Advanced Pharmaceutical Analysis

College of Pharmacy - University of Anbar / Fifth Year 2019 - 2020

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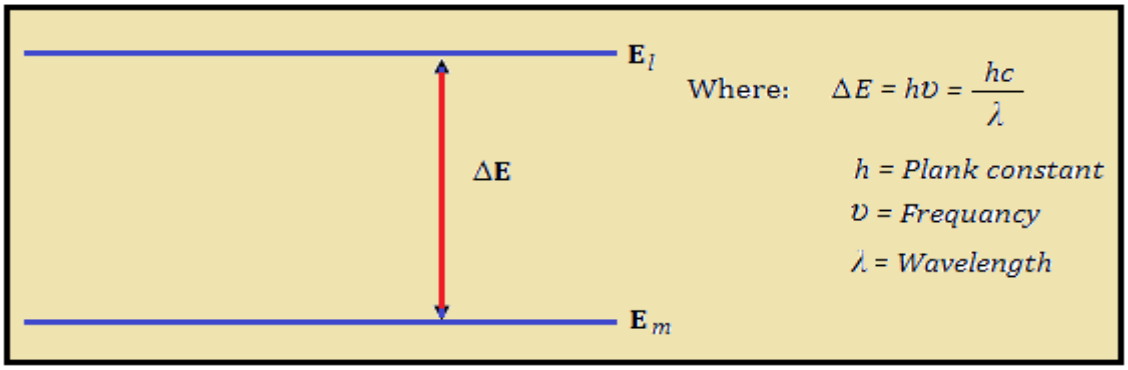
Chapter 1

UV/Visable Spectroscopy

1.1 Introduction

Spectroscopy is based, principally, on the study of the interaction between radiation and matter. This interaction causes in the atom an electronic transition from a lower energetic level, *m*, to a higher level, *l*, occurring energy absorption from the atom equal to the energy difference between both levels, E_l - E_m.



A plot of these latter processes as a function of radiation wavelength is known as spectrum that offers information about the difference of energy involved in each electronic transition. Different types of spectroscopy can be found depending on the wavelength of the incident radiation.

1.2 Definitions and Units

Radiation is a form of energy and we are constantly reminded of its presence via our sense of sight and ability to feel radiant heat. It may be considered in terms of a wave motion where the wavelength, λ, is the distance between two successive peaks. The frequency, *ν*, is the number of peaks passing a given point per second (Figure 1.1). These terms are related so that: $c = \nu\lambda$
where *c* is the velocity of light in a vacuum.

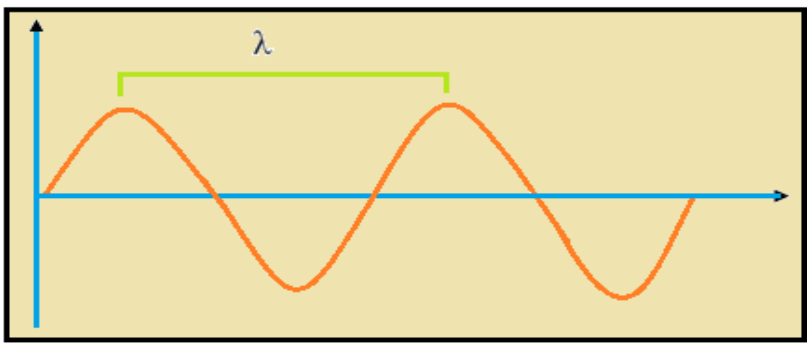


Figure 1.1 The wavelength λ of electromagnetic radiation.

The full electromagnetic radiation spectrum is continuous and each region merges slowly into the next. For spectroscopy purposes, we choose to characterize light in the ultraviolet and visible regions in terms of wavelength expressed in nanometers. Other units which may be encountered, but whose use is now discouraged, are the Angstrom (Å) and the millimicron (mμ).

$$1nm = 1m\mu = 10Å = 10^{-9} \text{ meters}$$

For convenience of reference, definitions of the various spectral regions have been set by the Joint Committee on Nomenclature in Applied Spectroscopy:

Region	Wavelength (nm)
Far ultraviolet	10-200
Near ultraviolet	200-380
Visible	380-780
Near infrared	780-3000
Middle infrared	3000-30,000
Far infrared	30,000-300,000
Microwave	300,000-1,000,000,000

The human eye is only sensitive to a tiny proportion of the total electromagnetic spectrum between approximately 380 and 780 nm and within this area we perceive the colors of the rainbow from violet through to red. The electromagnetic spectrum can offers information about the difference of energy involved in each electronic transition. Different types of spectroscopy can be found depending on the wavelength of the incident radiation as can be seen in Figure 1.2 and 1.3.

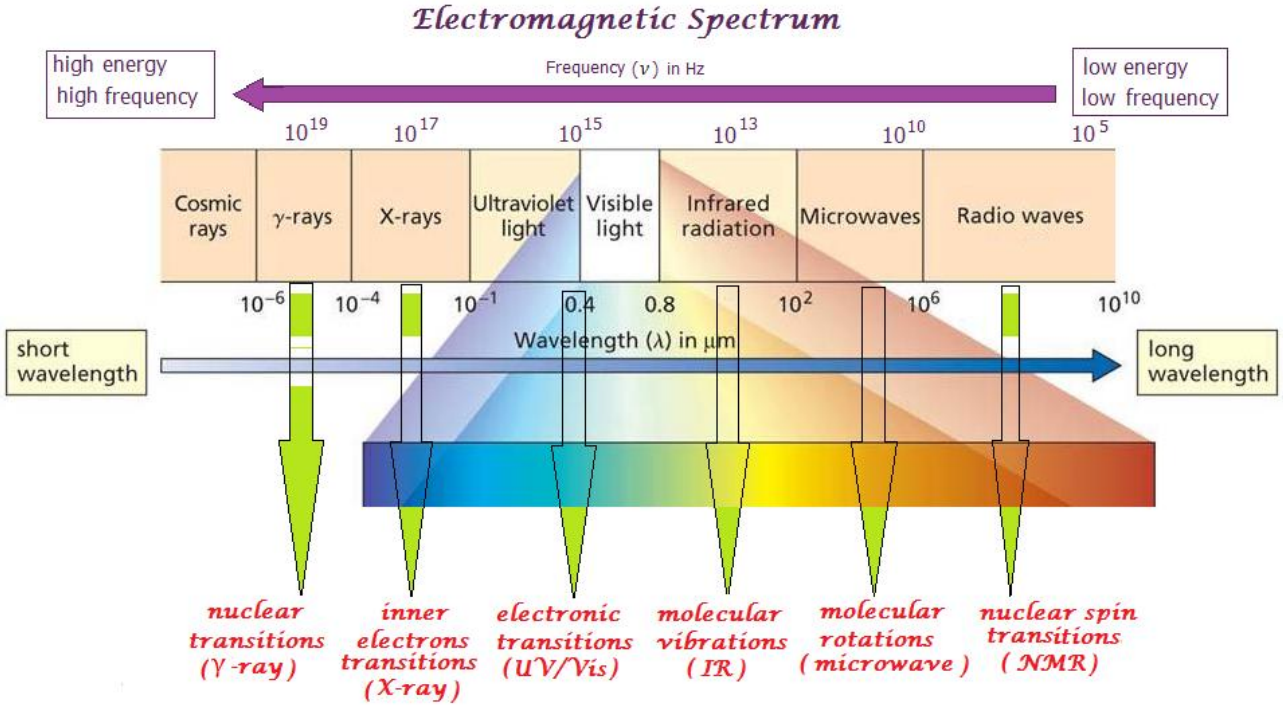


Figure 1.2 The electromagnetic spectrum.

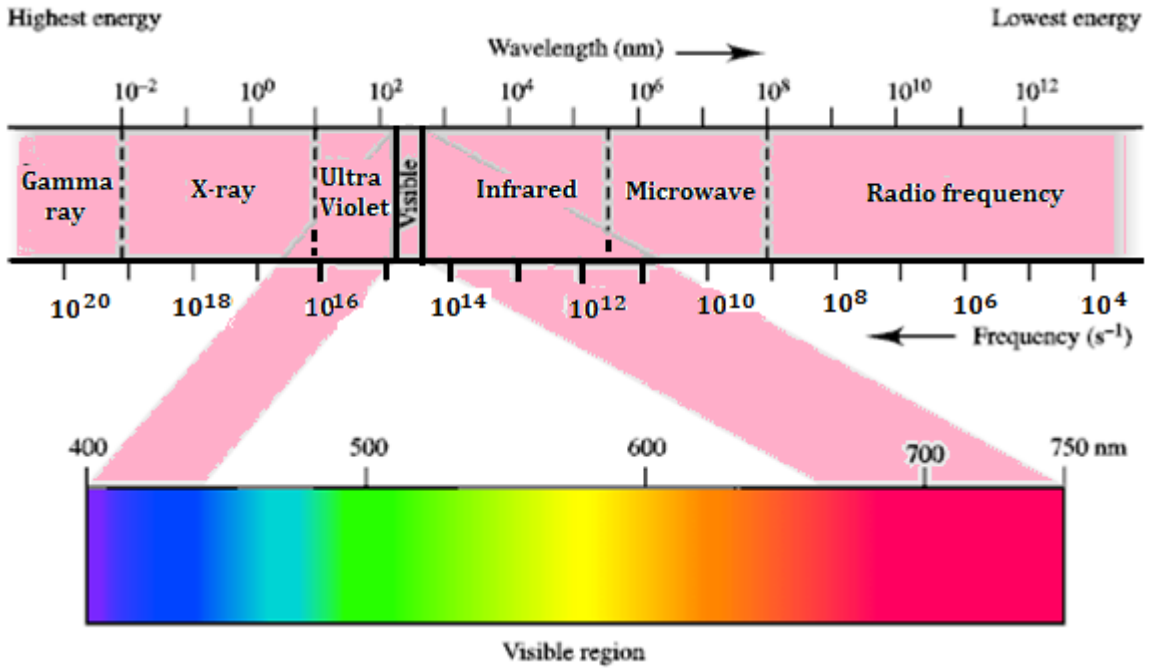


Figure 1.3 The Visible part in the electromagnetic spectrum.

Besides the sun, the most conveniently available source of visible radiation with which we are familiar is the tungsten lamp. If the current in the circuit supplying such a lamp is gradually increased from zero, the lamp filament at first can be felt to be emitting warmth, then glows dull red and the gradually brightens until it is emitting an intense white light and a considerable amount of heat.

UV-Visible spectroscopy offers information about the transition of the most external electrons of the atoms. Since atoms or molecules absorb UV-visible radiation at different wavelength, spectroscopy/spectrometry is often used in physical and analytical chemistry for the identification of substances through the spectrum emitted from or absorbed by them. This technique is also used to assess the concentration or amount of a given species using the Beer-Lambert law.

1.3 Beer-Lambert Law

The Beer-Lambert Law states that the concentration of a substance in solution is directly proportional to the 'absorbance', A, of the solution. It relates the absorption of a radiation to the properties of the material through which is passing through. In this case there is a logarithmic dependence between the transmission (or transmissivity), T, of light through a substance and the product of the absorption coefficient of the substance, α, and the distance the beam travels through the material (i.e. the path length), l. The absorption coefficient can, in turn, be written as a product of either a molar absorptivity of the absorber, ϵ (with units of $cm^{-1}\ M^{-1}$), and the concentration c of absorbing species in the material.

The transmission also can be expressed in terms of absorbance (A):

$$A = - \log T$$

$$A = - \log (I/I_o)$$

where I_o and I are the intensity of the incident and the transmitted beams, respectively.

Beer-Lambert equation can be written finally as:

$$A = \epsilon l c$$

Either transmittance or absorbance can be measured experimentally with the spectrometer. Thus, if the path length and the molar absorptivity are known and the absorbance is measured, the concentration of the substance (or the number density of absorbers) can be deduced. Commonly, both parameters are constant for a given set of experiments, thus, a plot of the sample absorbance against the concentration of the absorbing substance should be a straight line. In practice, a calibration curve is obtained by plotting the measured absorbance of a series of standard samples as a function of their concentration. If the absorbance of an unknown sample is then measured, the concentration of the absorbing component can be determined from this graph.

Example

An organic solution has an absorbance of 0.54 at 250 nm in a 0.5 cm length cuvette. What is the concentration of the solution if the absorption coefficient was 5.4×10^3 cm^{-1} M^{-1}?

$$A = \epsilon\, c\, l$$

$$0.54 = 5.4\times10^3 \times c \times 0.5$$

$$c = 0.0002\ M$$

Example

Calculate the absorbance A for a solution if the transmissivity T was 89% at 500 nm.

$$A = -\log T$$

$$A = -\log(89/100) = 0.050609$$

1.4 UV-Visible spectrometers

The basic parts of a spectrophotometer are a light source, a sample holder, a diffraction grating or monochromator to separate the different wavelengths of light, and a detector. The radiation source is often a Tungsten filament (300-2500 nm), a deuterium arc lamp which is continuous over the ultraviolet region (190-400 nm), and more recently light emitting diodes (LED) and Xenon Arc Lamps for the visible wavelengths. The detector is typically a photodiode or a CCD (Charge-Coupled-Device).

Photodiodes are used with monochromators, which permit only light of a single wavelength reaches the detector. Diffraction gratings are used with CCDs, which collects light of different wavelengths on different pixels.

A spectrophotometer can be either *single beam* or *double beam*. In a single beam instrument, all of the light passes through the same sample cell. First, the reference, I_o, (generally the solvent) must be measured before the sample. This was the earliest design, but is still in common use in both teaching and industrial labs.

In a double-beam instrument (Fig. 1.4), the light is split into two beams before it reaches the sample. One beam is used as the reference and the other as the sample. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beam are measured at the same time.

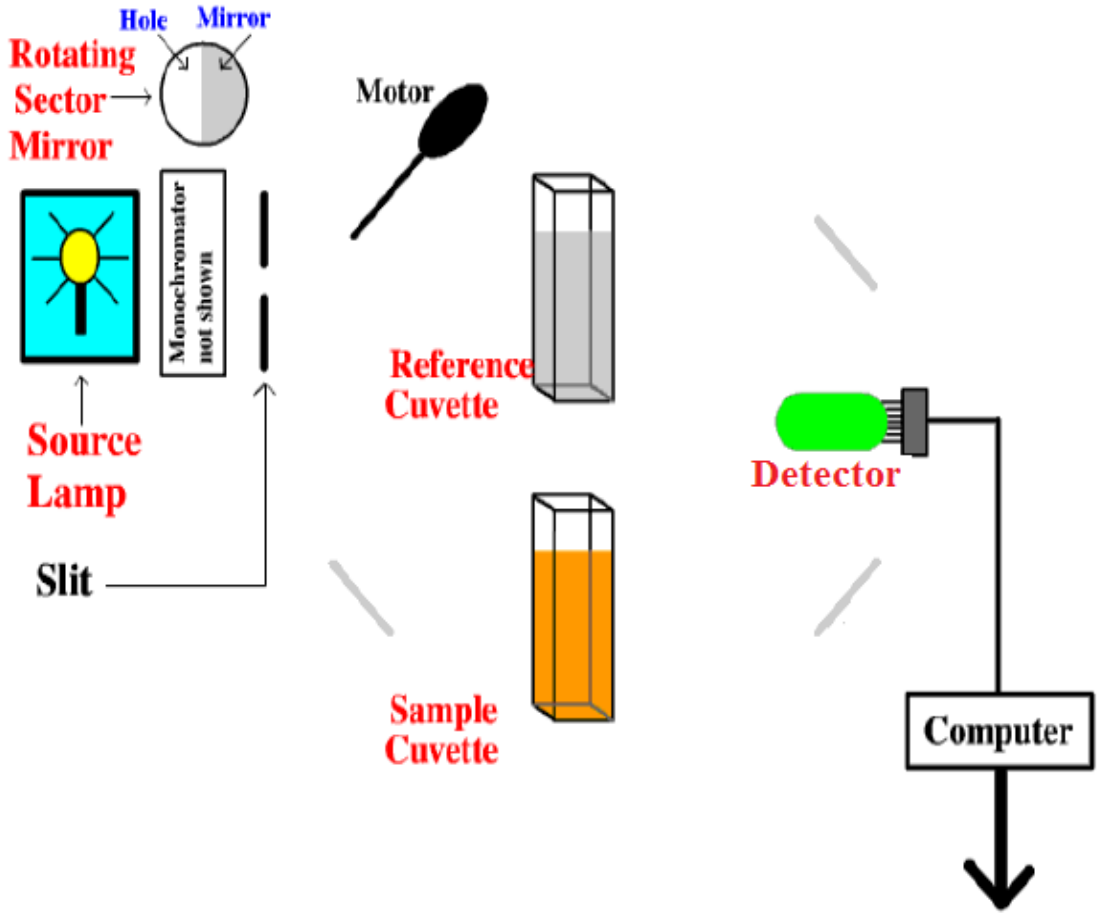


Figure 1.4 Schematic representations for a Double-Beam Spectrophotometer.

Samples for UV/Vis spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape, commonly with an internal width of 1 cm (this width becomes the path length, *l*, in the Beer-Lambert law). They must allow radiation to pass over the spectral region of interest. The most widely applicable cuvettes are made of quartz, glass, and plastic. Quartz or fused-silica cells are required when working at wavelengths of less than 300 nm where other materials show a significant absorption. The solution usually very dilutes and chosen so that it doesn't absorb any significant amount of light in the wavelength range we are interested in (200-800 nm). The reference cell just contains the pure solvent. Figure 1.5 shows the double-beam spectrophotometer instrument.

Figure 1.5 Double-Beam Spectrophotometer.

1.5 Theory

To gain an understanding of the origins of practical absorption spectrometry, a short diversion into quantum theory is necessary. For this purpose, it is best to think of radiation as a stream of particles known as photons instead of the waves considered earlier. Atoms and molecules exist in a number of defined energy states or levels and a change of level requires the absorption or emission of an integral number of a unit of energy called a quantum, or in our context, a photon. The energy of a photon absorbed or emitted during a transition from one molecular energy level to another is given by the equation:

$$e = h\nu$$

where h is known as Planck's constant and ν is the frequency of the photon. We have already seen that $c = \nu\lambda$, therefore, $E = hc/\lambda$.

Thus, the shorter the wavelength, the greater the energy of the photon and vice versa. A molecule of any substance has an internal energy which can be considered as the sum of the energy of its electrons, the energy of vibration between its constituent atoms and the energy associated with rotation of the molecule. The electronic energy levels of simple molecules are widely separated and usually only the absorption of a high energy photon, that is one of very short wavelength, can excite a molecule from one level to another.

In complex molecules the energy levels are more closely spaced and photons of near ultraviolet and visible light can affect the transition. These substances, therefore, will absorb light in some areas of the near ultraviolet and visible regions. The vibrational energy states of the various parts of a molecule are much closer together than the electronic energy levels. Light absorption due to only to vibrational changes occurs in the infrared region. The rotational energy states of molecules are so closely spaced that light in the far infrared and microwave regions of the electromagnetic spectrum has enough energy to cause these small changes.

For ultraviolet and visible wavelengths, one should expect from this discussion that the absorption spectrum of a molecule (i.e., a plot of its degree of absorption against the wavelength of the incident radiation) should show a few incident photon exactly very sharp lines. Each line should occur at a wavelength where the energy of an incident photons exactly matches the energy required to excite an electronic transition.

In practice it is found that the ultraviolet and visible spectrum of most molecules consists of a few humps rather than sharp lines. These humps show the molecule is absorbing radiation over a band of wavelengths. One reason for this band, rather than line absorption is that an electronic level transition is usually accompanied by a simultaneous change between the more numerous vibrational levels. Thus, a photon with a little too much or too little energy to be accepted by the molecule for a 'pure' electronic transition can be utilized for a transition between one of the vibrational levels associated with the lower electronic state to one of the vibrational levels of a higher electronic state.

Furthermore, each of the many vibrational levels associated with the electronic states also has a large number of rotational levels associated with it.

Thus a transition can consist of a large electronic component, a smaller vibrational element and an even smaller rotational change. The rotational contribution to the transition has the effect of filling in the gaps in the vibrational fine structure.

In addition, when molecules are closely packed together as they normally are in solution, they exert influences on each other which slightly disturb the already numerous, and almost infinite energy levels and blur the sharp spectral lines into bands. These effects can be seen in the spectra of benzene as a vapour and in solution. In the vapour, the transitions between the vibration levels are visible as bands superimposed on the main electronic transition bands.

In solution they merge together and at high temperature or pressure even the electronic bands can blur to produce single wide band such as that enclosed by the dotted line in Figure 1.6.

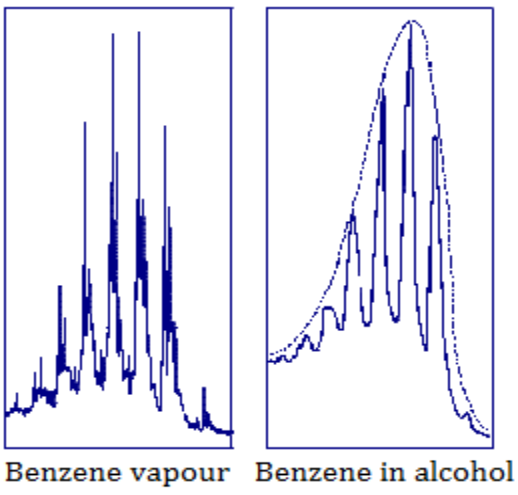


Figure 1.6 Vapor and solution spectra of Benzene.

1.6 Electronic Structure of Diatomic Molecules

According to the Molecular Orbital Theory, an atomic orbital is the space within which an electron belonging to the orbital spends 95% of its time. In molecular orbital theory orbitals embrace two or more nuclei. Electrons in a molecule are not tied to a particular atom, they are "scattered" throughout the entire molecule.

For a diatomic molecule we can write the two orbitals produced from the linear combination of the atomic orbitals as follow:

$$\psi_{m.o} = \psi_1 + \psi_2$$

$$\psi_{m.o} = \psi_1 - \psi_2$$

When atomic orbitals interact, the resulting molecular orbitals can be either bonding orbitals or antibonding orbitals. Bonding interactions between atomic orbitals are constructive (in-phase) interactions; they are lower in energy than the atomic orbitals that combine to produce them. Antibonding interactions between atomic orbitals are destructive (out-of-phase) interactions, with a nodal plane where the wavefunction of the antibonding orbital is zero between the two interacting atoms. They are higher in energy than the atomic orbitals that combine to produce them. Figure 1.7 shows the interaction between s orbitals.

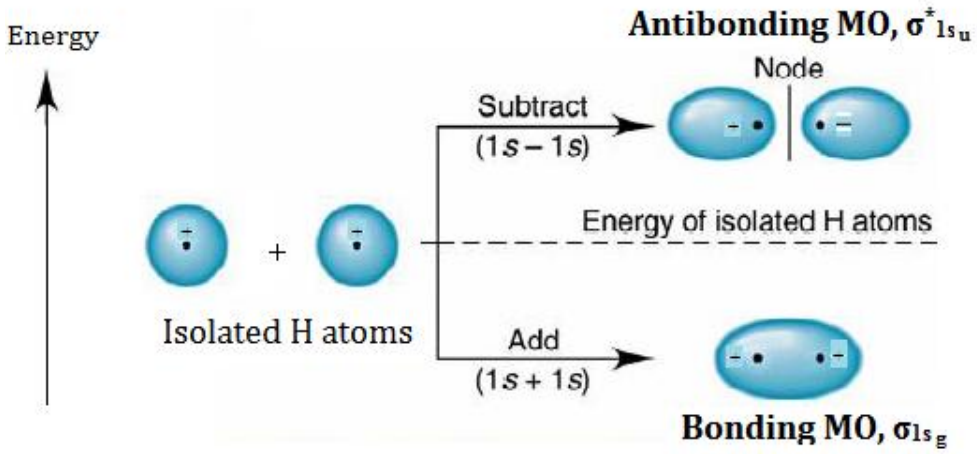


Figure 1.7 The Overlap of two s atomic orbitals.

If inversion through the center of symmetry in a molecule results in the same phases for the molecular orbital, then the MO is said to have gerade (g) symmetry, from the German word for even. If inversion through the center of symmetry in a molecule results in a phase change for the molecular orbital, then the MO is said to have ungerade (u) symmetry, from the German word for odd. For a bonding MO with σ-symmetry, the orbital is σ_g (s' + s'' is symmetric), while

an antibonding MO with σ-symmetry the orbital is σ_u, because inversion of s' – s'' is antisymmetric.

For p orbitals, the overlap takes place in two ways depending on the orientation of the orbitals in space. It is either head to head as it happen for p_z suborbital (Fig. 1.8).

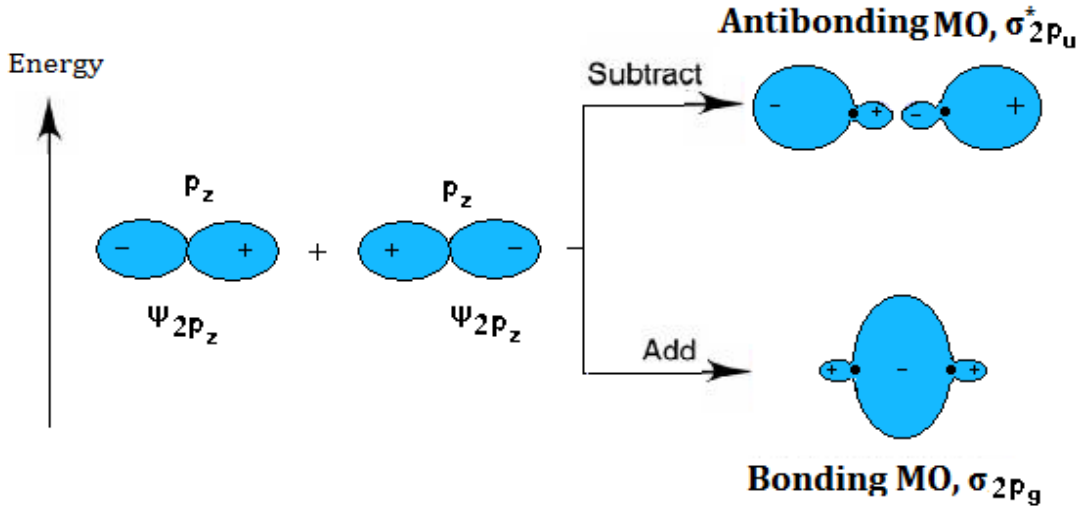


Figure 1.8 The Overlap of two p atomic orbitals head to head.

Or side to side as it happen for p_x and p_y (Fig. 1.9)

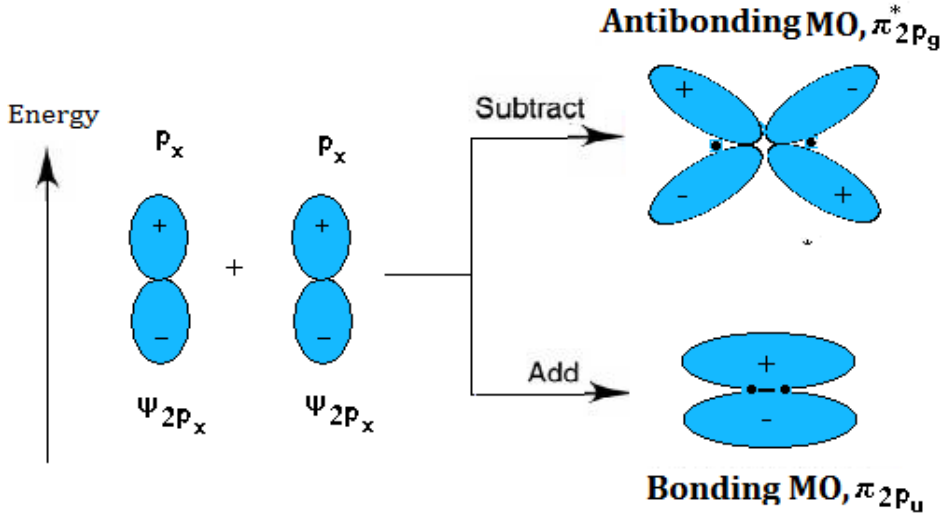


Figure 1.9 The overlap of two p atomic orbitals side to side.

The bonding strength in π orbitals is less than in σ. The energy diagram of the MOs is shown in Figure 1.10.

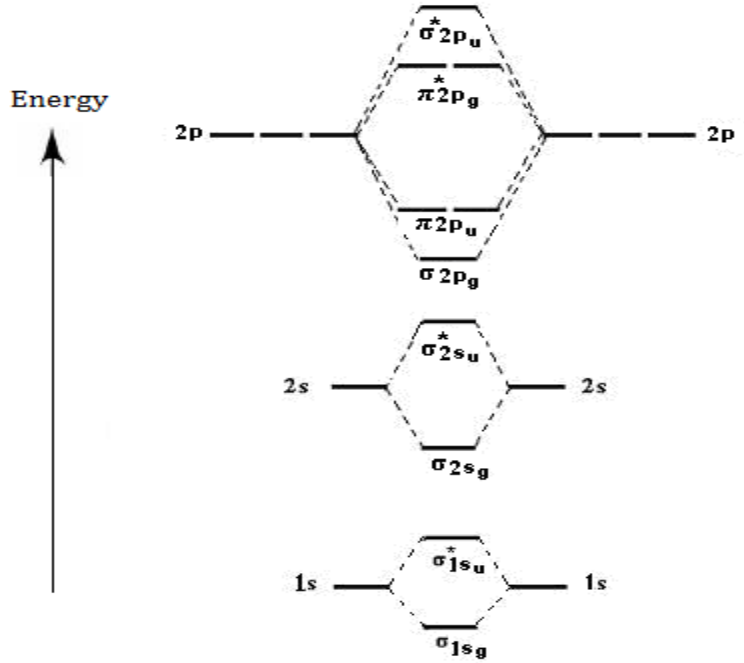


Figure 1.10 The energy diagrams of the MOs.

There is another type of electrons or orbitals called *n* orbitals or nonbonding orbitals. Nonbonding MOs are the result of no interaction between atomic orbitals because of lack of compatible symmetries. They have the same energy as the atomic orbitals of one of the atoms in the molecule as showing in the general Figure 1.11.

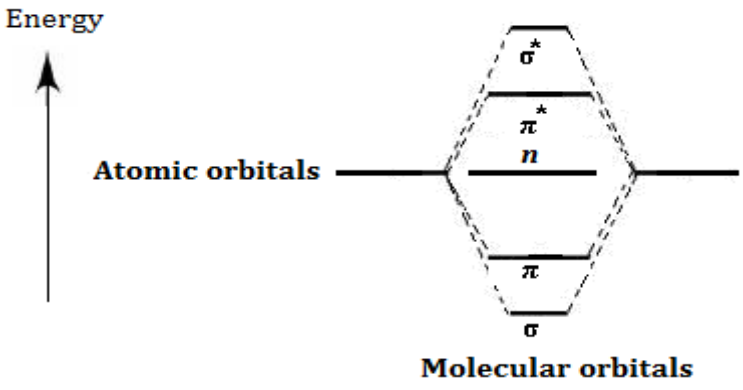


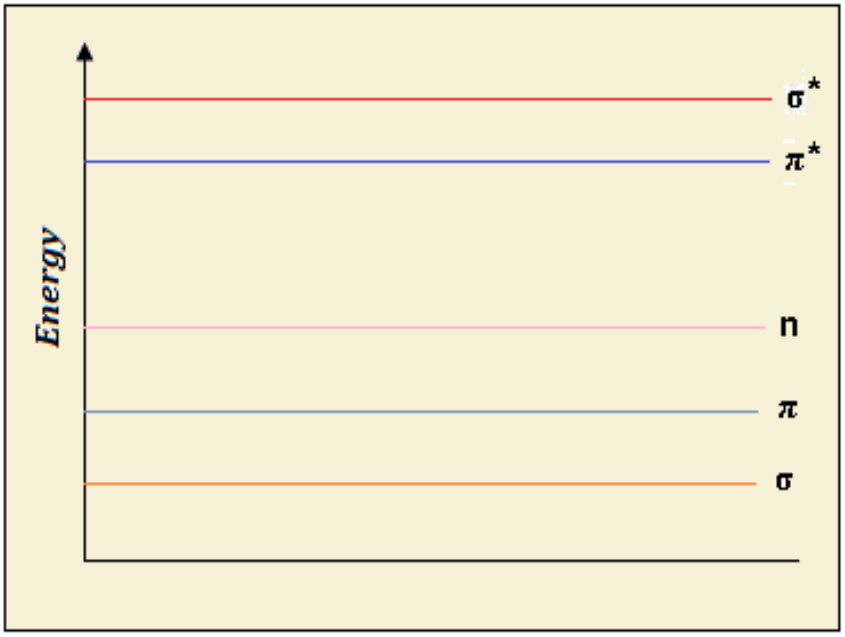
Figure 1.11 General energy diagrams of the MOs.

*1.7 Types of Transitions in Electronic Spectr*a

There are three types of electrons in molecules:

1- σ Electrons
2- π Electrons
3- Non-bonding *n* Electrons

The electronic arrangement for the electronic orbitals is as follow:



In general σ electrons are more attracted to the nuclei, and because of that it needs more energy to undergo electronic transition, while the two other types need less energy. More frequent *n* electrons need less energy than π electrons to undergo electronic transition.

The probable transitions are:

Allowed transitions: $g \rightarrow u$ *or* $u \rightarrow g$
Forbidden transitions: $g \rightarrow g$ *or* $u \rightarrow u$

- $\sigma \rightarrow \sigma^*$ *appears at* (less than 200 nm)
- $\pi \rightarrow \pi^*$ *and* $n \rightarrow \sigma^*$ *appears at* (about 200 nm)
- $n \rightarrow \pi^*$ *appears at* (about 200-400 nm) and (about 400-750 nm)

1.8 Absorption of UV-Visible Light

A group in a molecule that absorb light are known as chromophore. This is covalently unsaturated group responsible for electronic absorption. Or any group of atoms that absorbs light whether or not a color is thereby produced. e.g. C=C, C=O, NO_2. A compound containing chromophore is called chromogen.

The color of a molecule may be intensified by groups called auxochromes which generally do not absorb significantly in the 200-800 nm region, but will affect the spectrum of the chromophore to which it is attached. The most important auxochromic groups are OH, NH_2, CH_3 and halogen and their properties are acidic (phenolic) or basic.

Intensely absorbing compounds must be examined in dilute solution, so that significant light energy is received by the detector, and this requires the use of completely transparent (non-absorbing) solvents.

Because the absorbance of a sample will be proportional to its molar concentration in the sample cuvette, a corrected absorption value known as the molar absorptivity is used when comparing the spectra of different compounds.

Absorption spectrum consists of absorption bands corresponding to structural groups of molecules (Chromophores). Visible light that hits the chromophore can thus be absorbed by exciting an electron from its ground state into an excited state. The chromophore is a region in the molecule where the energy difference between two different molecular orbitals falls within the range of the visible spectrum.

The UV/Visible spectrum for compound usually obtained by drawing the relation between the wavelength and absorbance (Fig. 1.12).

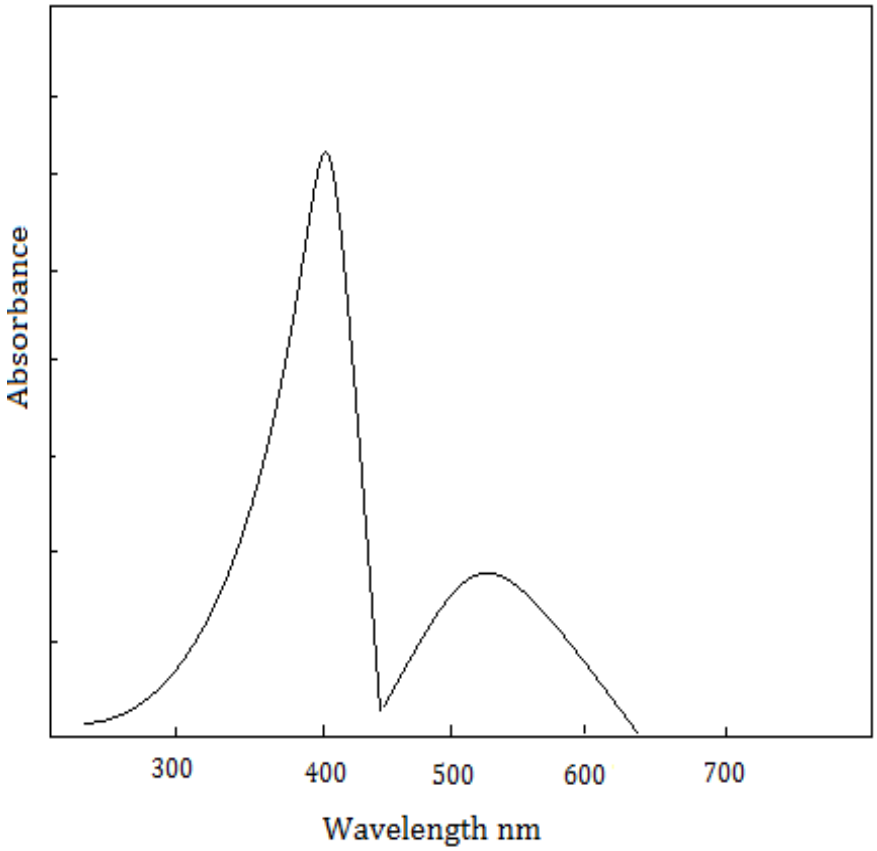


Figure 1.12 Typical UV/Visible diagram.

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Chapter 2

Infra-Red Spectroscopy

2.1 Introduction

Infrared (IR) radiation refers broadly to the electromagnetic spectrum between the visible and microwave region. Of greatest practical use to the organic chemist is the limited portion between 4000 and 400 cm^{-1}. There has been some interest in the near-IR (14,290-4000 cm^{-1}) and far-IR regions, 700-200 cm^{-1}.

Although the IR spectrum is characteristic of the entire molecule, it is true that certain groups give rise to bands at or near the same frequency regardless of the structure of the rest of the molecule. It is the persistence of these characteristic bands that permits the chemist to obtain useful structural information by simple inspection and reference to generalized charts of characteristic group frequencies. We shall rely heavily on these characteristic group frequencies.

2.2 Theory

Infrared radiation of frequencies less than about 100 cm^{-1} is absorbed and converted by an organic molecule into energy of molecular rotation. This absorption is quantized; thus a molecular rotation spectrum consists of discrete lines.

Infrared radiation in the range from about 10,000-100 cm^{-1} is absorbed and converted by an organic molecule into energy of molecular vibration. This absorption is also quantized, but vibrational spectra appear as bands rather than as lines because a single vibrational energy change is accompanied by a number of rotational energy changes. It is with these vibrational-rotational bands, particularly those occurring between 4000 and 400 cm^{-1}, that we shall be concerned. The frequency or wavelength of absorption depends on the ***relative masses of the atoms***, the ***force constants of the bonds***, and the ***geometry of the atoms.***

Band positions in IR spectra are presented here as wavenumbers ($\bar{\upsilon}$) whose unit is the reciprocal centimeter (cm^{-1}); this unit is proportional to the energy of vibration. Wavelength (λ) was used in the older literature in unites of micrometers (μm = 10^{-6} m; earlier called microns). Wavenumbers are reciprocally related to wavelength.

$$cm^{-1} = 10^4/\mu m$$

Note that wavenumbers are sometimes called frequencies. However, this is incorrect since wavenumbers ($\bar{\upsilon}$ in unites of cm^{-1}) are equal to $1\times10^4/\lambda$ in unites of μm, whereas frequencies (υ in Hz) are equal to C/λ in cm, C being the speed of light (3×10^{10} cm/s). The symbol $\bar{\upsilon}$ is called "nu bar". Our spectra are linear in cm^{-1} except for a few linear in μm.

Band intensities can be expressed either as transmittance (T) or absorbance (A). Transmittance is the ratio of the radiant power transmitted by a sample to the radiant power incident on the sample. Absorbance is the logarithm, to the base 10, of the reciprocal of the transmittance; $A = \log_{10}(1/T)$. Organic chemists usually report intensity in semiquantitative terms (s = strong, m = medium, w = weak).

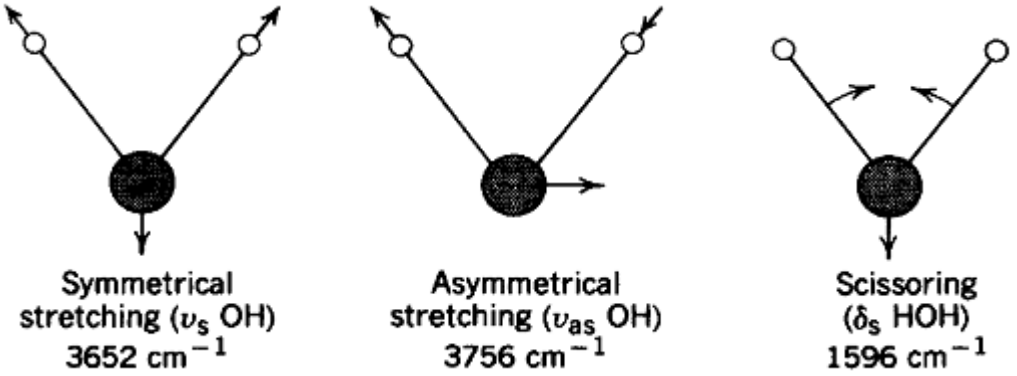
There are two types of molecular vibrations: stretching and bending. A stretching vibration is a rhythmical movement along the bond axis such that the interatomic distance is increasing or decreasing. A bending vibration may consist of a change in bond angle between bonds with a common atom or the movement of a group of atoms with respect to one another. For example, twisting, rocking, and torsional vibrations involve a change in bond angles with reference to a set of coordinates arbitrarily set up within the molecule.

Only those vibrations that result in the rhythmical change in the dipole moment of the molecule are observed in the IR. The alternating electric field, produced by the changing charge distribution accompanying a vibration, couples the molecule vibration with the oscillating electric field of the electromagnetic radiation.

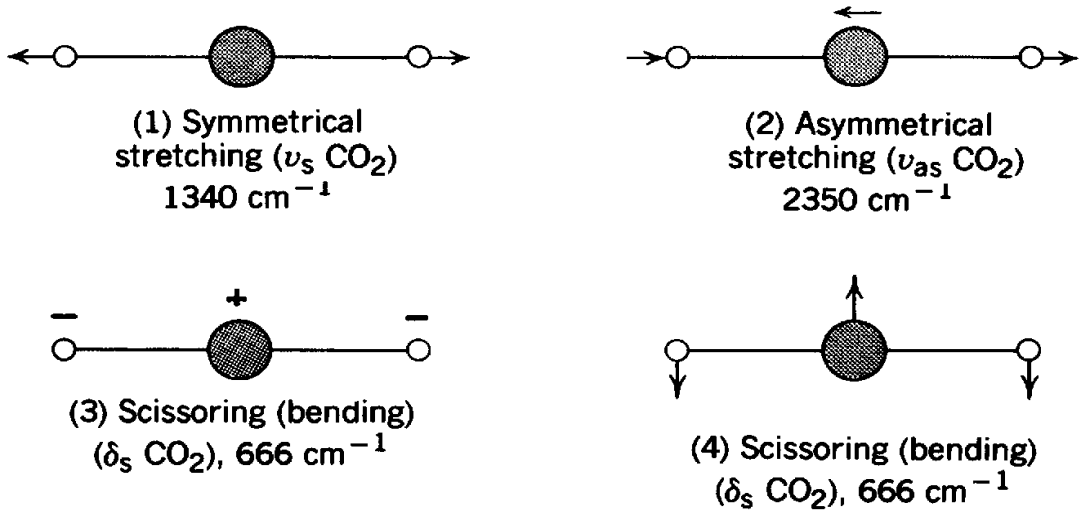
A molecule has as many degrees of freedom as the total degrees of freedom of its individual atoms. Each atom has three degree of freedom corresponding to the Cartesian coordinates (x, y, z) necessary to describe its position relative to other atoms in the molecule. A molecule of n atoms therefore has $3n$ degrees of freedom. For nonlinear molecules, three degrees of freedom describe rotation and three describe translation; the remaining $3n$-6 degrees of freedom are vibrational degrees of freedom or fundamental vibration. Linear molecules have $3n$-5 vibrational degrees of freedom, for only two degrees of freedom are required to describe rotation.

Fundamental vibrations involve no change in the center of gravity of the molecule.

The three fundamental vibrations of the nonlinear, triatomic water molecule can be depicted as follows:



The CO_2 molecule is linear and contains three atoms; therefore it has four fundamental vibrations [(3 × 3) – 5].



The symmetrical stretching vibration in (1) above is inactive in the IR since it produces no change in the dipole moment of the molecule. The bending vibrations in (3) and (4) above are equivalent and are the resolved components of bending motion oriented at any angle to the internuclear axis; they have the same frequency and are said to be doubly degenerate.

The various stretching and bending modes for an AX_2 group appearing as a portion of a molecule, for example, the CH_2 group in a hydrocarbon molecule, are shown in Figure 2.1. The 3*n*-6 rule does not apply since the CH_2 group represents only a portion of a molecule.

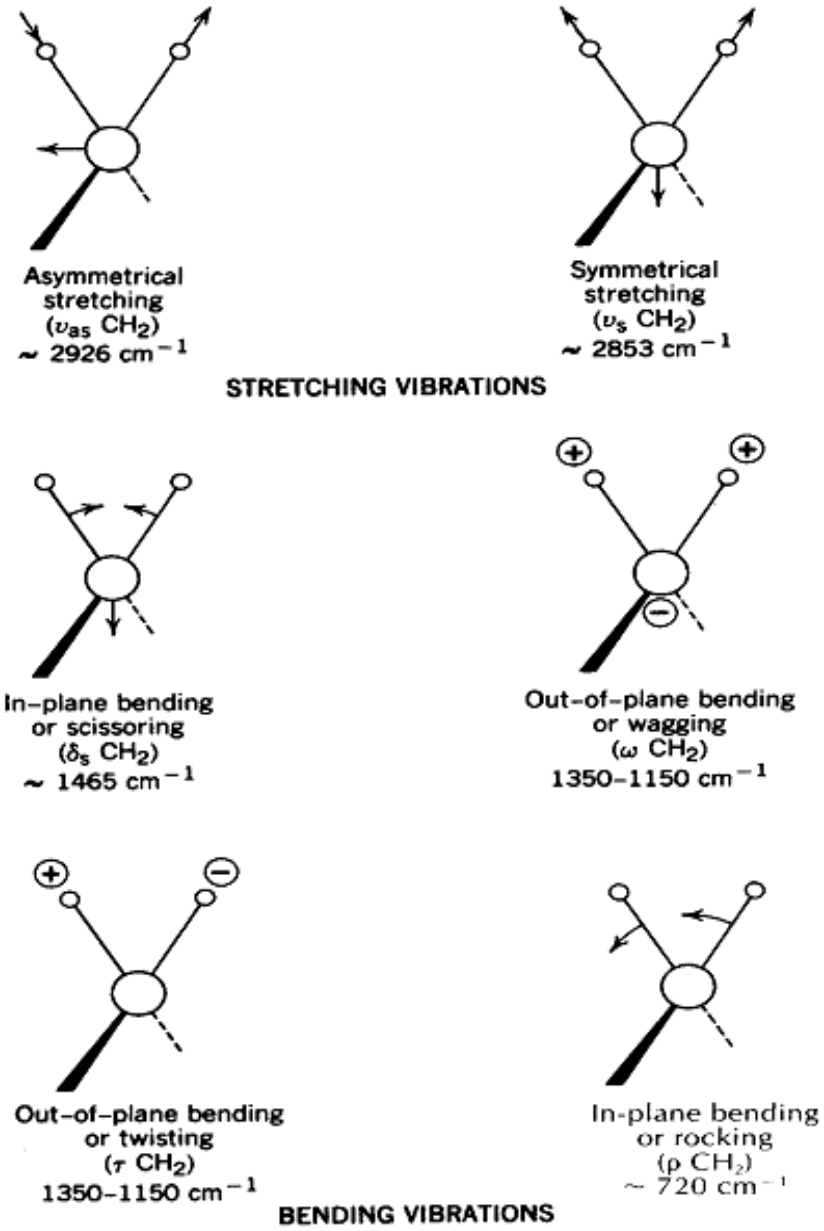


Figure 2.1 Vibrational modes for a CH_2 group.
(+ and – indicate movement perpendicular to the plane of the page)

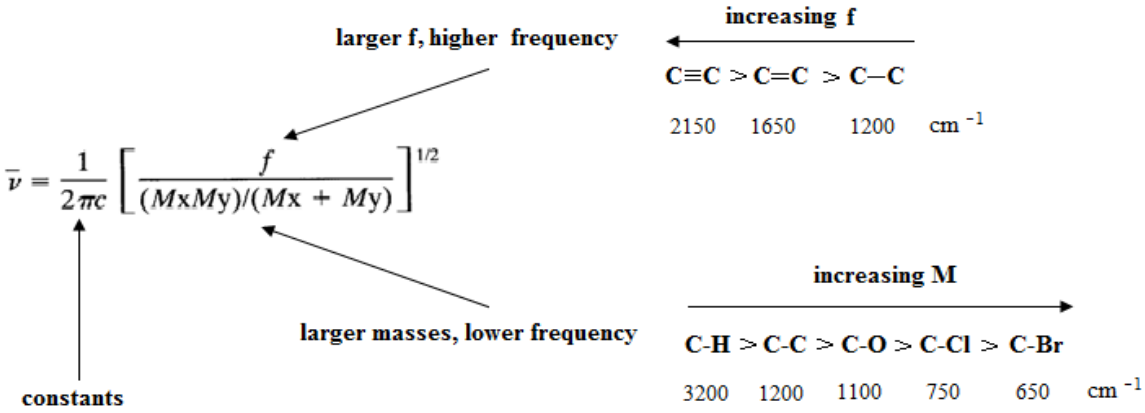
The theoretical number of fundamental vibrations (absorption frequencies) will seldom be observed because overtones (multiples of a given frequency, which results from exiting the vibrational mode from v=0 to v=2. Usually the fundamental transition from v=0 to v=1 or v=±1) and combination tones (sum of two other vibrations, which results when more than two or more fundamental vibrations are excited simultaneously) increase the number of bands, whereas other phenomena reduce the number of bands. The following will reduce the theoretical number of bands.

1- Fundamental frequencies that fall outside of the 4000-400 cm^{-1} region.
2- Fundamental bands that are too weak to be observed.
3- Fundamental vibrations that are so close that they coalesce.
4- The occurrence of a degenerate band from several absorption of the same frequency in highly symmetrical molecules.
5- The failure of certain fundamental vibrations to appear in the IR because of the lack of change in molecular dipole.

Assignments for stretching frequencies can be approximated by the application of Hooke's law. In the application of the law, two atoms and their connecting bonds are treated as a simple harmonic oscillator composed of two masses joined by a spring. The following equation, derived from Hooke's law, states the relationship between frequency of oscillation, atomic masses, and the force constant of the bond.

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}}$$

Where $\bar{\upsilon}$ = the vibrational frequency (cm^{-1})
C = velocity of light (cm/s)
K = force constant of bond
μ = masses of atoms



*M*x and *M*y = mass (g) of atom x and y, respectively.
K can be replaced by the symbol *f*

Example: The vibrational frequency of ^{127}HI found to be 2309.5 cm^{-1}. Calculate the value of force constant of the bond.

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}}$$

$$\mu = \frac{m_1 m_2}{m_1 + m_2}$$

$$= \frac{(1)\ (127)}{1 + 127\ \ (6.022 \times 10^{23})}$$

$$= 1.65 \times 10^{-24}\ gm$$

$$2309.5 = \frac{1}{2(3.14)\ (3 \times 10^{10})}\sqrt{\frac{k}{1.65 \times 10^{-24}}}$$

$$k = 312.38 \times 10^{3}\ g\ .\ sec^{-2}$$

The value of f is approximately 5×10^5 dyne/cm for single bonds and approximately two and three times this value for double and triple bonds, respectively.

Application of the formula to C—H stretching using 19.8×10^{-24} and 1.64×10^{-24} as mass value for C and H, respectively, places the frequency of the C—H bond vibration at 3040 cm^{-1}. Actually, C—H stretching vibrations, associated with methyl and methylene groups, are generally observed in the region between 2960 and 2850 cm^{-1}. The calculation is not highly accurate because effects arising from the environment of the C—H within a molecule have been ignored. The frequency of IR absorption is commonly used to calculate the force constants of bonds.

Calculations place the stretching frequencies of the following bonds in the general absorption regions indicated:

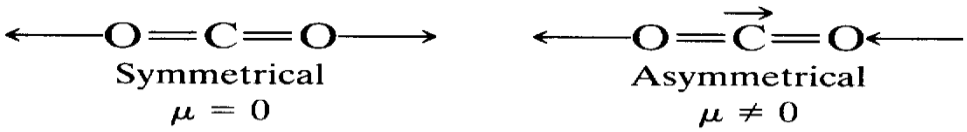
Bond Type	Absorption Region (cm^{-1})
C—C, C—O, C—N	1300–800
C=C, C=O, C=N, N=O	1900–1500
C≡C, C≡N	2300–2000
C—H, O—H, N—H	3800–2700

To approximate the vibrational frequencies of bond stretching by Hooke's law, the relative contributions of bond strengths and atomic masses must be considered. For example, a superficial comparison of the C—H group with the F—H group, on the basis of atomic masses, might lead to the conclusion that the stretching frequency of the F—H bond should occur at a lower frequency than that for the C—H bond. However, the increase in the force constant from left to right across the first two rows of the periodic table has a greater effect than the mass increase. Thus, the F—H group absorbs at a higher frequency (4138 cm^{-1}) than the C—H group (3040 cm^{-1}).

In general, functional groups that have a strong dipole give rise to strong absorptions in the IR.

2.2.1 Coupled Interactions

When two bond oscillators share a common atom, they seldom behave as individual oscillators unless the individual oscillation frequencies are widely different. This is because there is mechanical coupling interaction between the oscillators. For example, the carbon dioxide molecule, which consists of two bonds with a common carbon atom, has two fundamental stretching vibrations: an asymmetrical and a symmetrical stretching mode. The symmetrical stretching mode consists of an in-phase stretching or contracting of the bonds, and absorption occurs at a wavelength longer than that observed for the carbonyl group in an aliphatic ketone. The symmetrical stretching mode produces no change in the dipole moment (μ) of the molecule and is therefore "inactive" in the IR, but it is easily observed in the Raman spectrum near 1340 cm^{-1}. In the asymmetrical stretching mode, the two C=O bonds stretch out of phase; one C=O bond stretches as the other contracts. The asymmetrical stretching mode, since it produces a change in the dipole moment, is IR active; the absorption (2350 cm^{-1}) is at a higher frequency (shorter wavelength) than observed for a carbonyl group in aliphatic ketones.



This difference in carbonyl absorption frequencies displayed by the carbon dioxide molecule results from strong mechanical coupling or interaction. In contrast, two ketonic carbonyl groups separated by one or more carbon atoms show normal carbonyl absorption near 1715 cm^{-1} because appreciable coupling is prevented by the intervening carbon atom.

Coupling accounts for the two N—H stretching bands in the 3497-3077 cm^{-1} region in primary amine and primary amide spectra; for the two stretching bands in the 1818-1720 cm^{-1} region in carboxylic anhydride and imide spectra, and for the two C—H stretching bands in the 3000-2760 cm^{-1} region for both methylene and methyl groups.

Useful characteristic group frequency bands often involve coupled vibrations. The spectra of alcohols have a strong band in the region between 1260 and1000 cm^{-1} which is usually designated as the "C—O stretching band." In the spectrum of methanol this band is at 1034 cm^{-1}; in the spectrum of ethanol it occurs at1053 cm^{-1}. Branching and unsaturation produce absorption characteristic of these structures. It is evident that we are dealing not with an isolated C—O stretching vibration but rather a coupled asymmetric vibration involving C—C—O stretching.

Vibrations resulting from bond angle changes frequently couple in a manner similar to stretching vibrations. Thus, the ring C—H out-of-plane bending frequencies of aromatic molecules depend on the number of adjacent hydrogen atoms on the ring; coupling between the hydrogen atoms is affected by the bending of the C—C bond in the ring to which the hydrogen atoms are attached.

Interaction arising from coupling of stretching and bending vibrations is illustrated by the absorption of secondary acyclic amides. Secondary acyclic amides, which exist predominantly in the trans conformation, show strong absorption in the 1563-1515 cm^{-1} region; this absorption involves coupling of the N—H bending and C—N stretching vibrations.

The requirements for effective coupling interaction may be summarized as follows:

1- The vibrations must be of the same symmetry species if interaction is to occur.
2- Strong coupling between stretching vibrations requires a common atom between the groups.
3- Interaction is greatest when the coupled groups absorb, individually, near the same frequency.
4- Coupling between bending and stretching vibrations can occur if the stretching bond forms one side of the changing angle.
5- A common bond is required for coupling of bending vibrations.
6- Coupling is negligible when groups are separated by one or more carbon atoms and the vibrations are mutually perpendicular.

Fermi resonance is a common phenomenon in IR and Raman spectra, results in the splitting of two vibrational bands that have nearly the same energy and symmetry. It requires that the vibrational levels be of the same symmetry species and that the interacting groups be located in the molecule so that mechanical coupling is appreciable.

An example of Fermi resonance in an organic structure is the "doublet" appearance of the stretch of cyclopentanone under sufficient resolution conditions. Figure 2.2 shows the appearance of the spectrum of cyclopentanone under the usual conditions. With adequate resolution (Fig. 2.3), Fermi resonance with an overtone or combination band of an α-methylene group shows two absorptions in the carbonyl stretch region.

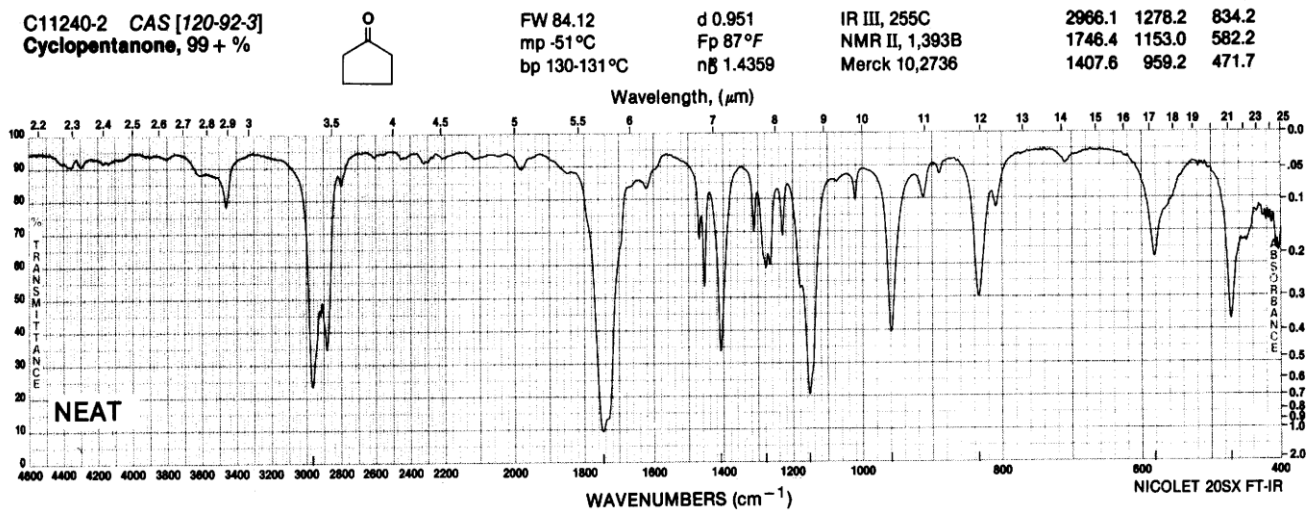


Figure 2.2 Cyclopentanone, thin film.

2.2.2 Hydrogen Bonding

Hydrogen bonding can occur in any system containing a proton donor group (X—H) and a proton acceptor (Y) if the s orbital of the proton can effectively overlap the *p* or *π* orbital of the acceptor group. Atoms X and Y are electronegative, with Y possessing lone pair electrons. The common proton donor groups in organic molecules are carboxyl, hydroxyl, amine, or amide groups.

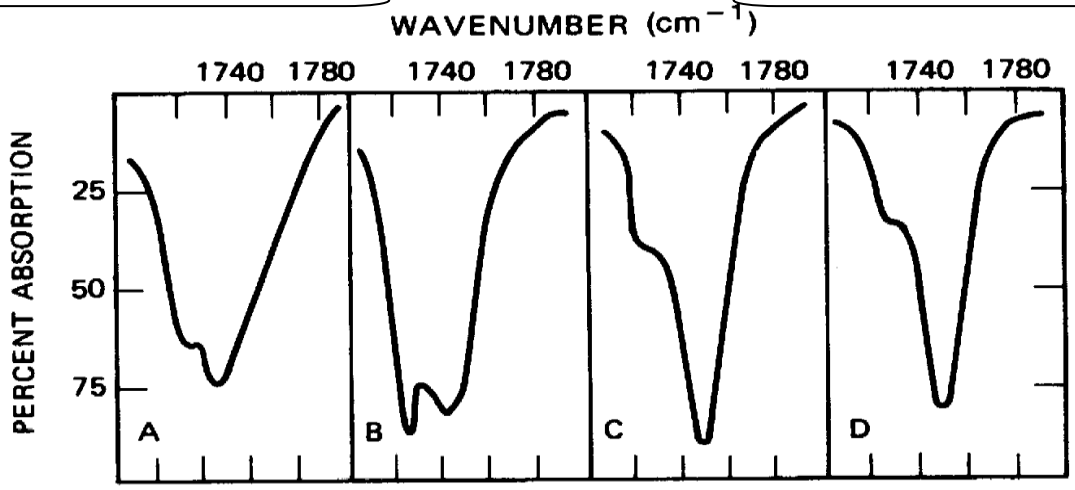


Figure 2.3 Infrared spectrum of cyclopentanone in various media. A. Carbon tetrachloride solution (0.15 *M*). B. Carbon disulfide solution (0.023 *M*). C. Chloroform solution (0.025 *M*). D. Liquid state (thin films). (Computed spectral slit width 2 cm^{-1}).

Common proton acceptor atoms are oxygen, nitrogen, and the halogens. Unsaturated groups, such as the C=C linkage, can also act as proton acceptor.

The strength of the hydrogen bond is at a maximum when the proton donor group and the axis of the lone pair orbital are collinear. The strength of the bond decreases as the distance between X and Y increases.

Hydrogen bonding alters the force constant of both groups; thus, the frequencies of both stretching and bending vibrations are altered. The X—H stretching bands move to lower frequencies (longer wavelengths) usually with increased intensity and band widening. The stretching frequency of the acceptor group, for example, C=O, is also reduced but to a lesser degree than the proton donor group. The H—X bending vibration usually shifts to a shorter wavelength when bonding occurs; this shift is less pronounced than that of the stretching frequency.

*Inter*molecular hydrogen bonding involves association of two or more molecules of the same or different compounds. Intermolecular bonding may result in dimer molecules (as observed for carboxylic acids) or in polymeric molecular chains, which exist in neat samples or concentrated solutions of monohydroxy alcohols. *Intra*molecular hydrogen bonds are formed when the proton donor and acceptor are present in a single molecule under spatial

conditions that allow the required overlap of orbitals, for example, the formation of a five- or six- membered ring. The extent of both inter- and intra molecular bonding is temperature dependent. The effect of concentration on intermolecular and intramolecular hydrogen bonding is markedly different. The bands that result from intermolecular bonding generally disappear at low concentrations (less than about 0.01 M in nonpolar solvents). Intramolecular hydrogen bonding is an internal effect and persists at very low concentrations.

The change in frequency between "free" OH absorption and bonded OH absorption is a measure of the strength of the hydrogen bond. Ring strain, molecular geometry, and the relative acidity and basicity of the proton donor and acceptor groups affect the strength of bonding. Intramolecular bonding involving the same bonding groups is stronger when a six-membered ring is formed than when a smaller ring results from bonding. Hydrogen bonding is strongest when the bonded structure is stabilized by resonance.

The effects of hydrogen bonding on the stretching frequencies of hydroxyl and carbonyl groups are summarized in Table 2.1.

An Important aspect of hydrogen bonding involves interaction between functional groups of solvent and solute. If the solute is polar, then it is important to note the solvent used and the solute concentration.

Table 2.1 Stretching Frequencies in Hydrogen Bonding.

	Intermolecular Bonding			Intramolceular Bonding		
X—H ··· Y Strength	Frequency Reduction (cm^{-1}) ν_{OH}	$\nu_{C=O}$	Compound Class	Frequency Reduction (cm^{-1}) ν_{OH}	$\nu_{C=O}$	Compound Class
Weak	300[a]	15[b]	Alcohols, phenols, and intermolecular hydroxyl to carbonyl bonding	<100[a]	10	1,2-Diols, α- and most β-hydroxy ketones; *o*-chloro and *o*-alkoxy phenols
Medium				100–300[a]	50	1,3-Diols; some β-hydroxy ketones; β-hydroxy amino compounds; β-hydroxy nitro compounds
Strong	>500[a]	50[b]	RCO_2H dimers	>300[a]	100	*o*-Hydroxy aryl ketones; *o*-hydroxy aryl acids; *o*-hydroxy aryl esters; β-diketones; tropolones

[a] Frequency shift relative to "free" stretching frequencies.
[b] Carbonyl stretching only where applicable.

2.3 Instrumentation

2.3.1 Dispersion IR Spectrometer

For many years, an infrared spectrum was obtained by passing an infrared beam through the sample and scanning the spectrum with a dispersion device (the familiar diffraction grating). The spectrum was scanned by rotating the diffraction grating; the absorption areas (peaks) were detected and plotted as frequencies versus intensities.

Infrared sources consist of an inert solid that is electrically heated to a temperature between 1,500 and 2,200 K. The heated material will then emit infra red radiation.

Figure 2.4 demonstrates a sophisticated double-beam dispersion instrument, operation of which involves splitting the beam and passing one portion through the sample cell and the other portion through the reference cell. The individual beams are then recombined into a single beam of alternating segments by means of the rotating sector mirror, *M*7, and the absorption intensities of the segments are balanced by the attenuator in the reference beam. Thus, the solvent in the reference cell and in the sample cell are balanced out, and the spectrum contains only the absorption peaks of the sample itself.

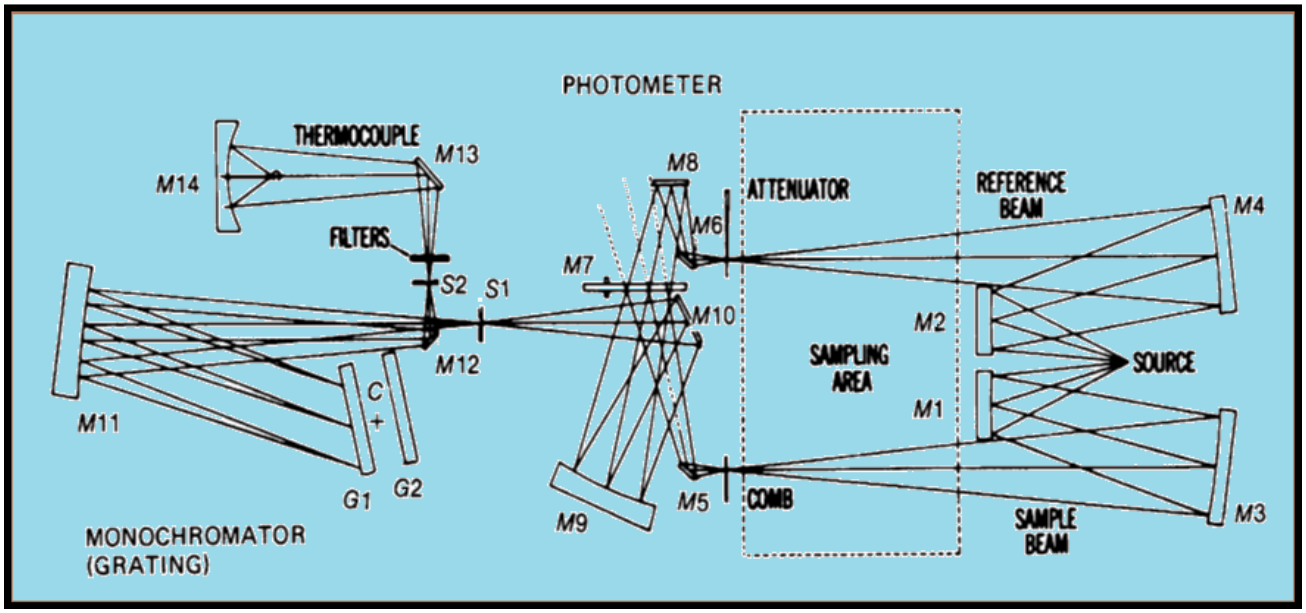


Figure 2.4 Optical System of double-beam IR spectrophotometer**.**

2.3.2 Fourier Transform Infrared Spectrometer (Interferometer)

Fourier transform infrared spectroscopy, also commonly known as FT-IR is a preferred method for infrared spectroscopy, which uses the mathematical Fourier transform process to convert the raw data "interferogram" into the actual spectrum.

An infrared beam containing various frequencies of light will pass through the sample, and the amount of IR light absorbed by the sample is measured. Then the process is repeated many times with different combinations of IR light frequencies. An Interferometer, a fixed mirror and a moving mirror are used to create an interferogram which has the unique property that every data point (a function of the moving mirror position) has the information about every infrared frequency which comes from the IR source. Then computer processing with Fourier transform takes place to convert the raw data (light absorption for each mirror position) into the desired result (light absorption for each wavelength). (Figure 2.5)

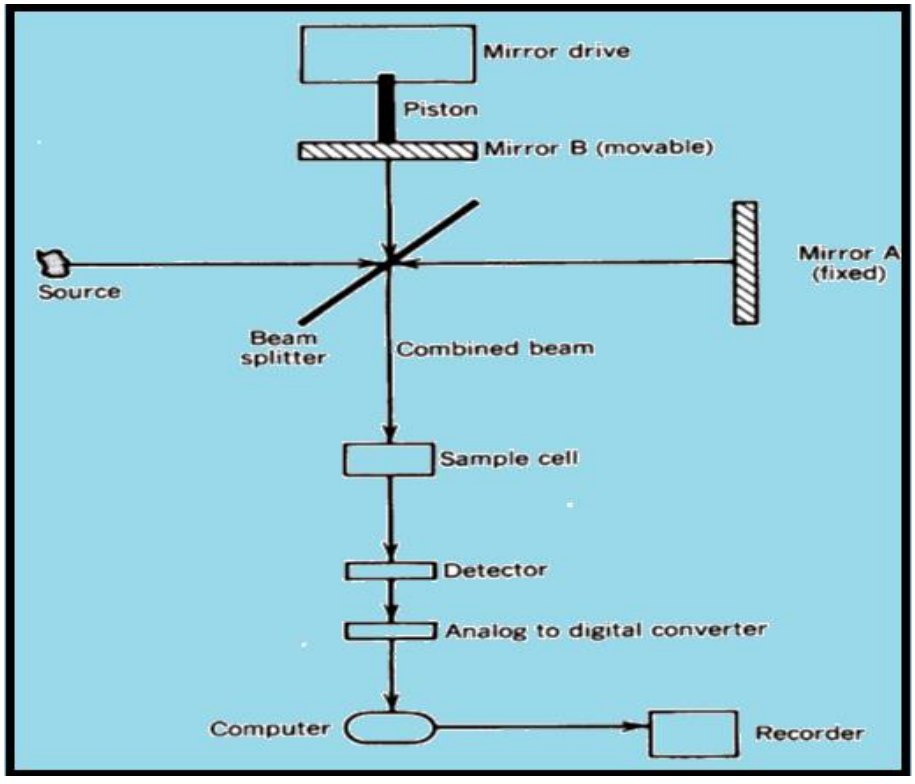


Figure 2.5 Schematic of an FT IR spectrometer.

An FT IR unit can be used in conjunction with HPLC or GC. As with any computer-aided spectrometer.

spectra of pure samples or solvents (stored in the computer) can be subtracted from mixtures. Flexibility in spectral printout is also available: for example, spectra linear in either wavenumber or wavelength can be obtained from the same data set.

2.4 Sample Handling

Infrared spectra may be obtained for gases, liquids, or solids.

* The spectra of gases or low-boiling liquids may be obtained by expansion of the sample into an evacuated cell. Gas cells are available in length of a few centimeters to 40 m. The sampling area of a standard IR spectrophotometer will not accommodate cells much longer than 10 cm; long paths are achieved by multiple reflection optics.

* Liquids may be examined neat or in solution. Neat liquids are examined between salt plates, usually without a spacer. Pressing a liquid sample between flat plates produces a film 0.01 mm or less in thickness, the plates being held together by capillary action. Samples of 1-10 mg are required. Thick samples of neat liquids usually absorb too strongly to produce a satisfactory spectrum. Volatile liquids are examined in sealed cells with very thin spacers. Silver chloride plates may be used for samples that dissolve sodium chloride plates.

Solutions are handled in cells of 0.1-1 mm thickness. Volumes of 0.1-1 mL of 0.05-10% solutions are required for readily available cells. A compensating cell, containing pure solvent, is placed in the reference beam. The spectrum thus obtained is that of the solute except in those regions in which the solvent absorbs strongly. For example, thick samples of carbon tetrachloride absorb strongly near 800 cm^{-1}; compensation for this band is ineffective since strong absorption prevents any radiation from reaching the detector.

The solvent selected must be dry and transparent in the region of interest. When the entire spectrum is of interest, several solvents must be used. A common pair of solvents is carbon tetrachloride ($CC1_4$) and carbon disulfide (CS_2). Carbon tetrachloride is relatively free of absorption at frequencies above

1333 cm^{-1} whereas CS_2 shows little absorption below 1333 cm^{-1}. Solvent and solute combinations that react must be avoided. For example, CS_2 cannot be used as a solvent for primary or secondary amines. Amino alcohols react slowly with CS_2 and $CC1_4$.

When only very small samples are available, ultramicrocavity cells are used in conjunction with a beam condenser. A spectrum can be obtained on a few micrograms of sample in solution. When volatility permits, the solute can be recovered for examination by other spectrometric techniques.

* Solids are usually examined as a mull, as a pressed disk, or as a deposited glassy film. Mulls are prepared by thoroughly grinding 2-5 mg of a solid in a smooth agate mortar. Grinding is continued after the addition of I or 2 drops of the mulling oil. The suspended particles must be less than 2 μm to avoid excessive scattering of radiation. The mull is examined as a thin film between flat salt plates. Nujol (a high-boiling petroleum oil) is commonly used as a mulling agent. When hydrocarbon bands interfere with the spectrum, Fluorolube (a completely halogenated polymer containing F and Cl) or hexachlorobutadiene may be used. The use of both Nujol and Fluorolube mulls makes possible a scan, essentially free of interfering bands, over the 4000-250 cm^{-1} region.

The pellet (pressed-disk) technique depends on the fact that dry, powdered potassium bromide (or other alkali metal halides) can be compacted under pressure in vacuum to form transparent disks. The sample (0.5-1.0 mg) is intimately mixed with approximately 100 mg of dry, powdered KBr. Mixing can be effected by thorough grinding in a smooth agate mortar or, more efficiently, with a small vibrating ball mill, or by lyophilization. The mixture is pressed with special dies under a pressure of 10,000-15,000 psi into a transparent disk.

The quality of the spectrum depends on the intimacy of mixing and the reduction of the suspended particles to 2 μm or less. Microdisks, 0.5-1.5 mm in diameter, can be used with a beam condenser. The microdisk technique permits examination of samples as small as 1 μg. Bands near 3448 and 1639 cm^{-1}, resulting from moisture, frequently appear in spectra obtained by the pressed-disk technique.

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