inorganic lanthanides ions like Eu^{3+} or Tb^{3+}, which also show strong fluorescence. Many drugs (e.g., morphine), some natural amino acids and cofactors (e.g., tyrosine, tryptophan, nicotinamide adenine dinucleotide, reduced form flavin adenine

dinucleotide, etc.) fluoresce. Some examples are given in Table 4-6 along with their characteristic excitation and emission wavelengths, which can be used for qualitative and quantitative analyses. Fluorescence detection and analysis has become a major tool in biopharmaceutical and chemical analysis, particularly in areas related to manipulation of biopolymers (nucleic acids and proteins) and imaging of biologic membranes and living organisms (cells, bacteria, etc.). For example, in peptides and proteins the UV absorbance for the $n \rightarrow \pi^*$ ($\varepsilon = 100$) and $\pi \rightarrow \pi^*$ ($\varepsilon = 7000$) transitions in the peptide bond occurs from about 210 to 220 and 190 nm, respectively.12 The absorbance of several side-chain transitions can often be obfuscated absorbance from peptide bonds as well as other species present in the solution, and therefore does not offer good specificity. Tryptophan (280 nm), tyrosine (274), phenylalanine (257 nm), and cystine (250 nm) have electronic

P.95

transitions that occur above the 220-nm range for the peptide bond, but these transitions can be obfuscated as well.12 However, tryptophan, phenylalanine, and tyrosine can also undergo fluorescence that can be used to discriminate peptides or proteins from biologic matrices. The use of other dyes and fluorophores for biologic applications is also an exciting and rapidly developing area. The student is advised to seek out Web sites of companies such as Molecular Probes (www.probes.com) to look at the wide scope and use of fluorescent labels and probes as well as modern instrumentation in the field. Schulman and Sturgeon18give a thorough review of the applications of photoluminescence to the analysis of traditional pharmaceuticals.

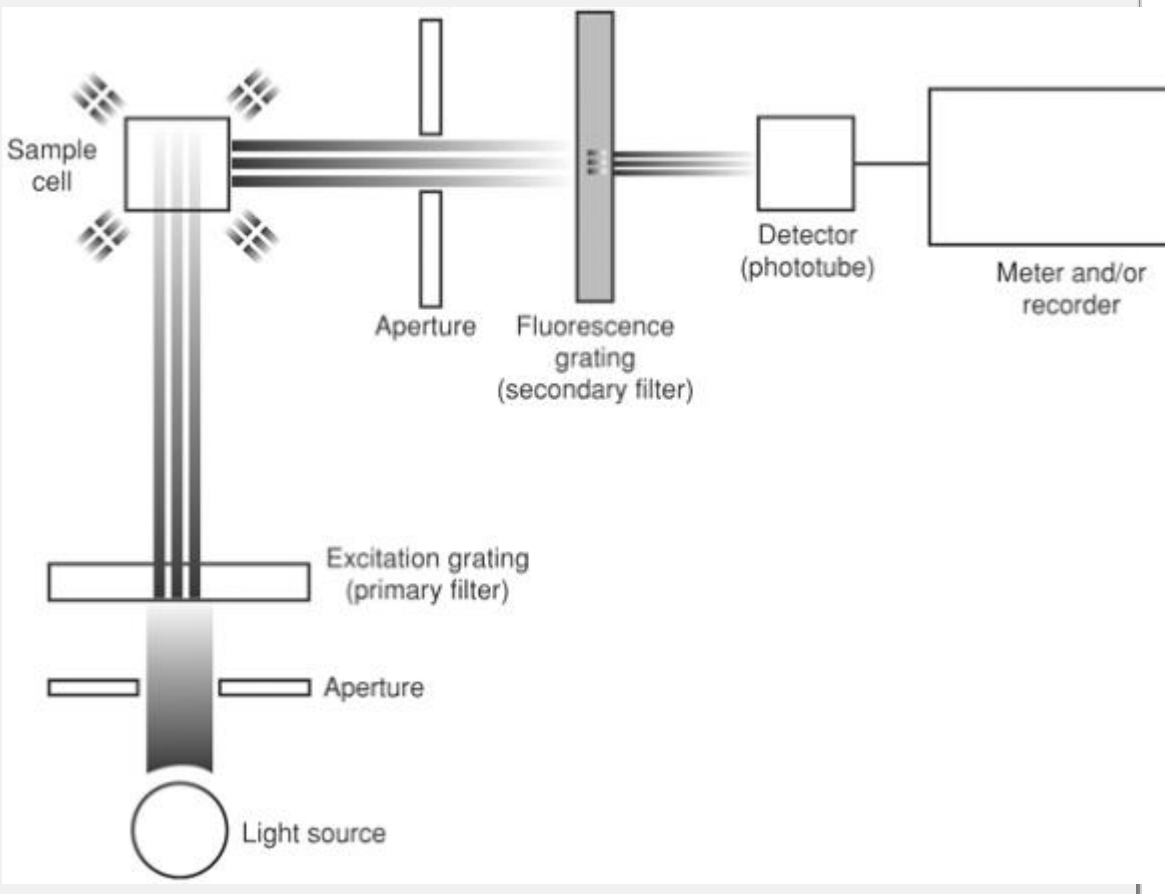

Fig. 4-12. Schematic diagram of a filter fluorometer. (From G. H. Schenk, *Absorption of Light and Ultraviolet Radiation*, Allyn and Bacon, Boston, 1973, p. 260. With permission.)

Table 4-6 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	Water (pH 2)
Riboflavin	455	520	Ethanol
Hydralazine	320	353	Concentrated H_2SO_4

Infrared Spectroscopy

The study of the interaction of electromagnetic radiation with vibrational or rotational *resonances* (i.e., the harmonic oscillations associated with the stretching or bending of the bond) 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 wave number, 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 may affect this symmetry, thereby shifting the dipole moment as found for the normal vibrational modes (2), (3), and (3′) for CO_2 in Figure 4-13. Other resonances, such as (1) for CO_2 inFigure 4-13, do not affect the dipole moment and therefore 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 4-14. 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 wave numbers). 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. Because 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.

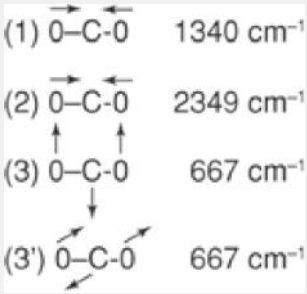

Fig. 4-13. The normal vibrational modes of CO_2 and their respective wave numbers, showing the directions of motion in reaching the extreme of the harmonic cycle. (Modified from W. S. Brey, *Physical Chemistry and Its Biological Applications*, Academic Press, New York, 1978, p. 316.)

Because the vibrational resonances of a complex molecule often can be attributed to particular bonds or groups, they behave as though they result 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. A typical infrared spectrum of theophylline is shown in Figure 4-15. 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* inFigure 4-15, is due to a carbonyl stretching vibration for theophylline.

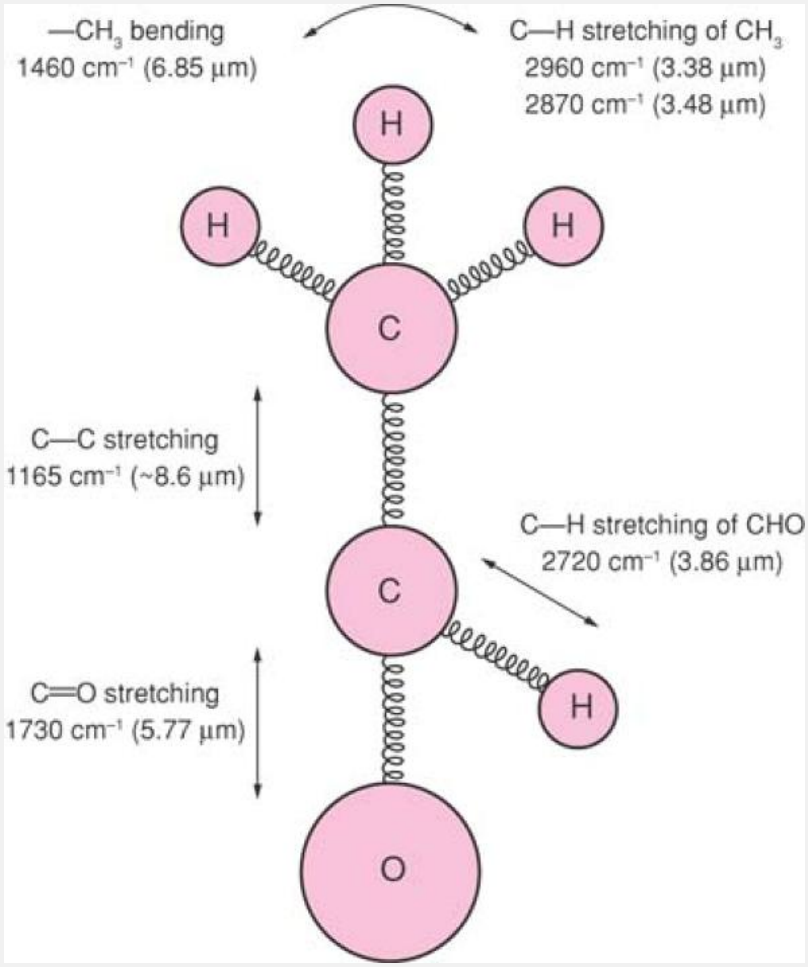

Fig. 4-14. Bending and stretching frequencies for acetaldehyde. (From H. H. Willard, L. L. Merritt, Jr., and J. A. Dean,*Instrumental Methods of Analysis*, 4th Ed., Van Nostrand, New York, 1968. With permission.)

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 depend 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 affect similar frequency shifts.

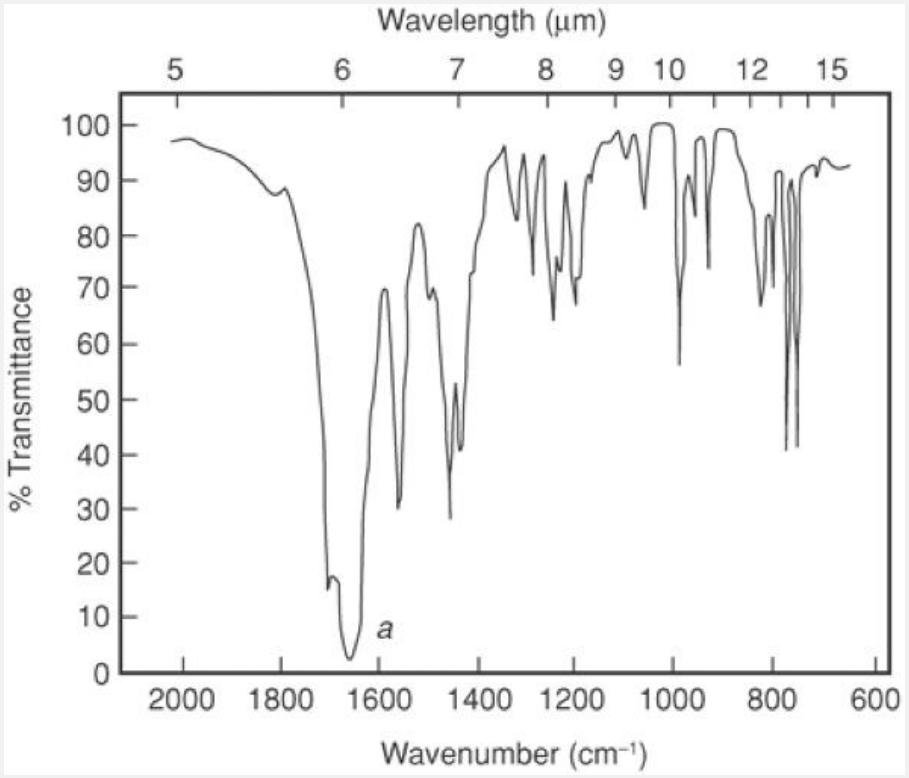

Fig. 4-15. Infrared spectrum of theophylline. (From E. G. C. Clarke, Ed., *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1969. With permission.)

The use of infrared spectroscopy in pharmacy has centered on its applications for drug identification, as described by Chapman and Moss.19 The development of Fourier transform infrared spectrometry20 has enhanced infrared applications for both qualitative and quantitative analysis of drugs owing to the greater sensitivity and the enhanced ability to analyze aqueous samples with Fourier transform infrared instrumentation. Thorough surveys of the techniques and applications of infrared spectroscopy are provided by Smith21 and Willard et al.22

Near-Infrared Spectroscopy

Near-IR spectroscopy is rapidly becoming a valuable technique for analyzing pharmaceutical compounds. The near-IR region ranges from approximately 10,000 to 4000 cm^{-1} and comprises mainly of mid-IR overtones and combinations of these overtones arising from heavy-atom electronic transitions, including O—H, C—H, and N—H stretching. Thus, near-IR spectroscopy is typically used for analyzing water, alcohols, and amines. In addition, near-IR bands arise from forbidden transitions; therefore, the bands are far less intense than the typical absorption bands for these atoms.23

Some advantages of using near-IR spectroscopy over other techniques include extremely fast analysis times, nondestructive analysis, lack of a need for sample preparation, qualitative and quantitative results, and the possibility of multicomponent analysis. Pharmaceutical applications include polymorph identification, water content analysis, and identification and/or monitoring of an active component within a tablet or other solid dosage form. Because of the nondestructive nature of the technique, online analysis for controlling large-scale processes is possible.

Electron Paramagnetic and Nuclear Magnetic Resonance Spectroscopy

Now we enter into the description of two widely used spectroscopic techniques in the pharmaceutical sciences and related fields of chemistry, biology, and medicine. Electron paramagnetic resonance and

NMR are based on the magnetic behavior of atomic particles such as electrons and nuclei in the presence of an external magnetic field. In the following, fundamentals of the techniques will be given, including differences between electrons and nuclei where applicable.

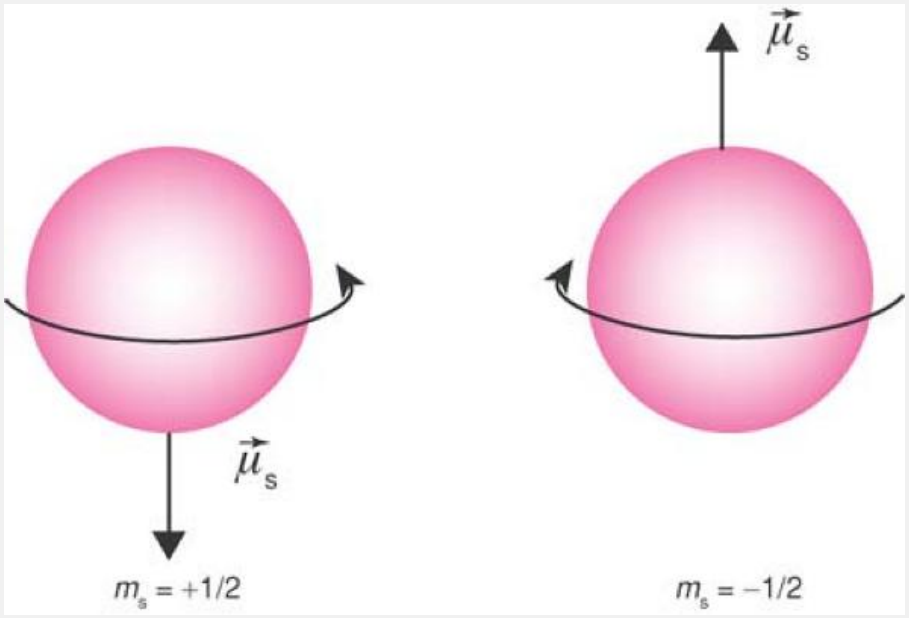

Fig. 4-16. Electron and nuclear rotation.

Charged particles such as electrons (negatively charged) and protons (positively charged) behave as if they were charged, spinning tops. Accordingly, they have magnetic moments associated with the movement around their axes, as shown in Figure 4-16 (i.e., they behave as small magnets). In paired electrons the spins are opposite, so the magnetic moments cancel each other out. However, single unpaired electrons have a magnetic moment (Bohr magneton, μ_{Bohr}) equal to

$$\mu_{\text{Bohr}} = \frac{eh}{4\pi m_e} = 9.274 \times 10^{-24} \text{ joule/T} \qquad \textbf{(4–18)}$$

where *e* is the elementary charge, *h* is Planck's constant, and m_e is the electron's mass.*
Similarly, nuclear motion in protons and certain nuclei also has an associated magnetic moment, whose magnitude can be calculated from an equation similar to(4-18) by replacing the mass of the particle; protons have a larger mass than electrons, so that the nuclear magneton is $\mu_N = 5.051 \times 10^{-27}$ joule/T.
When the electron (or the proton) is placed in an external magnetic field, its magnetic moment is oriented in two different directions relative to the magnetic field, *B*, parallel or antiparallel, giving rise to two new energy levels splitting from each other by a magnitude ΔE, which depends on the applied field *B* according to

$$\Delta E = g\,\mu_{\text{Bohr}}\,B \qquad \textbf{(4–19)}$$

g is termed the Landé *splitting factor*, or simply the *g* factor. 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. The *g factor* is also characteristic for certain metal complexes

with unpaired electrons, from which information about their environment can be obtained.

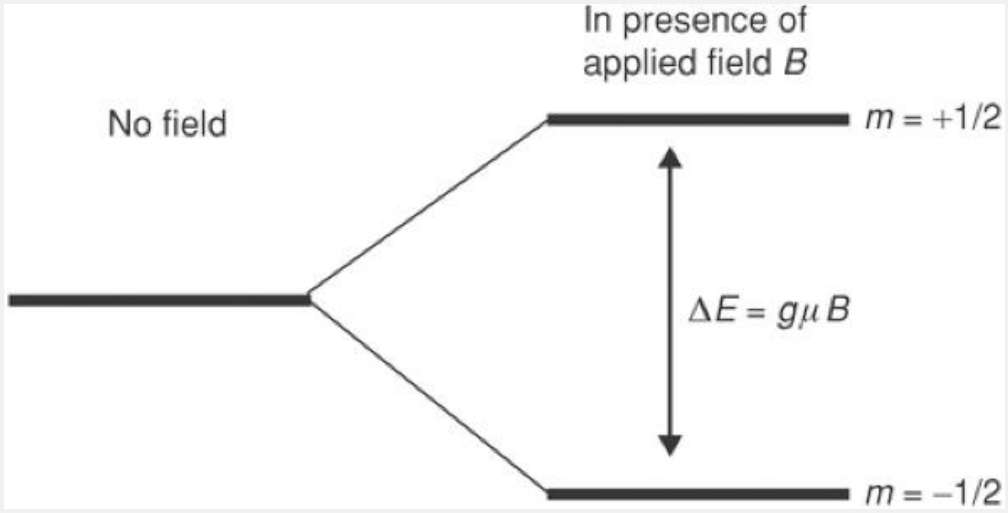

Fig. 4-17. Electron and nuclear spin splitting when subjected to an applied external magnetic field.

For an electron placed in a magnetic field of B = 1 T, $\Delta E = 18.54 \times 10^{-24}$ joule; according to equation **(**4-19**)**. Therefore, a simple calculation of the frequency that matches this energy difference, $v = \Delta E/h$, indicates that the electron can be excited with a radiation of 28 GHz, corresponding to the microwave region of the spectrum (see Table 4-4). A similar calculation for protons using

$$\Delta E = g_N \mu_N B \qquad \textbf{(4–20)}$$

with g_N = 5.586, affords an energy splitting of 2.82×10^{-26} joule, corresponding to an electromagnetic frequency of 42.58 MHz, also in the microwave region.

Transitions between electron spin energy levels give rise to the phenomenon of EPR, as indicated in Figure 4-17; the corresponding property of the nuclear spin levels leads to NMR. The term "resonance" relates to the classical interpretation of the phenomenon, because transitions occur only when the frequency v of the electromagnetic radiation matches the Larmor frequency of precession around the axis of the applied magnetic field, v_L, as displayed in Figure 4-18.

So far we have considered only electrons and protons, particles with spin ½, which have only two possible energy levels. Several nuclei also have magnetic moments and, therefore, NMR signals. Nuclei with odd mass numbers have total spin of half-integral value, whereas nuclei with odd atomic numbers and even mass numbers have total spin of integral value. Examples include ^{13}C, ^{19}F, and ^{31}P, which have spin ½, and ^{2}H and ^{14}N, which have spin 1.

By considering equations **(**4-19**)** and **(**4-20**)**, we can obtain the resonance signal by gradually varying the magnetic field strength, B, while keeping the radio frequency, v, constant. At some particular B value, a spin transition will take place that flips the electron or nuclei from one spin state to another (e.g., from I = -½ to +½, as in Figure 4-18). In some spectrometers, the experiment can be done in the reverse fashion: keeping B constant and varying v. This method is generally called*continuous-wave or field scan*, and the particular value of v depends linearly on B, as illustrated in Figure 4-19.

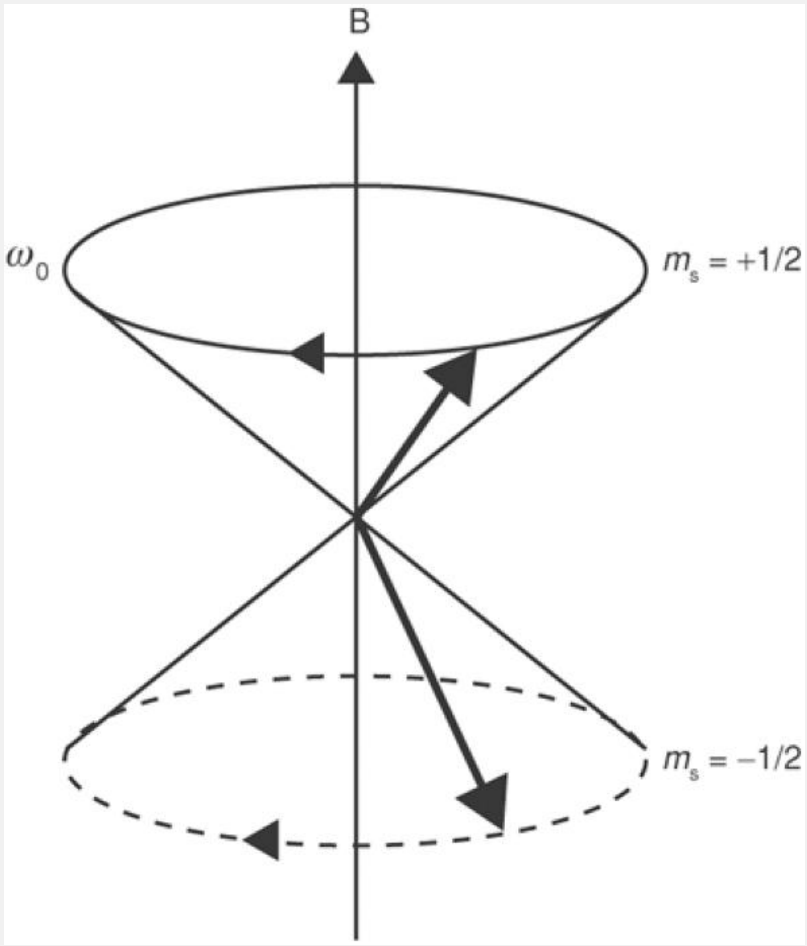

Fig. 4-18. Electronic or nuclear spin procession around an applied magnetic field.

In most modern spectrometers, particularly for NMR, all spins of one species in the sample are excited simultaneously by using a short, high-frequency pulse of electromagnetic radiation. Then their relaxation is measured as a function of time and transformed from the time domain to the frequency domain via fast Fourier transformation techniques. The signal-to-noise ratio can be significantly improved by the accumulation of a large number of measurements.

As we have seen, the theoretical basis of EPR spectroscopy is similar to that of NMR except that it is restricted to paramagnetic species (i.e., with unpaired electrons). Among thousands of types of substances constituting biologic systems with pharmacologic relevance, only very few, namely,

free radicals, including molecular oxygen (O_2) and nitric oxide (NO), and a limited number of transition metal ions reveal paramagnetic properties and may be observed by EPR spectroscopy. Nevertheless, EPR has many potential uses in biochemistry and medicine by the intentional introduction into the system of exogenous EPR-detectable paramagnetic probes (spin labels). This method often eliminates the necessity to purify biologic samples. Thus, EPR spectroscopy applied to biologic tissues and fluids can identify the changes in redox processes that contribute to disease. Electron paramagnetic resonance may also be used to characterize certain plant-derived products as potentially important to biotechnology by increasing the level of free radicals and other reactive species produced during light-induced oxidative stress of the cell. Several other applications can be found in references at the end of the chapter.[24,25,26]

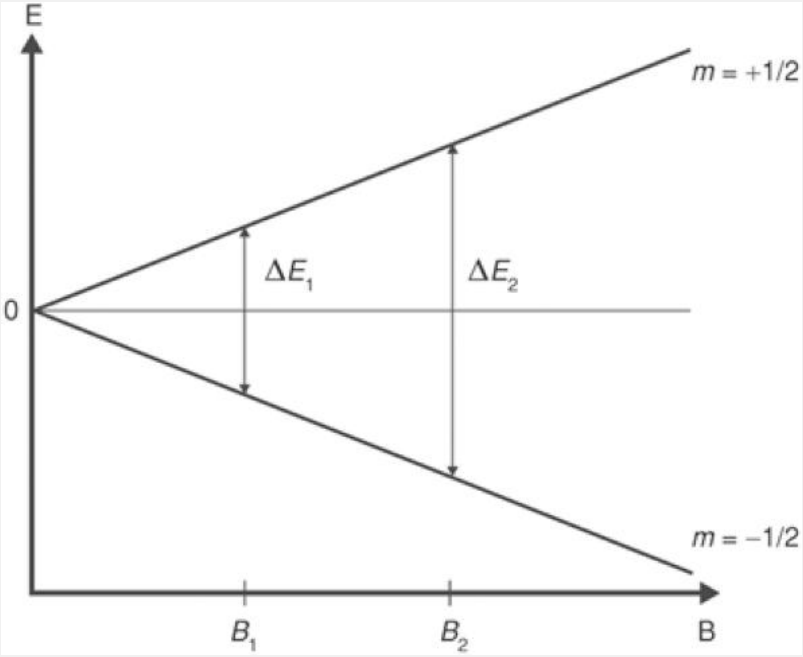

Fig. 4-19. Energy differences observed in electronic or nuclear spin processions as a function of magnetic field strengths.

Nuclear magnetic resonance is a powerful experimental method in chemistry and biology because the resonance frequency of a nucleus within a molecule depends on the chemical environment. The local field experienced by an atom in a molecule is less than the external magnetic field by an amount given by

$$B_{\text{effective}} = B\,(1-\sigma) \qquad \textbf{(4–21)}$$

where σ is the shielding constant for a particular atom (i.e., a measure of its susceptibility to induction from a magnetic field) and B is the external magnetic field strength. Instead of measuring the absolute value of σ, it is customary to measure the difference of σ relative to a reference.

Example 4-6
Nuclear Spin Energy

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_N, of 5.050824×10^{-27} joule/T in a magnetic field, B, of 1 T?

We can obtain $\Delta E = g_N\,\mu_N\,B_{\text{local}}$ for this particular nucleus, in which the corresponding local field experienced by the atom is given by equation (4-21). The local magnetic field B_{local} is replaced, and we obtain

$$\Delta E = g_N \mu_N B(1-\sigma) = (5.585691)(5.050824 \times 10^{-27}\ \text{joule/T}) \times (1\ \text{T})(1 + 7.25 \times 10^{-6}) = 2.821254 \times 10^{-26}\ \text{joule}$$

b. What is the radio frequency at which resonance will occur for this nuclear spin transition under the stated conditions?

$$\nu = \Delta E/h = (2.821254 \times 10^{-26}\ \text{joule}/6.626196 \times 10^{-34}\ \text{joule-sec}) = 4.2577289 \times 10^{7}\ \text{Hz}$$

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.

Example 4-7
Resonance of Tetramethylsilane

What is the radio frequency at which resonance occurs for TMS in a magnetic field of 1 T?
The shielding constant σ is 0.000, and $\Delta E = 2.821243 \times 10^{-26}$ joule for TMS, so

$$\nu = \Delta E/h = (2.82123 \times 10^{-26}\,\text{joule}/6.626196 \times 10^{-34}\,\text{joule-sec}) = 4.2576981 \times 10^{7}\,\text{Hz}$$

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 308 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.

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, B. Greater shielding will occur with higher electron density near a particular nucleus, and this reduces the frequency 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

$$\delta = (\sigma_r - \sigma_s) \times 10^6 \qquad \textbf{(4–22)}$$

where σ_r and σ_s are the shielding constants for the reference and the sample nucleus, respectively.
If the separation between the sample and reference resonance is ΔH or $\Delta \nu$, then

$$\delta = \frac{\Delta H}{H_R} \times 10^6 = \frac{\Delta \nu}{\nu_R} \times 10^6 \qquad \textbf{(4–23)}$$

where H_R and ν_R are the magnetic field strength and radio frequency for the nuclei, depending on whether field-swept or frequency-swept NMR is used.

Example 4-8
Proton Shift Reference

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 (4-23), we obtain the chemical shift:

$$\delta = \Delta\nu \times 10^6/\nu_R = 308 \times 10^6/42.57 \times 10^6 = 7.23\,\text{ppm}$$

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 7.24 ppm. The chemical shift of a nucleus provides information about its local magnetic environment and therefore can “type” a nuclear species. Table 4-7 lists some representative proton chemical shifts. Figure 4-20 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 δ = 2.0 is from the CH_3 group, whereas 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.

Table 4-7 Proton Chemical Shifts for Representative Chemical Groups or Compounds

Compound or Group	ppm
Tetramethylsilane (TMS), $(CH_3)_4Si$	0.00
Methane	0.23
Cyclohexane	1.44
Acetone	2.08
Methyl chloride	3.06
Chloroform	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

In Figure 4-20, 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 because each particular nucleus can be coupled by spin interactions to neighboring nuclei. If these neighboring nuclei are in different local magnetic environments, splitting of the bands can occur because of differences in electron densities. 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.

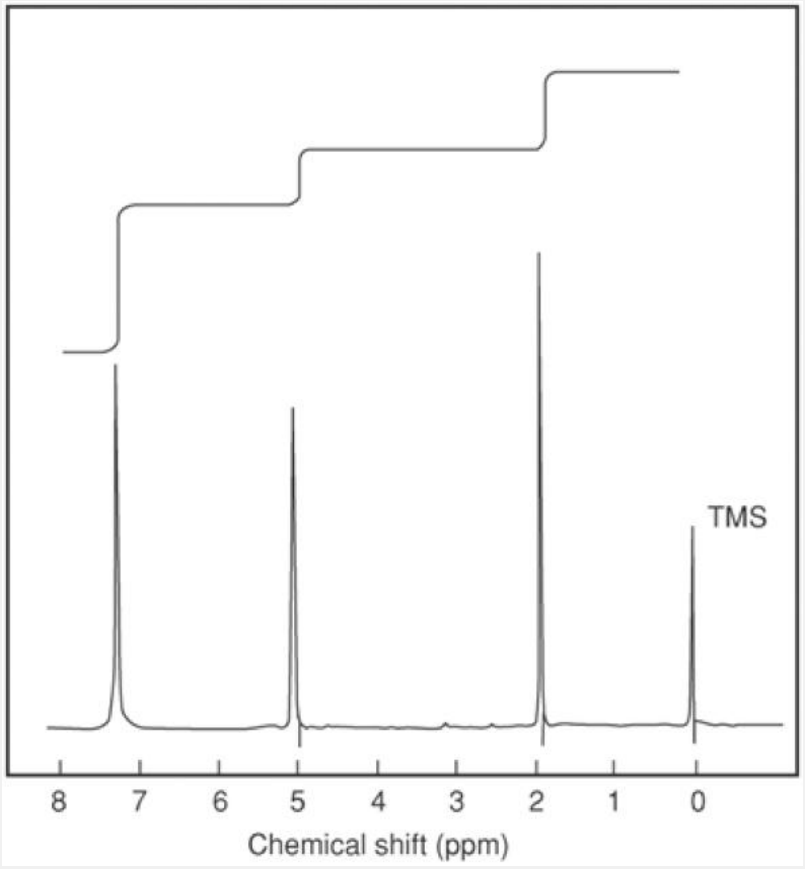

Fig. 4-20. Proton NMR spectrum of benzyl acetate with tetramethylsilane (TMS) as a reference. The TMS band appears at the far right. The upper curve is an integration of the spectral bands, the height of each step being proportional to the area under that band. (From W. S. Brey, *Physical Chemistry and Its Biological Applications*, Academic Press, New York, 1978, p. 498. With permission.)

Figure 4-21 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 the proportion 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, whereas 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, which can be obtained from Pascal's triangle:

$n = 0$				1				
$n = 1$			1		1			
$n = 2$		1		2		1		
$n = 3$	1		3		3		1	
$n = 4$	1		4		6		4	1

where *n* is the number of equivalent neighbor nuclei. Thus, a doublet presents intensities 1:1; a triplet, 1:2:1; and a quartet, 1:3:3:1. Further details of multiplicity and the interpretation of NMR spectra can be found in general texts on NMR.[27,28]

The typical range for NMR chemical shifts depends on the nucleus being observed: For protons, it is about 15 ppm with organic compounds, whereas it is about 400 ppm for ^{13}C and ^{19}F spectra. Table 4-8 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.

A widely used nucleus in characterization of new chemical compounds is ^{13}C. The development of ^{13}C NMR spectroscopy in recent years has been influenced by the application of *spin decoupling* techniques 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 broadband radio frequency 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). Because 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 3. These factors, proton spin decoupling and the NOE, have enhanced the sensitivity of ^{13}C NMR. They 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.

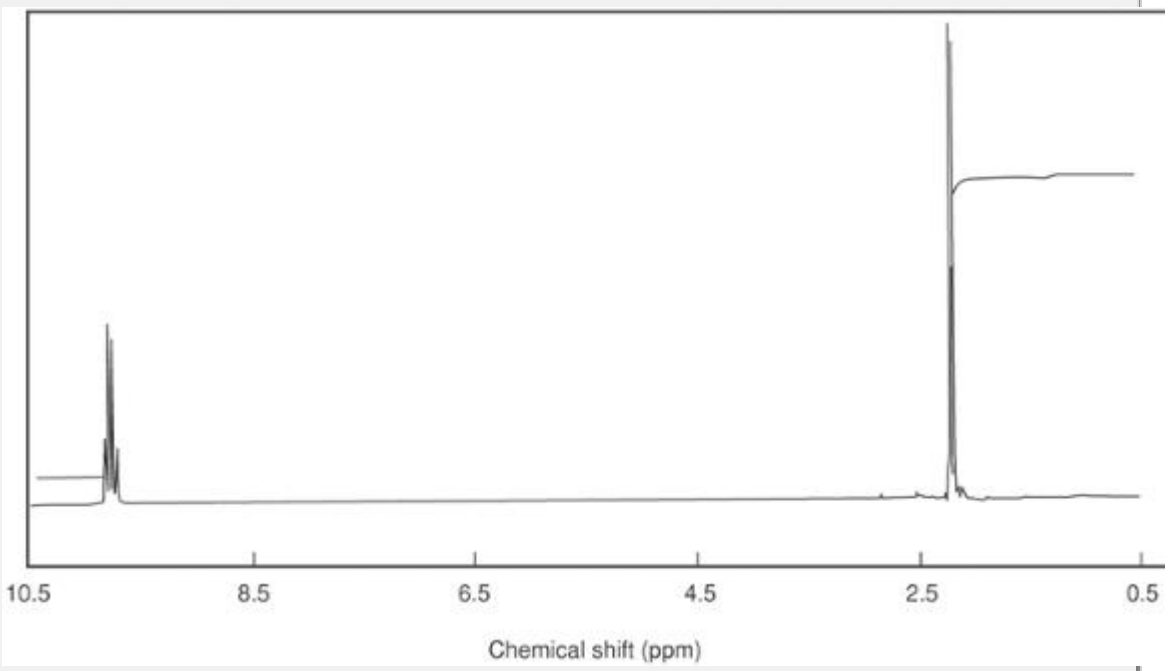

Fig. 4-21. Proton NMR spectrum of acetaldehyde. (From A. S. V. Burgen and J. C. Metcalfe, J. Pharm. Pharmacol. **22,** 156, 1970. With permission.)

A powerful extension of this enhancement of ^{13}C spectra involves systematically decoupling only specific protons by irradiating at particular radio frequencies rather than by broadband irradiation. Such systematic decoupling permits individual carbon atoms to show multiplet collapse and signal

intensification, as mentioned previously, 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 to produce what is termed *two-dimensional NMR spectrometry*. Farrar29 describes the techniques involved in these experiments.

Table 4-8 Basic Nuclear Magnetic ResonanceS (NMR) and Natural Abundance of Selected Isotopes*

Isotope	NMR Frequency (MHz) at Given Field Strength		Natural Abundance (%)
	At 1.0000 T	At 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. With permission.

Nuclear magnetic resonance is a versatile tool in pharmaceutical research. Nuclear magnetic resonance 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 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 Metcalfe30 describe applications of NMR to problems involving drug–membrane and drug–protein interactions. Illustrations of these interactions are analyzed by Lawrence and Gill31 using electron spin resonance (ESR) and by Tamir and Lichtenberg32 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. Rackham33 reviewed the use of NMR in pharmaceutical research, with particular reference to analytical problems.

Nuclear magnetic resonance characterization has become routine, and specialized techniques have rapidly expanded its application breadth. Two particularly exciting applications rely on the determination of the three-dimensional structure of complex biomolecules34 (proteins and nucleic acids) and the imaging of whole organisms (tomography). The paramount importance of these developments employing NMR has been recognized by the awarding of the Nobel Prize in Chemistry and Medicine in 2002 and 2003 for this work.

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 interact with the atoms in the substance at the interface and throughout the thickness of the substance. These interactions modify the light waves by absorbing energy, resulting in the waves being closer together by reducing the speed and shortening the wavelength, as shown in Figure 4-22. 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. However, in both cases there is an important observation: The light travels in the same direction after interaction as before it. In reflection, the light travels in the opposite direction after incidence with the medium. The relative value of the effect of refraction between two substances is given by the *refractive index, n*:

$$n = \frac{\sin i}{\sin r} = \frac{\text{Velocity of light in the first substance}}{\text{Velocity of light in the second substance}} = \frac{c_1}{c_2} \quad \textbf{(4–24)}$$

where sin *i* is the sine of the angle of the incident ray of light, sin *r* is the sine of the angle of the refracted ray, and c_1 and c_2 are the speeds of light of the respective media. Normally, the numerator is taken as the velocity of light in air and the denominator is that in 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 0.03% from that in a vacuum and is more commonly used.

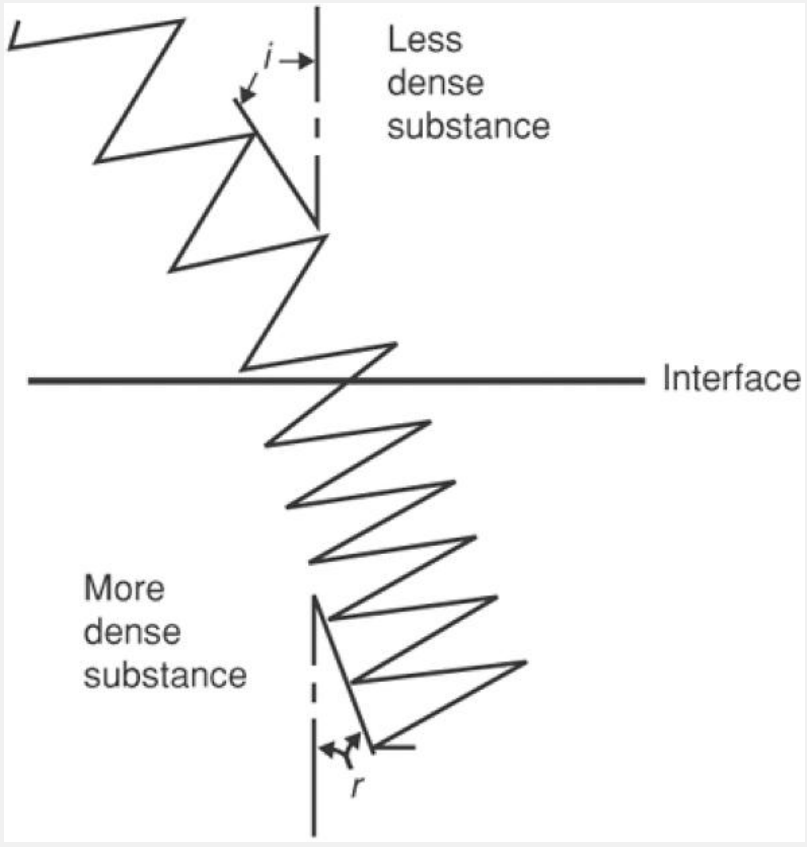

Fig. 4-22. Waves of light passing an interface between two substances of different density.

The refractive index varies with the wavelength of light and the temperature because both alter the energy of interaction. 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. As discussed in Chapter 2, temperature and pressure are related. The 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. Refractometers can be an important tool for studying pharmaceutical compounds that do not undergo extensive UV–Vis absorption due to their electronic structure.

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

$$R_m = \frac{n^2 - 1}{n^2 + 2}\left(\frac{M}{\rho}\right) \qquad \textbf{(4–25)}$$

where 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, and one should note the analogous equation to those utilized with dielectrics. 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/mole. 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 on a molecule induces vibrating dipoles due to energy absorption at the interface, where the greater the refractive index at a particular wavelength, the greater the dipolar induction. Simply stated, the interaction of light photons with polarizable electrons of a dielectric molecule causes a reduction in the velocity of light. The dielectric constant, a measure of polarizability, is greatest when the resulting 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

$$\varepsilon = n^2{}_\infty \qquad \textbf{(4–26)}$$

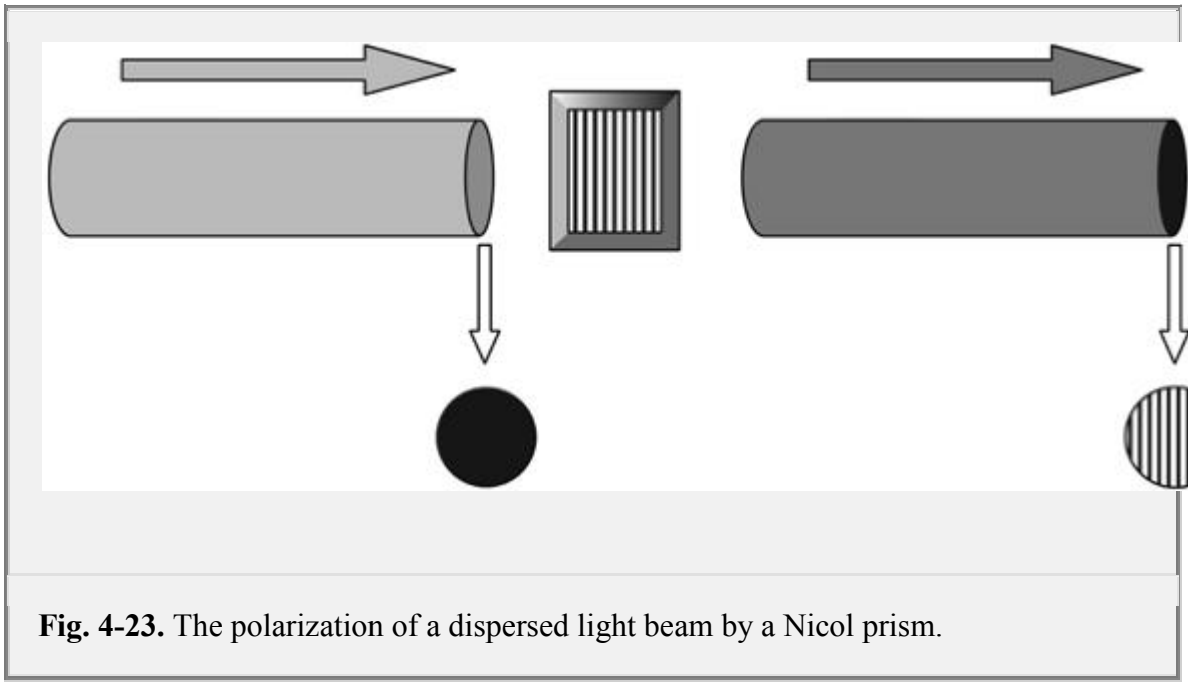

Fig. 4-23. The polarization of a dispersed light beam by a Nicol prism.

Molar polarization, P_i [equation (4-16)], can be considered roughly equivalent to molar refraction, R_m, and can be written as

$$P_i = \left(\frac{n_\infty^2 - 1}{n_\infty^2 + 2}\right)\frac{M}{\rho} = \frac{4}{3}\pi N\alpha_p \qquad (4\text{–}27)$$

From this equation, the polarizability, α_p, of a nonpolar molecule can 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

Electromagnetic radiation comprises two separate, sinusoidal-like wave motions that are perpendicular to one another, an electric wave and a magnetic wave (Fig. 4-4). Both waves have equal energy. A source will produce multiple waves of oscillating electromagnetic radiation at any given time, so that multiple electric and magnetic waves are emitted. These electromagnetic radiation waves travel in multiple orientations that are randomly dispersed in the resulting circular beam of radiation. Discussions of interactions with light sources are predominantly focused on the electric component of these waves, to which we restrict ourselves for the remainder of this section and in the Optical Rotatory Dispersion section and Circular Dichroism section.

Passing light through a polarizing prism such as a Nicol prism sorts the randomly distributed vibrations of electric radiation so that only those vibrations occurring in a single plane are passed (Fig. 4-23). The velocity of this *plane-polarized* light can become slower or faster as it passes through a sample, 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 in the planar light, as observed looking into the beam of polarized light, defines a substance as *dextrorotatory*. When the plane of light is rotated by the sample in a counterclockwise manner, the sample is defined as a *levorotatory* substance. A 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, whereas the levorotatory substance, which rotates the beam to the left, has an α that is defined as negative. Molecules that have an asymmetric center (chiral) and therefore lack symmetry about a single plane are optically active, whereas symmetric molecules (achiral) 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. Figure 4-24 illustrates how a polarimeter can be used to measure these phenomena.

Optical rotation, α, depends on the density of an optically active substance because 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 given by the equation

$$\{\alpha_\lambda^t\} = \frac{\alpha v}{lg} \qquad (4\text{–}28)$$

where 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 milliliter 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 rotation of a molecule is indicative of its molecular structure

and as such should not change when measured under the same conditions in any lab. Organic chemists use specific rotation as a tool to help confirm the identity of a synthesized compound whose molecular properties are already defined. Other measurements are often performed, but this is a quick test to check the identity and purity of a reaction product. The specific rotations of some drugs are given in Table 4-9. The subscript D on $[\alpha]$ 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 4-9, the concentration is often assumed to be 1 g per milliliter of solvent. However, $[\alpha]$ values should be reported at specific temperatures and concentration including solvent. Data missing this information should be treated with caution because the specific conditions are not known. The specific rotation of steroids,

carbohydrates, amino acids, and other compounds of biologic importance are given in the *CRC Handbook*.35

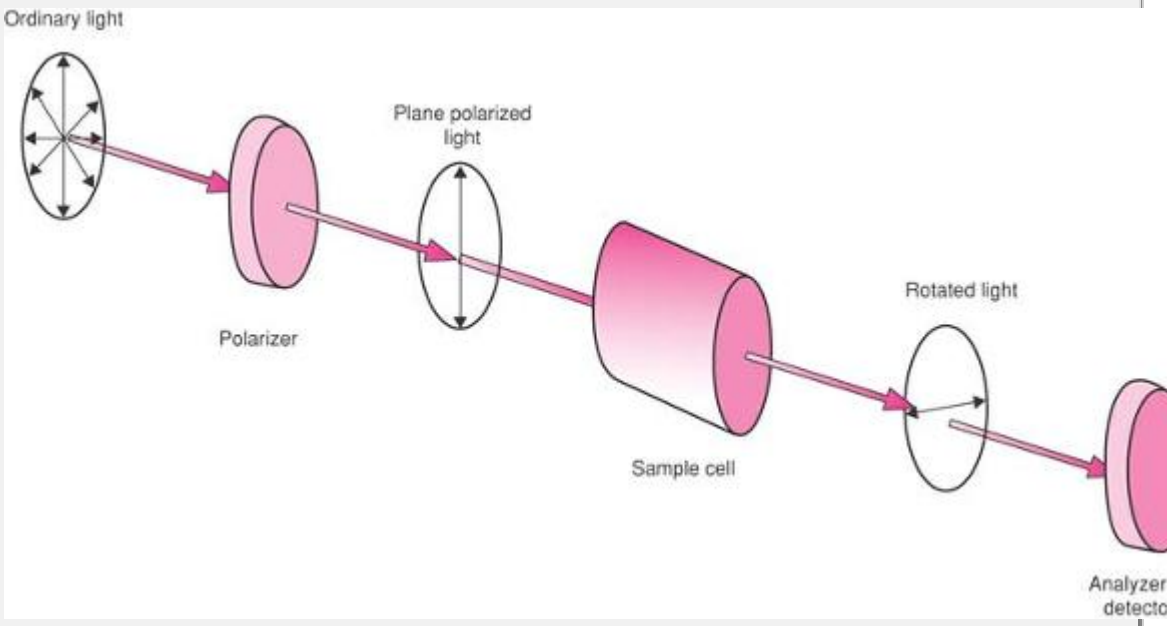

Fig. 4-24. Schematic view of a polarimeter.

Table 4-9 Specific Rotations

Drug	$[\alpha]_D$ (deg)	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 measure of the angle of rotation as a function of the wavelength. Recall that this is light in the UV–Vis range and that different wavelengths of light have different energies, which may result in changes in absorption patterns of molecules due to their

electronic structure. Varying the wavelength of light changes the specific rotation for an optically active substance because of the electronic structure of the molecule. 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 4-25. 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 4-26. In addition, ORD is often useful for the structural examination of organic compounds. Detailed discussions of ORD are given by Campbell and Dwek12 and Crabbe.36

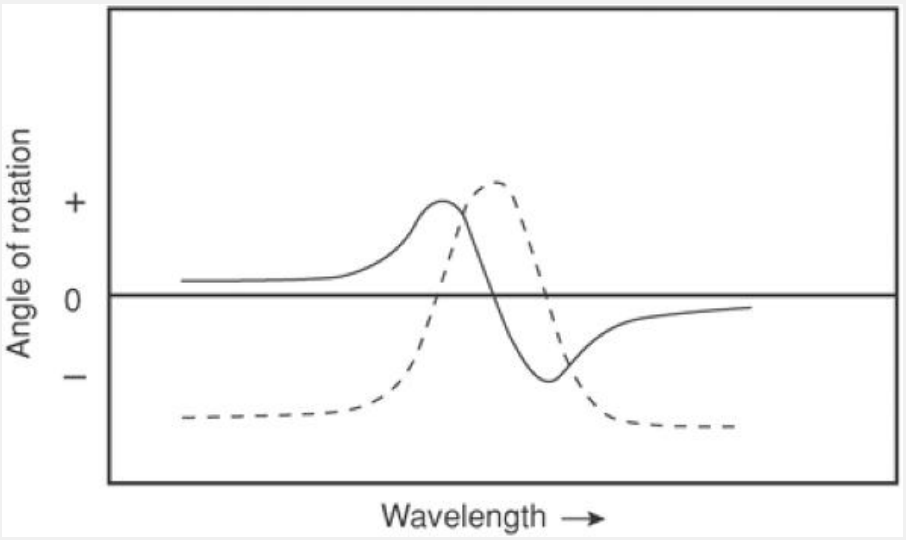

Fig. 4-25. The Cotton effect. Variation of the angle of rotation (solid line) in the vicinity of an absorption band of polarized light (dashed line). (Modified from W. S. Brey, *Physical Chemistry and Its Biological Applications*, Academic Press, New York, 1978, p. 330.)

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 spiraling in the opposite direction, a right-handed and a left-handed component. Handedness is used as a convention to conceptualize circularly polarized light. To visualize handedness, take both hands and hold them with the fingers pointed straight up and each thumb pointed toward your nose. Now curl the fingers around in a loose fist, holding the thumbs steady. The thumb on each hand indicates the light traveling toward your nose, and the curling of the fingers shows how the light vectors are spiraling into two separate vectors, the left and right circular directions. For an optically active substance, the values of the index of refraction, *n*, of the two vectors cannot be the same.

Because of chirality, one of the two vectors may be absorbed differently at one wavelength of light, whereas the converse may be true at another wavelength. 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. Conceptually, it is important to realize that at the source, the circularly polarized light is traveling at the same speed in a circle. When one component of light, say the left-handed component, has energy absorbed, it will slow down in contrast to the right-hand component, stretching the circle into an ellipse. The converse is true as well. This can also be illustrated by using the hand convention. When circularly polarized light is caused to become elliptically polarized, it is termed *circular dichroism* (CD).12,37

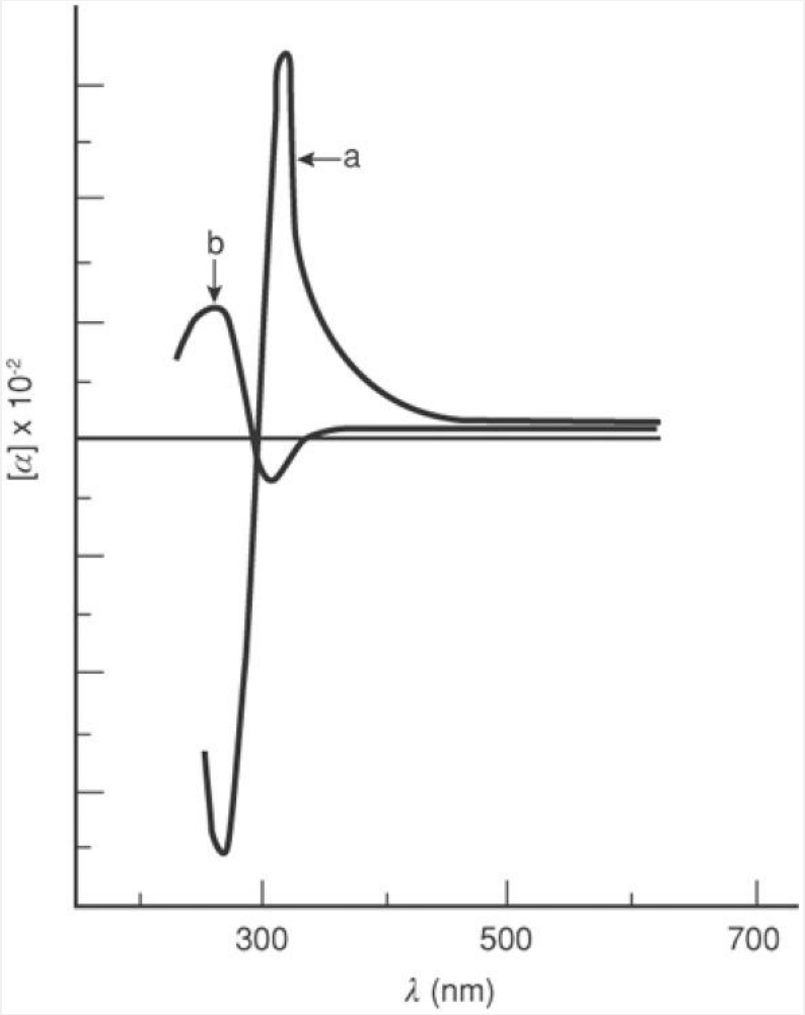

Fig. 4-26. Optical rotary dispersion curves for (a) *cis*-10-methyl-2-decalone and (b) *trans*-10-methyl-2-decalone. Note the positive effect in (a) and the negative effect in (b). (From C. Djerassi, *Optical Rotatory Dispersion*, McGraw-Hill, New York, 1960. With permission.)

The Cotton effect is the name given to the unequal absorption (elliptic effect) of the two components of circularly polarized light by a molecule 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. By traditional convention, the molar ellipticity is given by

$$[\theta] = \frac{[\psi]M}{100} = 3300(\varepsilon_L - \varepsilon_R) \text{ deg liter mole}^{-1} \text{ dm}^{-1} \quad \textbf{(4–29)}$$

where [ψ] is the specific ellipticity, analogous to the specific rotation, M is the molecular weight, and $\varepsilon\varepsilon_L$ and $\varepsilon\varepsilon_R$ are the molar absorptivities for the left and right components

of circularly polarized light at a selected wavelength. Perrin and Hart38 review the applications of CD to interactions between small molecules and macromolecules, and the theory is described by Campbell and Dwek.12 Circular dichroism has its greatest application in the characterization of chiral macromolecules, in particular proteins that are utilized in biotechnology-based therapeutics. Secondary

structure in proteins can easily be defined by close investigation of CD spectra.[37,39,40,41] The magnitude of the absorption of the components of circularly polarized light is distinctly different for α-helices, β-sheets, and β-turns. By measuring the CD spectrum of proteins whose secondary structure is known, one can compile database and use it to deconvolve the secondary structural composition of an unknown protein. The CD spectra for a protein can also serve as standard and be used to determine whether effects during production or formulation are altering the proteins' folding patterns, which could dramatically decrease its activity.

Circular dichroism has also been applied to the determination of the activity of penicillin, as described by Rasmussen and Higuchi.[42] The activity was measured as the change in the CD spectra of penicillin after addition of penicillinase, which enzymatically cleaves the β-lactam ring to form the penicillate ion, as shown for benzylpenicillin in Figure 4-27. Typical CD spectra for benzylpenicillin and its hydrolysis product are shown in Figure 4-27**.** 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.[43] The penicillins all have single positive CD bands with maxima at 230 nm, whereas 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.

Electron and Neutron Scattering and Emission Spectroscopy

Electrons can flow across media in response to charge separation, and do so at certain frequencies and wavelengths depending on the magnitude of the charge separation. Neutrons can also be generated and flow in a similar manner. The exact mathematical and theoretical description of this flow is well beyond the scope of this text. However, the concept that they can flow is important to the discussion of the following subjects. It must also be noted that whereas electron and neutron waves are not traditionally thought of as electromagnetic radiation, they can be used to determine important molecular properties and are included in this text. The main distinction between electrons and neutrons versus electromagnetic radiation is that electrons and neutrons have a finite resting mass, in contrast to electromagnetic radiation, which has a zero finite resting mass.[12]

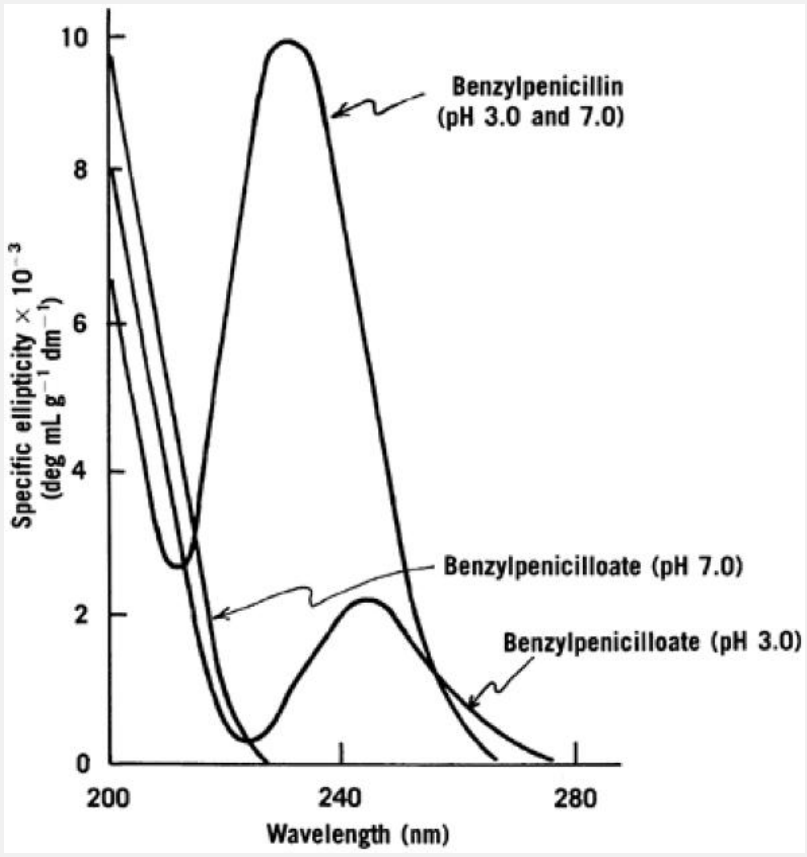

Fig. 4-27. Circular dichroism spectra of benzylpenicillin and its penicilloic acid derivative. (From C. E. Rassmussen and T. Higuchi, J. Pharm. Sci. **60,** 1616, 1971. With permission.)

Several techniques that are pharmaceutically relevant also employ electron or neutron beams, including small-angle neutron scattering and scanning and transmission electron microscopy (SEM and TEM, respectively). Scattering of energy waves results when a loss of energy occurs through interaction with a medium, as occurs in refraction. Scattering is usually measured at an angle θ from the incident beam of energy and does not measure transmittance of the energy wave. Both electron and neutron waves play an important role in identifying some of the molecular properties of compounds and excipients. They are discussed briefly here.

Scanning and transmission electron microscopy are two techniques that are routinely employed in pharmaceutical and biologic sciences to produce high-resolution images of surfaces. Let us consider SEM. In general, the image generated with SEM is produced by electron wave emission from a filament and bombardment of the sample, where the electrons undergo refraction after interaction with the sample surface. As the electrons pass through the sample surface, they continuously undergo refraction until they are refracted back out of the sample surface at a lower energy and are detected. In the case of refraction, the incident electron beam interacts with electrons in the sample's atomic electron cloud, which results in alteration of its path after collision. For heavier atoms, the electron cloud is dense, and the incident electrons from the beam undergo greater refraction. In SEM techniques, the sample is often sputter coated (sprayed) with gold to provide a greater electron density at the surface and prevent the beam from passing through the sample. In TEM, the electrons are transmitted through the sample. The refracted electrons

generate a high-resolution snapshot of the sample surface and provide information about its contour and size; however, it does not offer molecular information.

Molecular (atomic) information can be obtained from monitoring the energy, which is in the form of x-rays emitted when the electrons interact with the molecules. In general, upon an inelastic collision the incidental electron beam can also knock out ground-state electrons in the molecules. This is known as Auger electron emission. The outer-shell electrons in each atom then relax to the ground state, which results in the emission of energy in the form of x-ray fluorescence. For each atom, the atomic structure is different, and the relaxation energies change on the basis of the individual atoms. The emission of these x-rays can be captured using techniques such as energy-dispersive x-ray analysis (EDAX) and wavelength-dispersive x-ray analysis. These techniques provide information about the molecular composition by detecting the different x-ray frequencies and wavelengths emitted during an SEM run. Energy-dispersive x-ray analysis captures the frequency patterns of the emitted x-ray waves, whereas wavelength-dispersive x-ray analysis measures the x-ray wavelengths from the atoms present. These emissions can be measured across a linear region of the sample or in the whole sample. They are qualitative but can be quantitative in nature, depending on the type of analysis performed.

A good example of the utility of this technique is the examination of a coated tablet that contains an iron-based drug in its core (Fig. 4-28). If an SEM and a linear EDAX analysis are performed across the surface of a split tablet, EDAX demonstrates that the iron is only present in the center of the tablet and not in the coated region. SEM provides a picture of the surface of the split tablet that can be used to look at formulation conditions. If a similar tablet is then placed in water for 1 hr and subjected to the same analysis, one might observe a lower amount of iron in the core, and iron present in the coating, with an image that shows the difference in the tablet's core structure upon water exposure. This could provide very valuable information to a formulator, who may want to compare several different coatings to control the release of the iron-based drug or look at how different polymorphs (Chapter 2) of the compound might behave in the same formulation. In fact, this type of analysis is routinely performed for many types of formulations including micro- and nanospheres. The information about physical properties of state (Chapter 2) and molecular properties is very important. There are also many other techniques employing similar methods that are being widely incorporated into the industrial characterization and formulation development of drugs.

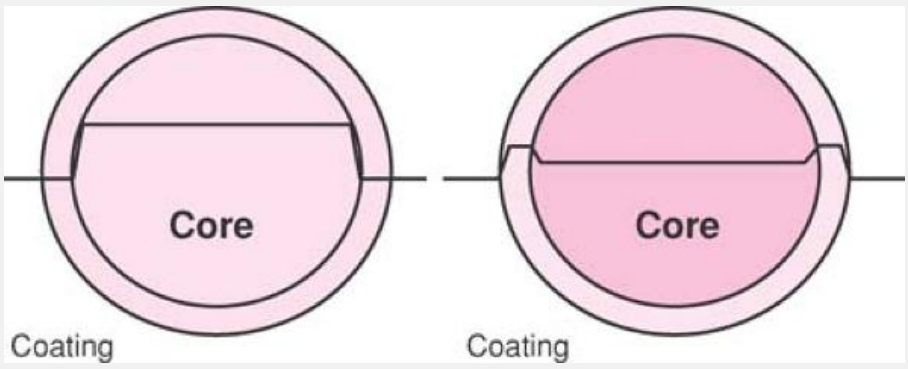

Fig. 4-28. A coated iron-containing tablet that was split, showing initially a smooth surface (*left*) and then, after exposure to water for 1 hr, erosion and a rougher surface (*right*). The line through the tablets shows how a hypothetical scanning electron

microscope image combined with an energy-dispersive x-ray analysis (EDAX) linear profile focused on the iron x-ray fluorescence emission frequency is performed across the tablet. In the first tablet, the iron is uniformly contained in the center and the magnitude of the deviation of the line implies a higher iron concentration in the core. The image shows a smooth surface. After exposure to water for 1 hr, the iron and some of the excipients have migrated from the core, leaving a rough contour and a lower EDAX level. The coating has a higher level of iron by this scan, which could be due to dissolution and the effect of diffusion rates, as discussed in later chapters.

Small-angle neutron scattering is another technique that can be employed to illustrate the physical properties of a molecule. It is related to techniques like small-angle x-ray scattering and small-angle light scattering. Collectively, these techniques can be used to give information about the size (even molecular weights for large polymeric and protein molecules), shape, and even orientation of components in a sample.44 Small-angle neutron scattering is primarily used for the characterization of polymers, in particular dendrimers, and colloids.

Chapter Summary

The goal of this chapter was to provide a foundation for understanding the physical properties of molecules including methods to make those determinations. Students should understand the nature of intra- and intermolecular forces, molecular energetics of these forces, and their relevance to different molecules. Another important aspect that was covered was the determination of these physical properties using a variety of atomic and molecular spectroscopic techniques. The student should also appreciate the differences in the techniques with respect to the identification and detection of pharmaceutical agents.

Practice problems for this chapter can be found at thePoint.lww.com/Sinko6e.

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Chapter Legacy

Fifth Edition: published as Chapter 4. Updated by Gregory Knipp, Dea Herrera-Ruiz, and Hugo Morales-Rojas.
Sixth Edition: published as Chapter 4. Updated by Patrick Sinko.