IR

CHAPTER 2

Infrared Spectroscopy

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2.1 INTRODUCTION

The literature dealing with the infrared spectroscopy of organic compounds continues to expand at a very rapid rate. Numerous excellent references (e.g., Refs. 1–8) have appeared in recent years, and if an expansive treatment of the subject is desired, the reader is directed to these references. The purpose of this chapter is to give a basic knowledge of the subject and to offer illustrations of a pharmaceutical nature. Although an emphasis has been placed on the determination of the frequencies at which functional groups characteristically absorb, numerous studies have concerned themselves with

TABLE 2.1: Infrared Spectrophotometric Identity Tests of the USP XVII[a]

Acetazolamide *and* Tablets (KBr)
Bemegride *and* Injection (mineral oil)
γ-Benzene Hexachloride (KBr)
Betazole Hydrochloride *and* Injection (mineral oil)
Bishydroxycoumarin (KBr)
Calcium Disodium Edetate (mineral oil)
Calcium Pantothenate and Racemic Calcium Pantothenate (KBr)
Chlorambucil *and* Tablets (CS_2)
Chlorcyclizine Hydrochloride *and* Tablets (CS_2)[b]
Chlorpheniramine Maleate *and* Tablets (CS_2)[b]
Chlorpromazine Hydrochloride (KBr)
Chlorpropamide (KBr) *and* Tablets ($CHCl_3$)
Cholecalciferol (KBr)
Cyclizine Hydrochloride (KBr) *and* Tablets (CS_2)[b]
Desoxycorticosterone Acetate (KBr)
Diethyltoluamide *and* Solution (CS_2)
Dihydrotachysterol (KBr)
Dimenhydrinate *and* Syrup *and* Tablets (CS_2)[b]
Diphenhydramine Hydrochloride *and* Capsules *and* Elixir and Injection (CS_2)[b]
Disodium Edetate *and* Injection (KBr)
Ergocalciferol (KBr)
Estradiol Valerate (KBr)
Ethinyl Estradiol (KBr)
Guanethidine Sulfate (mineral oil)
Hydrochlorothiazide (KBr)
Hydrocortisone *and* Tablets *and* Acetate (KBr)
Sterile Hydrocortisone Acetate Suspension (KBr)
Hydrocortisone Sodium Succinate (KBr)
Hydrocortisone Sodium Succinate for Injection (KBr)
Hydroxystilbamidine Isethionate (KBr)
Sterile Hydroxystilbamidine Isethionate (KBr)
Mecamylamine Hydrochloride *and* Tablets (KBr)
Meclizine Hydrochloride (KBr)
Medroxyprogesterone Acetate *and* Tablets *and* Sterile Suspension (KBr)
Meglumine Iothalamate Injection (KBr)
Methazolamide *and* Tablets (KBr)
Methotrexate (KBr)
Metyrapone (mineral oil)
Phenobarbital *and* Elixir *and* Tablets (KBr)
Phentolamine Mesylate (KBr)
Phentolamine Mesylate for Injection (KBr)
Prednisolone *and* Tablets *and* Acetate *and* Sterile Suspension (KBr)
Prednisone *and* Tablets (KBr)
Primidone (KBr)
Procaine Hydrochloride *and* Injection (KBr)
Sterile Procaine Hydrochloride (KBr)
Prochlorperazine Edisylate *and* Injection (CS_2)[b]
Prochlorperazine Maleate *and* Tablets (CS_2)[b]
Promethazine Hydrochloride *and* Injection *and* Syrup (CS_2)[b]
Propoxyphene Hydrochloride *and* Capsules ($CHCl_3$)

TABLE 2.1 (continued)

Pyrimethamine (KBr)
Pyrvinium Pamoate (KBr)
Sodium Acetazolamide (KBr)
Sodium Iothalamate Injection (KBr)
Sodium Phenobarbital *and* Injection (KBr)
Sterile Sodium phenobarbital (KBr)
Sodium Warfarin (KBr)
Sodium Warfarin for Injection (KBr)
Spironolactone *and* Tablets (KBr)
Sulfamerazine (KBr)
Sulfamethazine (KBr)
Sulfamethoxypyridazine *and* Tablets (KBr)
Testosterone Cypionate (KBr)
Testosterone Propionate (KBr)
Thiotepa ($CHCl_3$)
Thiotepa for Injection ($CHCl_3$)
Tolbutamide *and* Tablets (mineral oil)
Tripelennamine Citrate (CS_2)[b]
Tripelennamine Hydrochloride *and* Tablets (CS_2)[b]
Tubocurarine Chloride (KBr)

[a] Solvent or dispersing agent is given in parentheses.
[b] The USP "Identification-Organic Nitrogenous Bases" test is employed in these examples. See also practical exercise 2.2.

the application of infrared spectroscopy to pharmaceutical analysis. The review by Price[9] on this subject covers the period up to 1954; Carol's review[10] was published in 1961. Other reviews are also worthy of mention, especially the series on analytical methods used for pharmaceuticals and related drugs which have appeared regularly for many years in *Analytical Chemistry*. Many of the references contained therein are devoted to spectrophotometric methods. The most recent review[11] in this series covers the literature through June 1966. The series of papers (e.g., Refs. 12–14) by Warren, Eisdorfer, Thompson, and Zarembo illustrate the value of infrared spectrophotometry in the identification of organic medicinal compounds. The current editions of the *United States Pharmacopeia* (USP XVII) and the *British Pharmacopoeia* (BP 1963) suggest the extensive use of infrared spectrophotometry as a means of identifying a large number of substances and preparations (Tables 2.1 and 2.2). The USP XVII describes, for the first time, a few assay processes based on infrared absorption spectra (Table 2.3). This number will certainly increase. Reference will be made to typical examples later in this chapter. It is unfortunate that neither the USP nor the BP are consistent in their use of infrared spectroscopy in the identification of medicinal compounds. Some, but not all of the USP official sulfa drugs, for example, are identified by means of their infrared spectra. The BP confines this application of infrared spectroscopy to the identification of steroids, but not all the official BP steroids are identified in this way.

TABLE 2.2: Infrared Spectrophotometric Identity Tests of the BP 1963[a]

Cortisone Acetate *and* Injection *and* Tablets
Deoxycortone Acetate *and* Implants
Deoxycortone Trimethylacetate *and* Injection
Dexamethasone *and* Acetate *and* Tablets
Digoxin
Dimethisterone *and* Tablets
Ethinyloestradiol
Ethisterone *and* Tablets
Fludrocortisone Acetate *and* Tablets
Fluoxymesterone *and* Tablets
Hydrocortisone
Hydrocortisone Acetate *and* Injection
Hydrocortisone Hydrogen Succinate
Hydrocortisone Sodium Succinate *and* Injection
Methylprednisolone *and* Tablets
Methyltestosterone *and* Tablets
Norethandrolone *and* Tablets
Norethisterone
Prednisolone *and* Acetate *and* Tablets
Prednisolone Trimethylacetate *and* Injection
Prednisone *and* Acetate *and* Tablets
Progesterone
Testosterone *and* Implants *and* Propionate

[a] Methods of preparation of substance are listed in BP, Appendix IV. Any of these methods can be used.

TABLE 2.3: Infrared Spectrophotometric Assays of the USP XVII[a]

Acetazolamine (pyridine)
Diethyltoluamide *and* Solution (CS_2)
Nitroglycerin Tablets (CS_2)
Propoxyphene Hydrochloride Capsules ($CHCl_3$)
Thiotepa for Injection ($CHCl_3$)

[a] Solvent is given in parentheses.

2.2 ABSORPTION SPECTROSCOPY

Absorption spectroscopy, or spectrophotometry, may be defined as the analysis of chemical substances by the measurement of the amount of radiation absorbed by these substances. An infrared absorption spectrum is obtained by passing electromagnetic radiation of the appropriate frequency range through a transparent layer of the substance being examined. The substance may be a solid, a liquid, a gas, or a solution of these. Some of the

radiation is absorbed selectively by the substance at certain frequencies. The energy not absorbed, i.e., the transmitted energy, can be analyzed by means of a suitable instrument (the spectrometer, or, if it incorporates a photocell, the spectrophotometer) and it will vary according to the frequency of the incident radiation. In this way, an absorption spectrum over a certain frequency range is obtained. When the molecule under investigation absorbs radiation, some electrons are raised to higher energy levels which are quantized. The high energy or excited states of the molecule are usually short-lived. The absorbed energy is rapidly released in the form of heat. Thus the temperature of the substance or its solution increases while the spectrum is being recorded. If all molecules of a substance were identical, the infrared spectrum would take the form of a series of *lines*. However, a group of molecules exists in a number of different vibrational and rotational states (see later), each state differing from another by a relatively small amount of energy. A group of molecules, therefore, absorbs energy over small wavelength ranges and gives rise to absorption *bands* or *peaks*.

A. UNITS OF MEASUREMENT

Electromagnetic radiation is characterized by either its wavelength (λ) or by its frequency (ν). These quantities are related, $\lambda\nu = C$, where C is the velocity of light, and the energy of radiation is given by the Plank equation, $E = h\nu$. In infrared spectroscopy, the common wavelength unit is the micron (μ; $1\ \mu = 10^{-4}$ cm). Wave numbers (σ or $\bar{\nu}$) are also used, where $\bar{\nu} = \lambda^{-1}$, (λ measured in centimeters). Wave numbers are therefore expressed in cm^{-1} units (reciprocal centimeters or Kaysers) and are a measurement of frequency. The position of an absorption peak in an infrared spectrum can be expressed, therefore, in wavelength (measured in microns) or in frequency units (measured in wave numbers). Interconversion is facile:

$$\bar{\nu}(cm^{-1}) = \frac{10^4}{\lambda(\mu)}$$

B. INFRARED REGION

The range of the electromagnetic spectrum extending from 0.8 to 200 μ is called the "infrared." The infrared region thus extends from just outside the visible region to the microwave region of the electromagnetic spectrum. The infrared region is subdivided. The range 0.8–2.5 μ is the *near infrared* region; the 2.5 to 16 μ is the *infrared* region; the range 16–200 μ is the *far infrared* region. The range 2.5 to 50 μ is sometimes called the "*midinfrared*" region. The range most useful to the organic medicinal chemist stretches from 2.5 to 16 μ, corresponding to 4000 to 625 cm^{-1}. Some infrared spectrophotometers produce tracings which are linear in wavelength units and therefore nonlinear in terms of wave numbers. Most modern instruments, however,

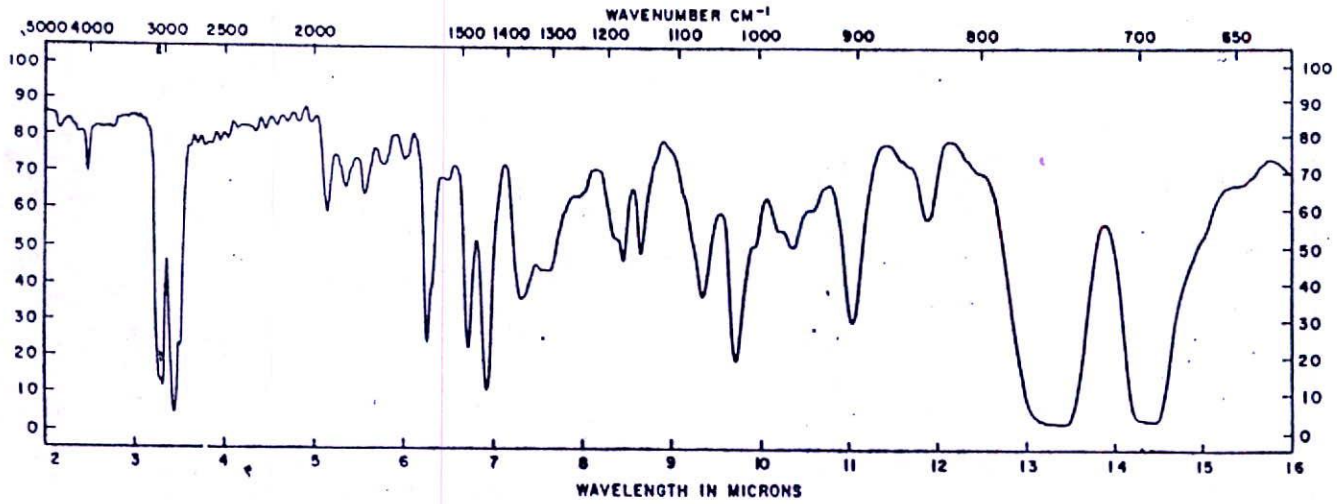


FIGURE 2.1: Infrared spectrum of a polystyrene film recorded on a Beckman IR5A spectrophotometer (linear in wavelength units).

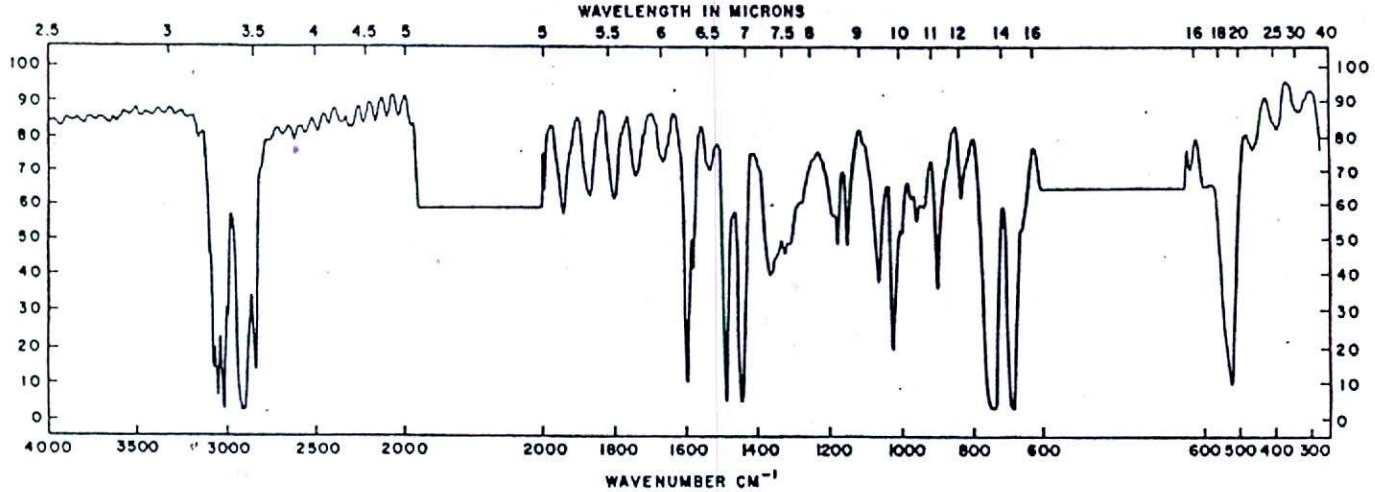


FIGURE 2.2: Infrared spectrum of a polystyrene film recorded on a Beckman IR10 spectrophotometer (linear in frequency units).

produce spectra linear in wave numbers, and so it is common to see positions of maximum absorption quoted in reciprocal centimeters rather than in wavelength. The spectrum of a polystyrene film (commonly used to calibrate infrared spectra) has been recorded on the Beckman IR5A spectrophotometer (linear in wavelength units) and on the Beckman IR10 spectrophotometer (linear in frequency units) for comparison purposes (Figs. 2.1 and 2.2, respectively).

C. ENERGY CHANGES

Whereas ultraviolet and visible electromagnetic radiation results in changes in electronic as well as vibrational and rotational energies of a molecule, the weaker energy of infrared radiation affects only the vibrational and rotational energy levels associated with the ground electronic energy state of the irradiated molecule. If the source of radiation is the far infrared or microwave region, only the rotational energy of the molecule is affected, whereas when the energy supplied is on the 2.5–16 μ range, changes in the vibrational as well as the rotational energies of the molecule result. For all intents and purposes however, the 2.5–16 μ region of the electromagnetic spectrum can be considered to affect only the vibrational energy levels of the irradiated molecule. Rotational transitions are rarely seen when solids or solutions are being examined. When they do occur, for example, when molecules in the vapor phase are examined, they appear as fine structure associated with the vibrational absorption bands. For this reason, it is assumed in the discussion which follows that the irradiation of a molecule by means of infrared radiation causes vibrational transitions only.

A popular illustration of *stretching and bending vibrations* is to think of the molecule as a system suspended in space in which the component atoms are visualized as balls and the bonds connecting them as springs. The system is imagined to be in constant motion—the springs are stretching and contracting, or bending. These motions are referred to, respectively, as *stretching* and *bending vibrations*, and the frequency with which these vibrations occur depends on the nature of the atoms joined by the bond and on the bond's location in the molecule. In a spring and ball system, if the spring connecting two balls is struck, it vibrates, and this vibration, in turn, influences the rest of the system and is influenced by the rest of the system. In a similar way, if a chemical bond vibrating at a certain frequency is struck by infrared radiation of that same frequency, the vibration of that bond will increase by absorption of the infrared radiation, which causes an electron to be raised to a higher (quantized) vibrational energy level. Because the energy levels are quantized, the electron will be promoted only when the frequency of the infrared radiation corresponds exactly to that of the bond's vibration. If, then, a substance is irradiated by infrared radiation the wavelength of which is constantly changing, different bonds will absorb energy at different frequencies. In this way, an infrared absorption spectrum is obtained in which

the percentage intensity of the transmitted infrared light is plotted against the wavelength (or wave number) of that light.

D. VIBRATIONAL MODES

A molecule of N atoms has $3N$ degrees of freedom, which means that the total number of coordinates required to specify the positions of all the atomic nuclei is $3N$. Three of these coordinates are required to indicate the position of the molecule's center of mass, and an additional three (two in linear molecules) describe the rotational motion of the molecule about its center of mass. It follows that to describe the vibrational motions of the atoms relative to one another, $3N$-6 coordinates are required ($3N$-5 if the molecule is linear). A nonlinear molecule containing N atoms, therefore, is said to possess $3N$-6 vibrational degrees of freedom. Another way of expressing this concept is to say that the nonlinear molecule has $3N$-6 possible *normal* or *fundamental vibrational modes* which can be responsible for the absorption of infrared radiation. Thus, the ethanol molecule possesses 21 vibrational modes each of which can, theoretically, give rise to an absorption band. Additional factors, however, must be considered. Molecules displaying partial or total symmetry give rise to simpler spectra than might be anticipated. This is due to the fact that, for a particular vibration to absorb infrared energy, a change in the dipole moment of the molecule must result. The double bonds in symmetrical alkenes (C=C) and in symmetrical azo compounds (N=N), for example, do not absorb infrared radiation for this reason. Such bonds are said to be *infrared inactive.*

1. Nonfundamental Vibrations

Absorption bands can occur in addition to those predicted by the number of degrees of freedom possessed by the molecule. These are *nonfundamental absorption bands* and occur for at least two reasons. *Overtones* are sometimes observed at twice the frequency of a strong band. The spectrum of a molecule with a carbonyl group which absorbs strongly near 1700 cm^{-1} may also have a weak intensity band at twice this frequency (near 3400 cm^{-1}). *Combination tones* are also weak bands which appear occasionally at frequencies that are the sum or difference of two or more fundamental bands. Transitions in which the vibrational quantum number changes by unity are permitted to be *infrared active.* Overtones and combination tones require a vibrational quantum change of greater than this value and, therefore, should not be expected. They do occur, however, which indicates that the vibrational motion is not truly harmonic. If an overtone or combination tone occurs near a fundamental frequency, the intensity of the overtone or combination tone is anomalously enhanced. This confusing situation is the result of quantum mechanical resonance (*Fermi resonance*) occurring between the two excited vibrational levels.

A vibrational spectrum is therefore highly characteristic of the particular molecule, but although each molecule has its own particular spectrum, certain *groups of atoms* give rise to absorption bands at or near the same frequencies even though the remaining portions of the molecules are quite different. Most literature references are concerned with studies to establish the ranges within which such *characteristic group frequencies* occur. To predict the presence of a particular group in a molecule is perhaps the most important application of infrared spectrophotometry. Studies in this field, however, can often be complicated by the phenomenon known as "coupling." Sometimes when two strongly absorbing bonds of the same symmetry are closely located within a molecule and absorb in the same region, coupling is said to occur and the absorption bands are shifted outside their characteristic frequency range.

Not only do different bonds or groups of atoms absorb at different frequencies, the *intensity* of the absorption also varies. It has been stated that for a vibrating molecule to absorb infrared radiation, the magnitude of the molecule's dipole moment must change. Generally speaking, the greater the magnitude of this dipole moment change, the greater is the intensity of the absorption band. Other factors, such as Fermi resonance, contribute to the intensity of the absorption band.

2. Fundamental Vibrations

If a group of atoms, $—AB_2$, is represented by

then stretching and bending vibrations can be illustrated as shown (Fig. 2.3).

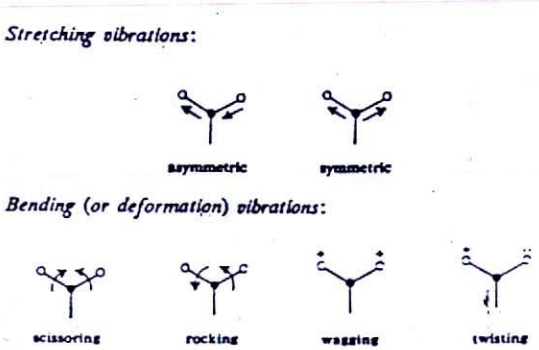
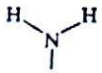


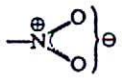
FIGURE 2.3: Stretching and bending vibration modes. The arrows indicate the direction of the vibration; the + and − signs represent, respectively, vibrations above and below the plane of the paper.

Typical examples of such a group of atoms include the methylene group

the amino group



the nitro group



and the carboxylate group

Diagrams similar to those shown in Fig. 2.3 can be used to illustrate stretching and bending in other systems, such as the methyl group (Fig. 2.4). The wavelength at which a stretching vibration occurs is dependent on the strength of

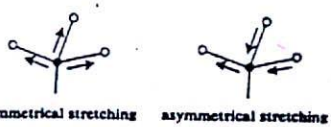


FIGURE 2.4: Stretching vibrations of the methyl group.

the bond joining the two atoms; the shorter the bond, the shorter is the wavelength (the larger the wave number) of the absorption band (Fig. 2.5). When, however, hydrogen is one of the atoms, stretching vibrations occur at frequencies much higher than those of other single-bond stretching vibrations. Deformation vibrations require less energy and for this reason occur at longer wavelengths (lower wave numbers) than stretching vibrations. Common deformation frequency ranges are shown in Fig. 2.5.

E. EXAMINATION OF AN INFRARED SPECTRUM

A common practice is to examine separately three different areas:

a. The region above 1400 cm^{-1} (1400–4000 cm^{-1}). The presence or absence of many groups in the molecule, including C=O, NH, OH, C≡C,

C=N, etc., is usually readily confirmed. In this region, however, there is a danger of making erroneous assignments. Nonfundamental absorption bands (overtones, combination tones) are often located here and should not be confused with O—H or N—H bands.

b. The region below 900 cm^{-1}. This is generally taken to mean the 900–600 cm^{-1} region. The infrared spectra illustrated in this chapter cover the range 600–250 cm^{-1}, but it is often not possible to determine the origin of the bands

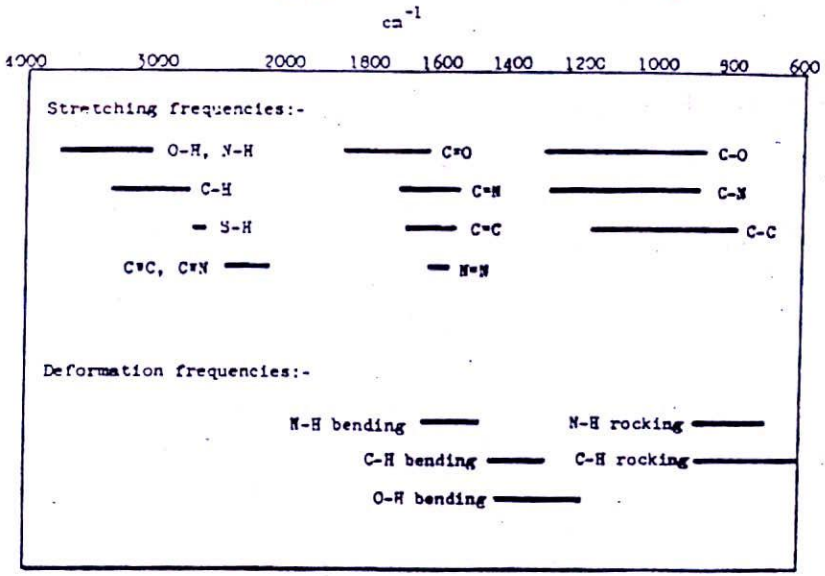


FIGURE 2.5: Common stretching and deformation vibrational frequencies.

in this region because of the lack of information available in the literature. Generally speaking, not many bands are located in the 900–600 cm^{-1} region and the ones that are can usually be assigned to particular transitions.

c. The region 900–1400 cm^{-1}. This is termed the "finger-print region," where many absorption bands are located, especially those due to bending vibrations as well as C—C, C—O, and C—N stretching vibrations. This area of the spectrum is usually complex, and it is often far from easy to determine the origin of every band. As there are more bending than stretching vibrations in a molecule, the fingerprint region contains many absorption bands of varying intensities which makes this region of particular importance in establishing the identity of a compound by comparison with an authentic sample. Two extremely similar molecules often have virtually identical vibrations in the other two regions (600–900; 1400–4000 cm^{-1}), but almost invariably, there are differences, sometimes slight, in the fingerprint region.

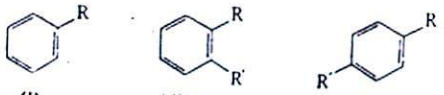
1. Group Frequencies

Numerous authors have constructed detailed tables of characteristic group frequencies. These are readily available, and it would be superfluous to construct yet another series of tables of this type here. Much information can be obtained from correlation charts such as those in the text book by Cross,[3] and by reference to one of the various forms of the Colthup chart, originally published in 1950,[15] modifications of which appear in most textbooks. More comprehensive group frequency correlation charts of this type are published by Beckman. One example is reproduced here* (Table 2.4). Another excellent reference for routine use is the bulletin by Jones,[16] which does not attempt to cover the subject completely, but which includes correlations most commonly used in the identification of naturally occurring organic compounds and their degradation products. With such a wealth of information available, a summary only of the principal absorption bands associated with the more frequently encountered functional groups is presented here (Table 2.5). The frequency of a band and its intensity vary with the nature of the molecule and according to whether a solid or a solution spectrum is recorded.

2. Aromatic Compounds

The presence of an aromatic nucleus in a molecule can usually be readily confirmed by means of its infrared spectrum. Three regions are of particular importance: (a) 1000–625 cm^{-1} (10–16 μ), (b) 1670–1430 cm^{-1} (~6–7 μ), and (c) 2000–1670 cm^{-1} (~5–6 μ).

A number of C—H bending vibrations appear in the 10–16 μ region; they vary in intensity. The pattern of absorption depends on the number of adjacent hydrogen atoms possessed by the aromatic nucleus. Of particular interest to the pharmaceutical chemist are the spectra of monosubstituted (I), *o*-disubstituted (II), and *p*-disubstituted (III) benzene derivatives. All monosubstituted benzenes exhibit strong absorption near 14.3 μ (700 cm^{-1}). Such is the importance of this strong band that it is true to say that if a molecule does not absorb strongly in this region, it is *not* a monosubstituted benzene. Many, but not all, monosubstituted benzenes also absorb strongly near



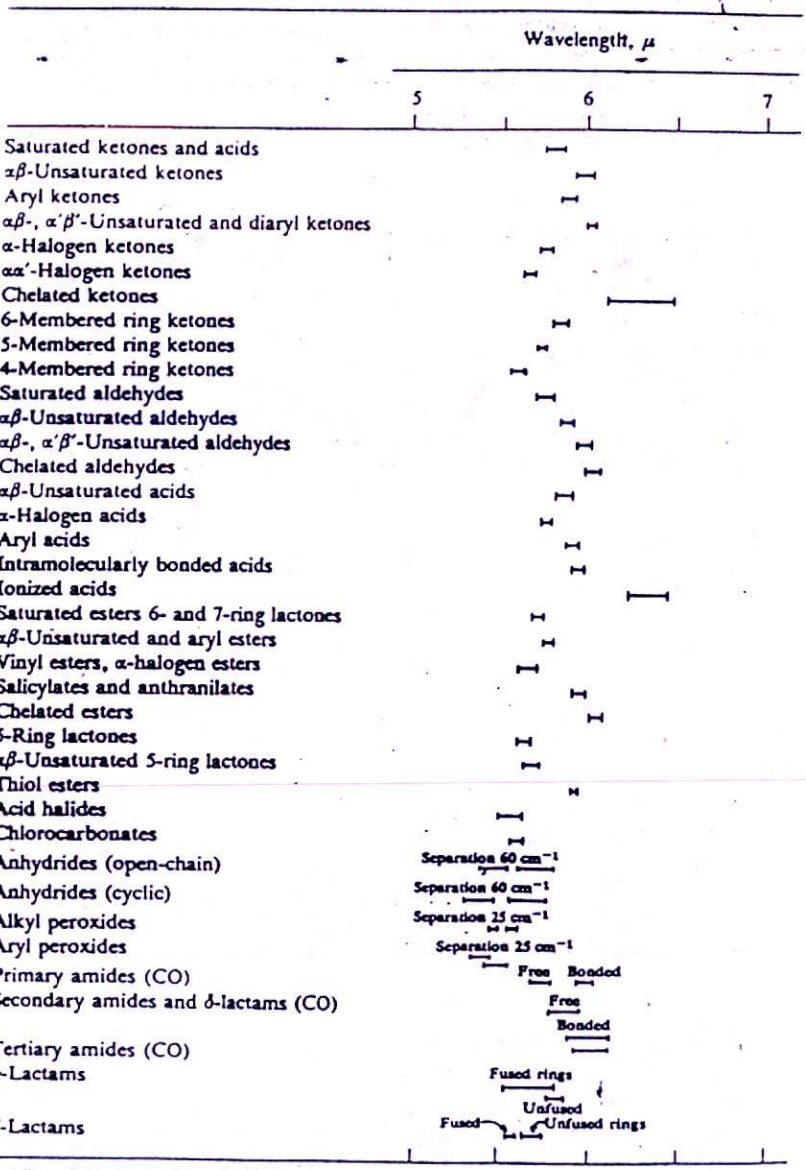
(I) (II) (III)

13.3 μ (750 cm^{-1}). The spectra of benzoic acid and many benzoates, for example, do not show a strong band near 750 cm^{-1}.

Ortho-disubstituted benzenes absorb strongly near 13.3 μ (750 cm^{-1}) only. A band near 700 cm^{-1} is not observed. Para-disubstituted benzenes, and other phenyl compounds with two adjacent hydrogen atoms on the phenyl ring (i.e., 1,2,4-trisubstituted and 1,2,3,4-tetrasubstituted benzenes), have a strong band in the 860–800 cm^{-1} region, usually located close to

* Reproduced with the kind permission of Beckman Instruments, Inc.

TABLE 2.4*: Carbonyl Vibrations (all strong bands)



* Based on work done by Bellamy.

TABLE 2.5: Principal Absorption Bands of Selected Functional Groups

	Frequency range, cm^{-1}	Wavelength range, μ	Intensity
C—H stretching.			
Alkanes Alkanes Alkynes Aromatic hydrocarbons Aldehyde C—H	Located in the 2850–3300 cm^{-1} (3.51–3.03 μ) region. Intensity[a] m to s. C≡C—H stretching characteristically located near 3300 cm^{-1} (3.03 μ) (s) Characteristic. Two bands 2800–2900 cm^{-1} (3.57–3.45 μ) (w) and 2700–2780 cm^{-1} (3.70–3.60 μ) (w)		
C—H bending			
Alkanes	1340–1480	7.46–6.75	m–s
Alkenes	1300–1420	7.70–7.04	s
	800–1000	12.5–10.0	s
Alkynes	630	15.9	s
Aromatic[a]	700–900	14.3–11.1	v
C—C double- and triple-bond stretching			
Alkenes	1620–1680	6.17–5.95	w–m
Alkynes[a]	2100–2300	4.76–4.35	w–m
Aromatic[a]	1450–1600	6.90–6.25	w–m
Carbonyl C=O stretching			
Aldehydes[a]			
Saturated aliphatic	1720–1740	5.81–5.75	s
α,β-Unsaturated aliphatic	1680–1705	5.95–5.87	s
Aryl	1680–1715	5.95–5.83	s
Ketones			
Saturated aliphatic	1705–1750	5.87–5.71	s
α,β-Unsaturated aliphatic	1660–1685	6.02–5.94	s
Aryl	1680–1700	5.95–5.88	s
Esters			
Saturated	1735–1750	5.76–5.71	s
α,β-Unsaturated	1715–1730	5.83–5.78	s
Aryl	1715–1730	5.83–5.78	s
Saturated γ-lactones	1760–1780	5.68–5.62	s
Esters			
Saturated β-lactones	~1820	~5.50	s
α,β-Unsaturated γ-lactones	1740–1760	5.75–5.70	s
β,γ-Unsaturated γ-lactones	~1800	~5.56	s
β-Ketoesters	~1650	~6.06	s
Carboxylic acids[a]			
Saturated	1700–1725	5.88–5.80	s
α,β-Unsaturated	1690–1715	5.92–5.83	s
Aryl	1680–1700	5.95–5.88	s

TABLE 2.5 (continued)

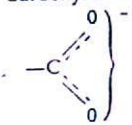
	Frequency range cm^{-1}	Wavelength range, μ	Intensity
Carboxylate ion			
	1550–1610	6.45–6.21	s
	1300–1400	7.69–7.15	s
Amides[a]			
Solid or concentrated solution	1630–1680	6.14–5.95	s
Dilute solution	1670–1700	5.99–5.88	s
Lactams	1680–1780	5.95–5.62	s
Ureas	1660–1720	6.02–5.81	s
Imides (two bands)	1700–1730	5.88–5.78	s
	1670–1700	5.99–5.88	s
Alcohols and phenols			
O—H stretching			
Free OH	3590–3650	2.79–2.74	Sharp bands (v)
Hydrogen-bonded OH	3450–3570	2.90–2.80	Sharp bands (v)
Intramolecular—no change on diluting solution; Intermolecular—change on diluting solution.			
Chelate compounds	2500–3200	4.00–3.10	Broad peak (v)
O—H bending and C—O stretching			
1° and 2° alcohols	1050–1100	9.50–9.10	s
	1310–1410	7.60–7.10	s
Amines			
N–H stretching			
1° (two bands)	~3400	~2.94	Sharp (m)
	~3500	~2.86	Sharp (m)
2°	3300–3500	3.03–2.86	m
3°	—	—	
Amine salts	3000–3150	3.33–3.18	m
N–H bending			
1° and 2°	1550–1650	6.45–6.06	m–s
Salts (two bands)	1570–1600	6.37–6.25	s
	~1500	~6.67	s
C—N stretching			
Aliphatic (two bands)	1000–1200	10.0–8.30	w
	~1400	~7.14	w
Aromatic	1250–1350	8.00–7.41	s
Nitriles and Isocyanides			
C≡N stretching			
Nitriles	2220–2280	4.50–4.38	m
Isocyanides	2050–2220	4.87–4.50	m

TABLE 2.5 (continued)

	Frequency range cm^{-1}	Wavelength range, μ	Intensity
	Nitro compounds		
N—O stretching			
Aromatic, asymmetric	1500–1570	6.67–6.37	s
Aromatic, symmetric	1300–1370	7.70–7.30	s
Aliphatic, asymmetric	1500–1570	6.45–6.37	s
Aliphatic, symmetric	1370–1380	7.30–7.25	s
C—N stretching			
Aromatic	~860	~11.6	m
Aliphatic	usually ~915	~10.9	m
	Halogen compounds[a]		
C—F stretching	1000–1400	10.0–7.10	s
C—Cl stretching	600–800	16.6–12.5	s
C—Br stretching	500–650	20.0–15.4	s
C—I stretching	500–600	20.0–16.6	s
	Sulfur compounds		
Thiols; S—H stretching	2550–2600	3.92–3.85	w
Thiocarbonyl compounds;			
C=S stretching	1050–1200	9.52–8.33	s
Sulfones, including sulfonamides;			
S=O stretching (two bands)	1140–1180	8.77–8.48	s
	1300–1350	7.69–7.41	s

[a] Intensity: s, strong; m, medium; w, weak; v, variable.

[b] Position varies with number of adjacent hydrogen atoms. This subject is considered in more detail on p. 71.

[c] If —C≡C—H, characteristic C—H stretching also.

[d] Usually four bands. This subject is considered in more detail below.

[e] Also display characteristic C—H stretching.

[f] O—H stretching also—several bands 2500–2700 cm^{-1} (4.00–3.70 μ) (w).

[g] Position of band varies appreciably depending on whether the spectrum is of the solid, of a concentrated solution, or of a dilute solution. Also seen is NH_2 and NH stretching, similar to that of amines.

[h] These bands are strong, but very variable in position.

810 cm^{-1} (12.35 μ). Once again, the presence of a band in these regions does not confirm that the molecule is an ortho- or a para-disubstituted benzene. The absence of strong absorption in these regions is more informative. Substituted benzenes other than those just mentioned are more difficult to characterize by means of infrared spectrophotometry. In these instances, bands due to C—H bending vibrations are weaker and more variable in position.

Aromatic compounds have four characteristic absorption bands in the 6–7 μ (1667–1429 cm^{-1}) region due to C=C stretching vibrations. These bands are often partially masked by other strong absorbing groups. Absence of strong absorption by a compound in the 6–7 μ region is indicative that the substance is not aromatic.

benzylamine
reduction
benzonitrile
benzamide
H_2O
oxidation
toluene
HNO_3
p-nitrotoluene
oxidation
benzoic acid
benzaldehyde
reduction
p-nitrobenzoic acid
reduction
esterification
benzyl alcohol
$LiAlH_4$
n-propyl benzoate
esterification
p-aminobenzoic acid
via diazotization
benzocaine
esterification
p-hydroxybenzoic acid
n-propyl p-hydroxybenzoate

SCHEME 2.1

Low [illegible] observed in the [illegible] is aromatic. Wi[illegible] ber and the pos[illegible] e to overtone or combination bands, is cm^{-1}) region if the molecule being examined .tives, it has been shown that both the num- nds depend on the substitution pattern of

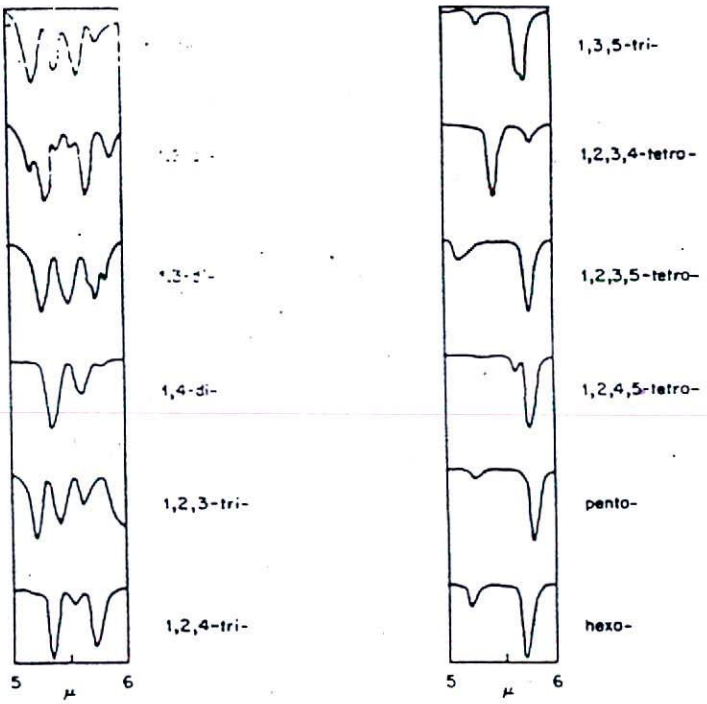


FIGURE 2.6: Diagrammatic representation of absorption bands in the 5–6 μ region for substituted benzenoid compounds.

the benzene ring (Fig. 2.6). A fairly concentrated solution of the substance (e.g., a 5% solution in a suitable solvent; 0.1-mm cells) must be examined if these bands are to be observed.

3. Infrared Spectra of Selected Compounds

An excellent way of gaining some knowledge of the frequencies at which functional groups characteristically absorb is to follow spectrophotographically the course of a reaction sequence in which one functional group is successively replaced by another. The reaction scheme chosen (Scheme 2.1)

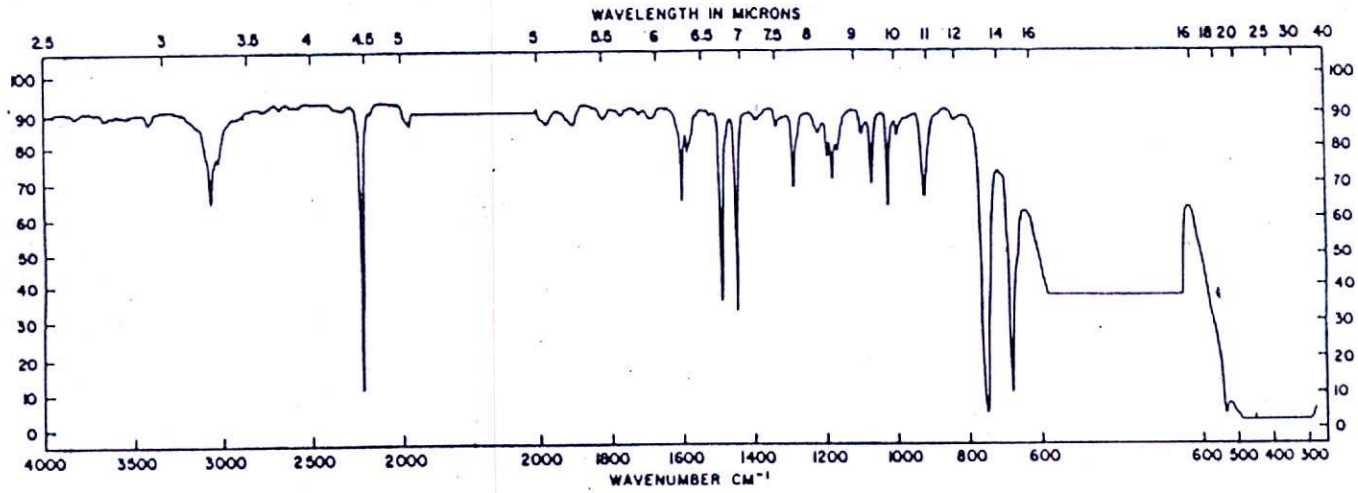


FIGURE 2.7: Infrared spectrum of benzonitrile (thin film).

has been selected for its simplicity and because it includes some examples of the many compounds of medicinal importance which contain substituted benzene rings. In addition, the scheme also illustrates the frequencies at which the absorption bands of carbonyl groups in different chemical environments occur. Brief interpretive comments are made on these spectra, but no attempt is made to identify each peak. The spectra of solids were recorded in the form of KBr disks, and liquids as thin films.

The spectrum of *benzonitrile* (Fig. 2.7) possesses features which suggest readily a monosubstituted benzene nucleus. Characteristic C—H bending occurs at 682 (s) and 752 (s) cm^{-1}, and the absorption pattern in the range 1670–2000 (w) cm^{-1} is expected for such a system. Also characteristic of an aromatic compound are the C—C stretching bands in the 1430–1670 (s or m) cm^{-1} region and the C—H stretching at 3065 (w) cm^{-1}. The strong band at 2220 cm^{-1} is very characteristic of C≡N stretching. The absorption band is noticeably lost on reduction of benzonitrile (to benzylamine) or its hydrolysis (to benzamide or benzoic acid). For the spectrum of *benzylamine* (Fig. 2.8) assignments similar to those described for benzonitrile can be made. Absorption bands due to C—H bending and stretching and aromatic C—C stretching occur at similar frequencies [692 (s), 735 (s); 1670–2000 (w); 1430–1670 (s) cm^{-1}]. The strong bands in the 2900–3100 cm^{-1} region are due to ethylenic and aromatic C—H stretching. A noticeable feature of this spectrum is the occurrence of two sharp bands of medium intensity at 3300 and 3380 cm^{-1}. Such absorption is characteristic of N—H stretching of a primary amino group. The spectrum of *benzamide* (Fig. 2.9) can be interpreted in a similar way. The major feature of this spectrum differing from the previous two is the presence of the strong band at 1655 cm^{-1}. Carbonyl stretching vibrations of primary amides occur in this region in solid-phase spectra. (In a dilute solution spectrum, this band would be located near 1690 cm^{-1}). The band at 1655 cm^{-1} is referred to as the amide I band. The strong band at 1620 cm^{-1} (amide II band) is due to N—H deformation and C—N stretching of a primary amide. The spectrum of *benzaldehyde* (Fig. 2.10) also shows the bands expected of a monosubstituted benzene nucleus though the characteristic substitution pattern in the 1670–2000 cm^{-1} region is partly masked by the strong band at 1700 cm^{-1} (aldehyde C=O stretching). Another noticeable feature of this spectrum is the occurrence of two bands of medium intensity at 2725 and 2810 cm^{-1}. These are very characteristic of aldehydes and arise from symmetric and asymmetric C—H stretching of the aldehyde group.

A single strong peak at 702 cm^{-1} and an ill-defined substitution pattern between 1750 and 2000 cm^{-1} in the spectrum of *benzoic acid* (Fig. 2.11) suggest, once again, a monosubstituted benzene nucleus. Aromatic C—C stretching (1400–1650 cm^{-1}) and C—H stretching ($\sim$ 3000 cm^{-1}) are present. The strong peak at 1698 cm^{-1} in the result of C=O stretching of a conjugated carbonyl group, and the absorption pattern which occurs in the 2500–2900

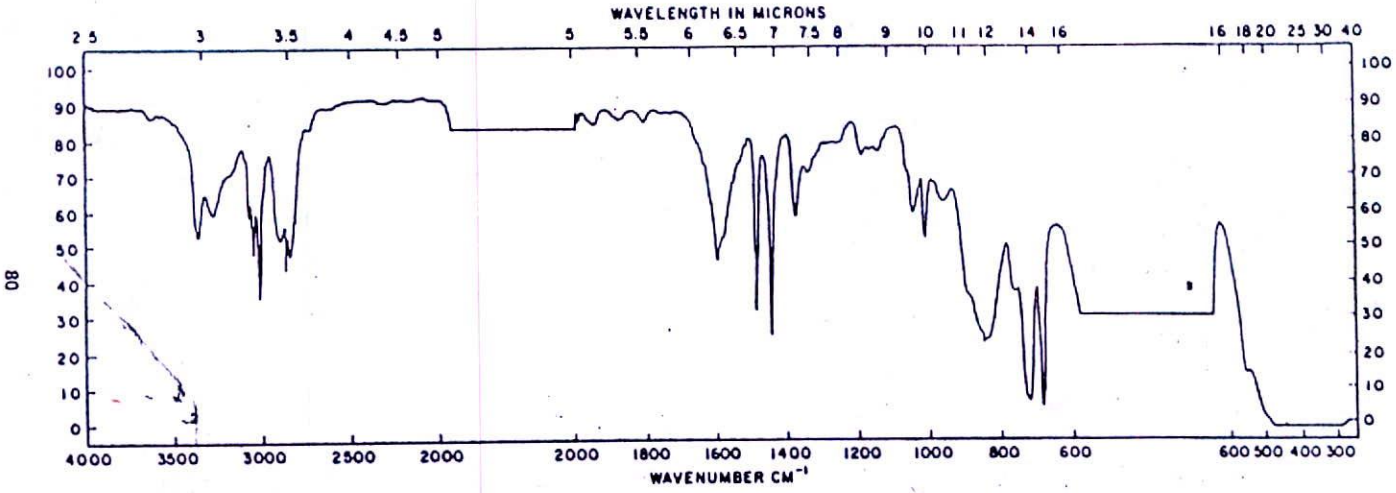


FIGURE 2.8: Infrared spectrum of benzylamine (thin film).

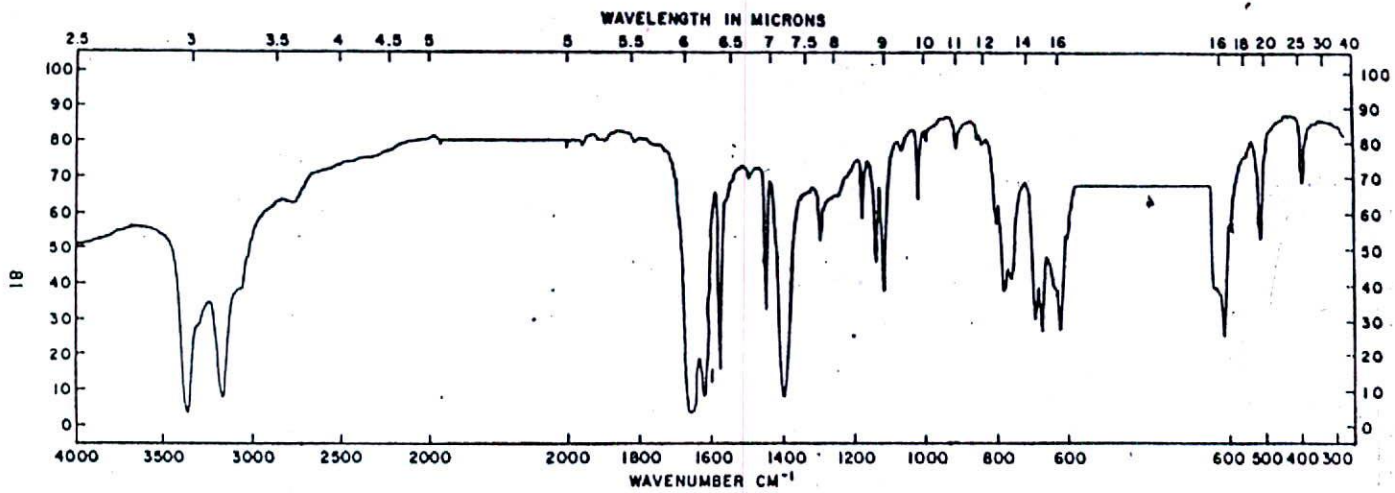


FIGURE 2.9: Infrared spectrum of benzamide (KBr disk).

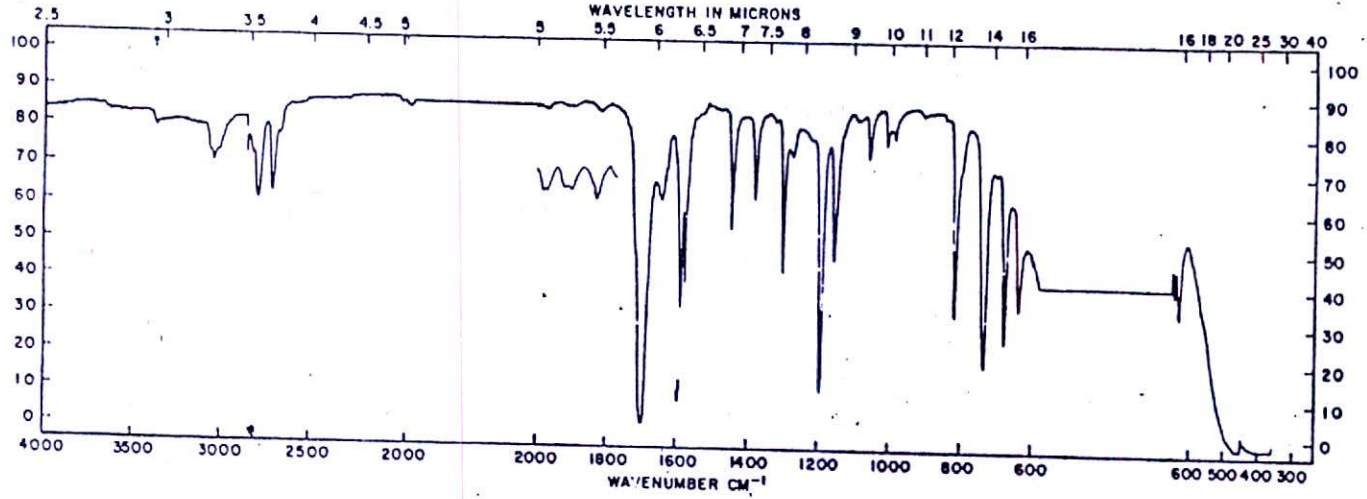


FIGURE 2.10: Infrared spectrum of benzaldehyde (thin film).

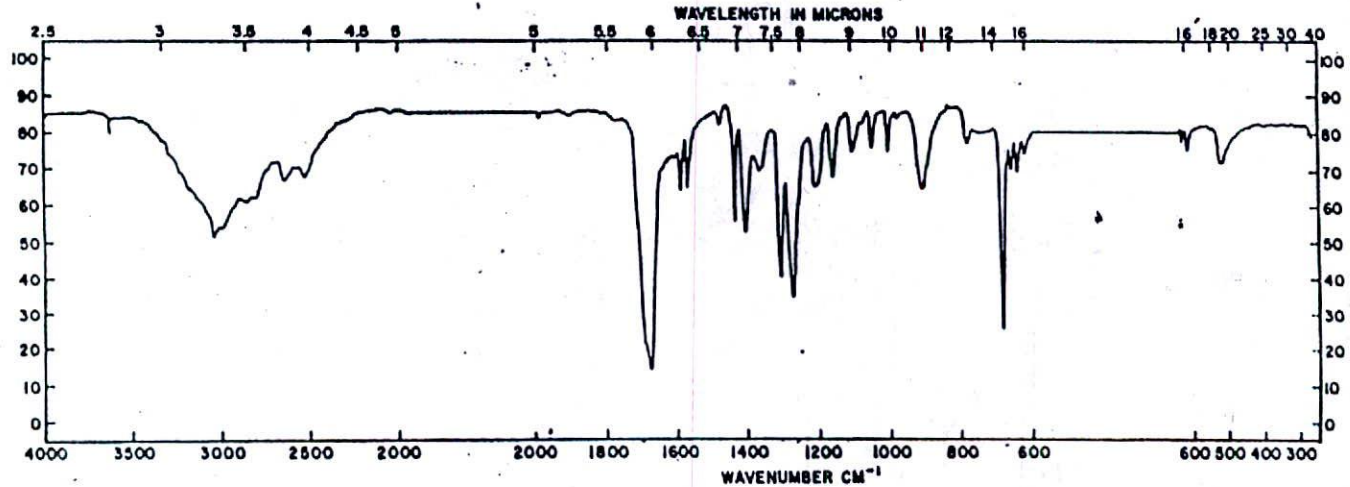


FIGURE 2.11: Infrared spectrum of benzoic acid (KBr disk).

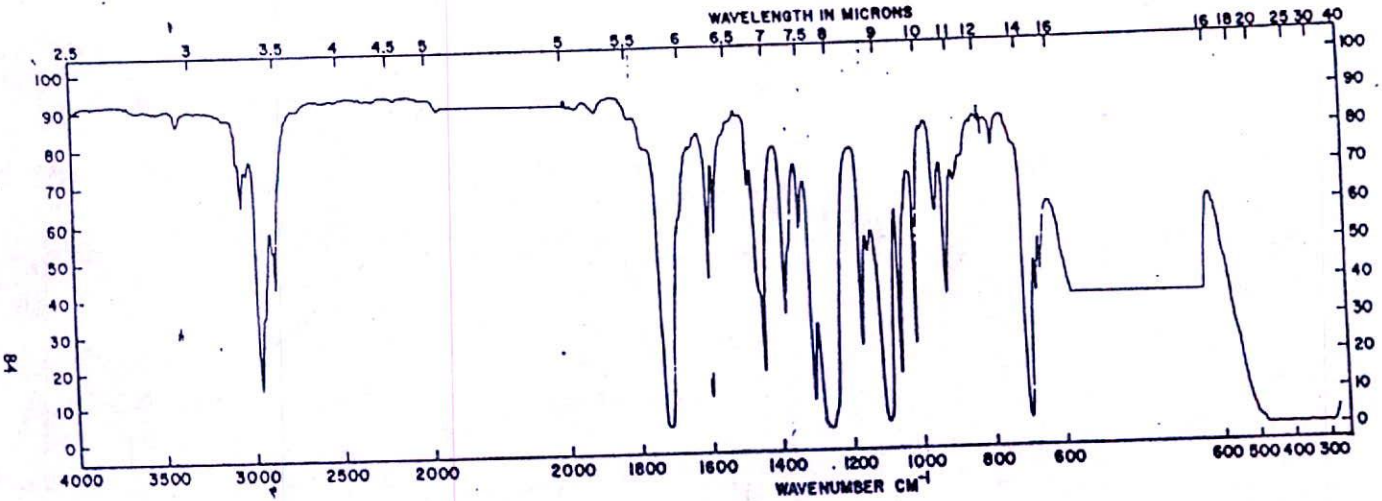
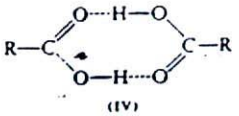


FIGURE 2.12: Infrared spectrum of *n*-propyl benzoate (thin film).

cm^{-1} region is very characteristic of a carboxylic acid dimer. Except in dilute solution, carboxylic acids exist as dimeric species (IV). The weak absorption in this region is attributable to bonded O—H stretching, a characteristically broad absorption. (In very dilute solution, O—H stretching is located near 3550 cm^{-1}; the molecule is then in monomeric form.)



(IV)

It is of interest to compare the spectrum of *n-propyl benzoate* (Fig. 2.12) with that of the corresponding acid. Both spectra are very similar in most regions, but are noticably different in the carbonyl and in the 2500–3200 cm^{-1} region. The carbonyl stretching vibration of the ester (1720 cm^{-1}) occurs at a slightly shorter wavelength (higher wave number) than that of the acid. The broad absorption of the acid in the region >2400 cm^{-1} has been replaced by relatively narrow bands attributable to aliphatic and aromatic C—H stretching. The substitution patterns in the 1750-2000 cm^{-1} and ~800 cm^{-1} regions of the spectrum of *p-nitrobenzoic acid* (Fig. 2.13) are consistent with a *p*-disubstituted benzene nucleus. This compound is obviously aromatic (1400–1650 cm^{-1}) and possesses a carboxylic acid group (cf. spectrum of benzoic acid). Two strong absorption peaks are present at 1535 and 1340 cm^{-1} due, respectively, to aromatic asymmetric and symmetric N—O stretching. A weaker peak at 865 cm^{-1} is due to aromatic C—N stretching.

It is informative to compare the spectrum of *benzyl alcohol* (Fig. 2.14) with that of benzaldehyde to see the effect of reducing an aldehyde to the corresponding primary alcohol. The obvious additional feature in the spectrum of the former is the strong, broad absorption band located ~3300 cm^{-1} associated with intermolecular hydrogen bonded (polymeric association) O—H stretching. This band has replaced the C=O and C—H absorption bands of the aldehyde.

The reader is left to interpret along similar lines the spectra of toluene, benzocaine, *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, *p*-nitrotoluene, and *n*-propyl *p*-hydroxybenzoate (Figs. 2.15 to 2.20, respectively). In particular, the effect on the spectrum of replacing one group with another should be rationalized.

All the spectra just mentioned are simple compounds and their spectra are relatively easy to interpret. This is not so with more complex substances with which it is often possible to confirm only the presence of functional groups. With more complex substances, group interaction can occur with resultant displacement of absorption bands. A few examples are now considered. The spectrum of *ethyl (o-nitrophenylthio)acetoacetate* (Fig. 2.21) has a strong band at 730 cm^{-1}, consistent with *o*-disubstitution, but little can be

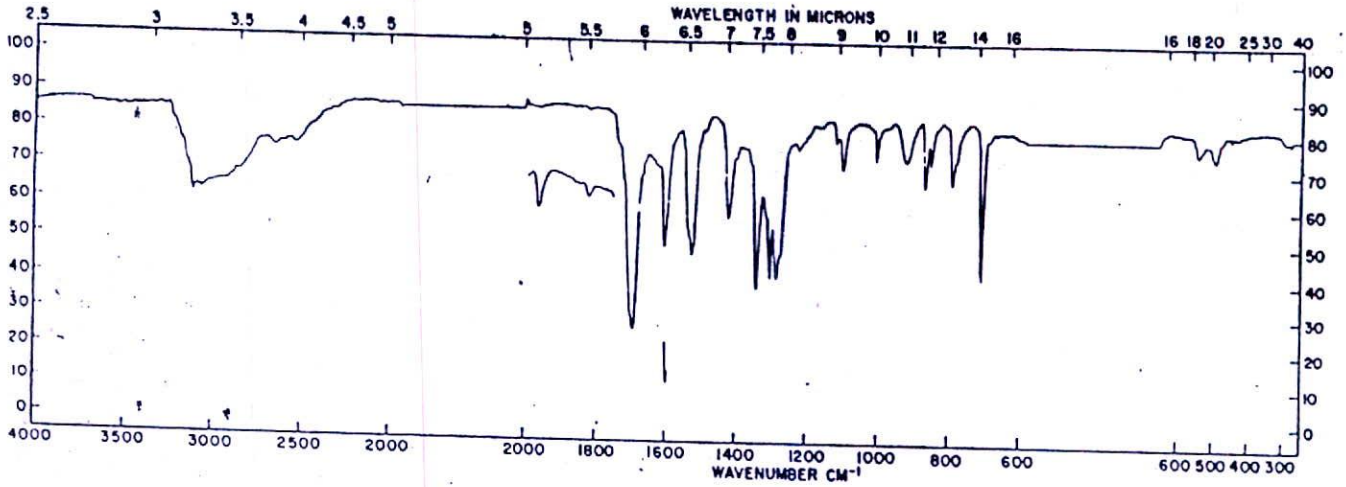


FIGURE 2.13: Infrared spectrum of *p*-nitrobenzoic acid (KBr disk).

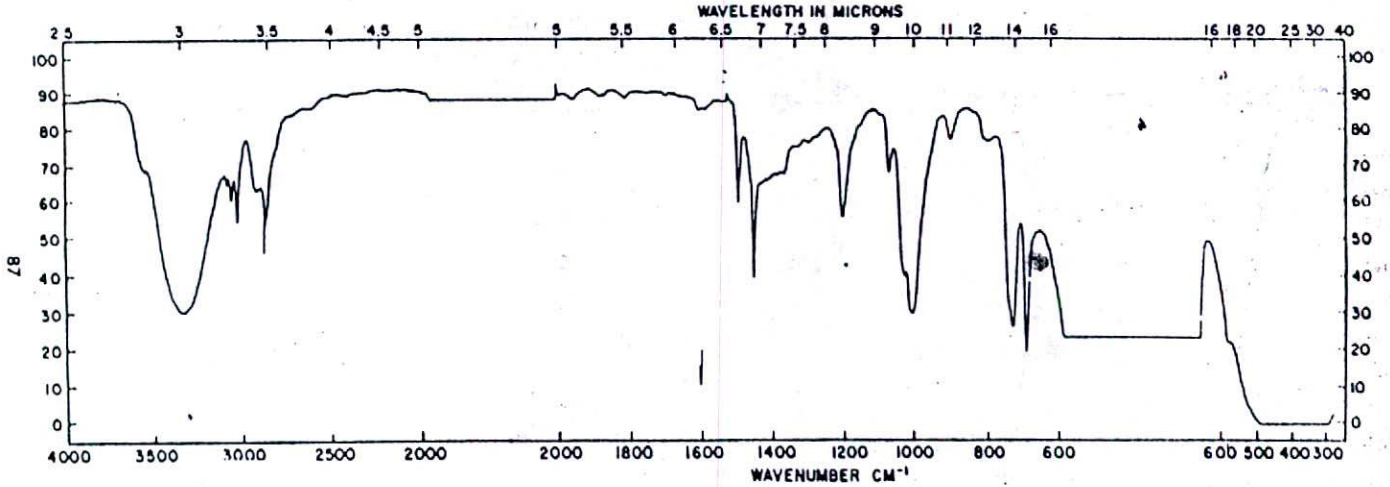


FIGURE 2.14: Infrared spectrum of benzyl alcohol (thin film).

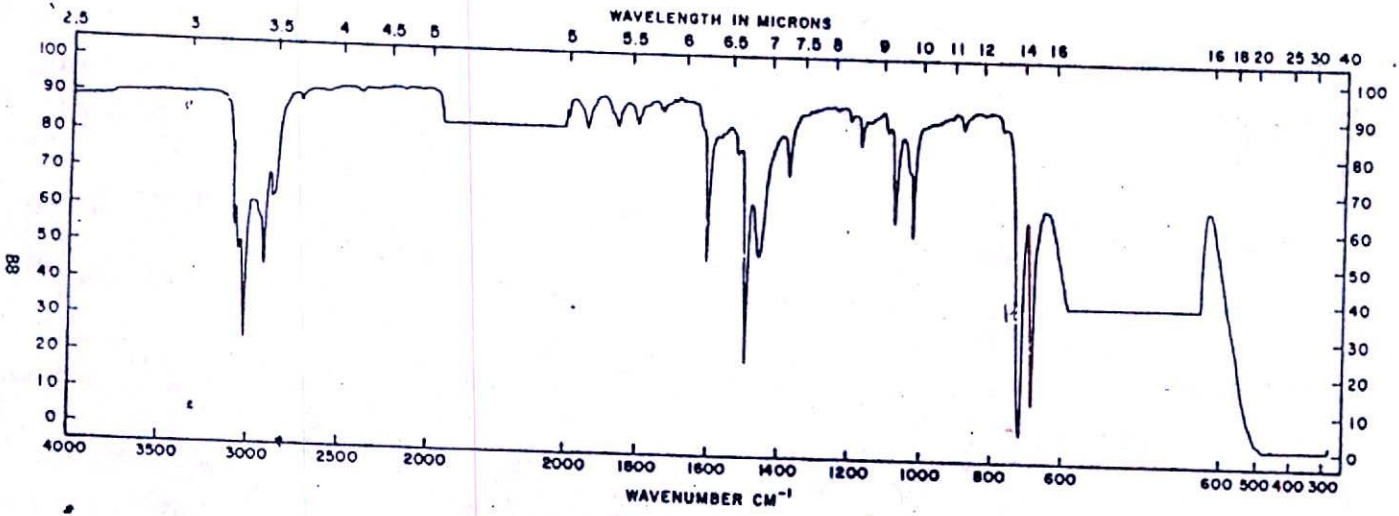


FIGURE 2.15: Infrared spectrum of toluene (0.02-mm thin film).

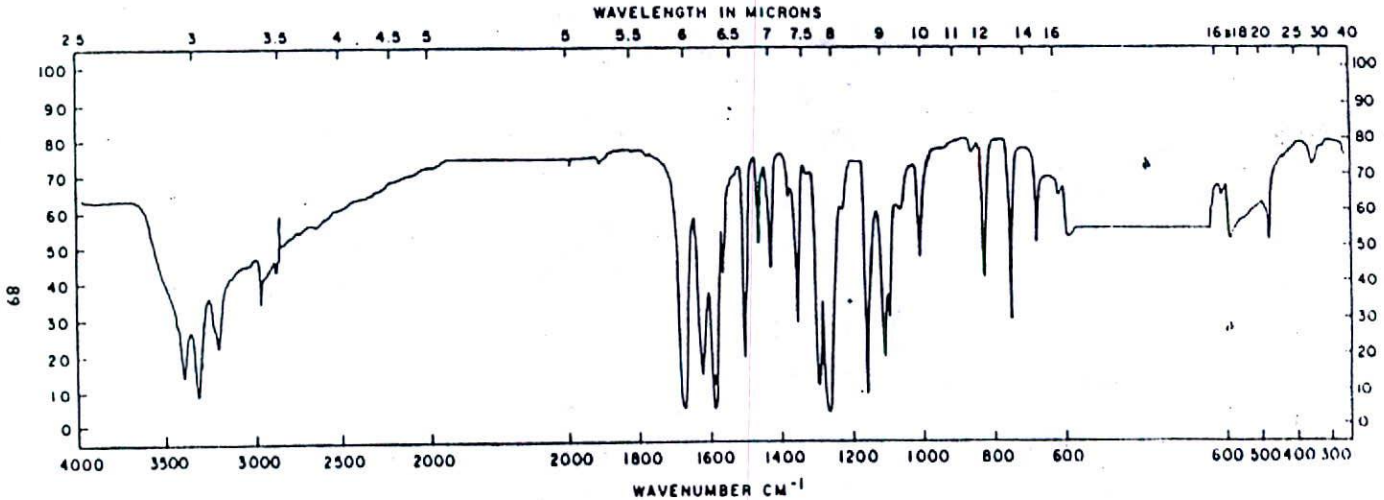


FIGURE 2.16: Infrared spectrum of benzocaine (KBr disk).

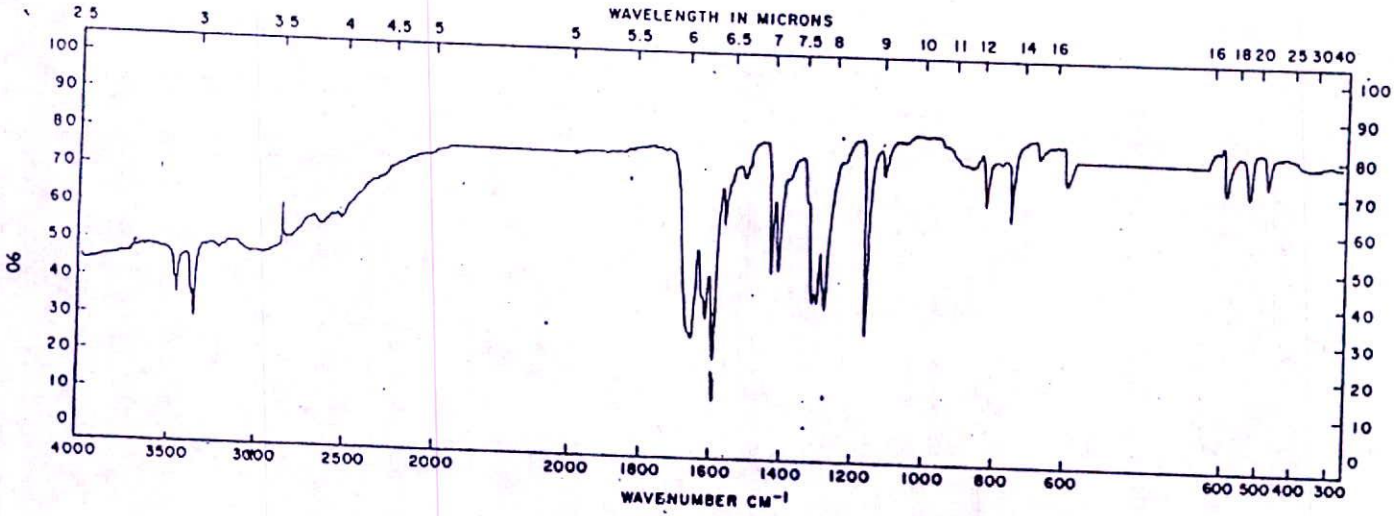


FIGURE 2.17: Infrared spectrum of *p*-aminobenzoic acid (KBr disk).

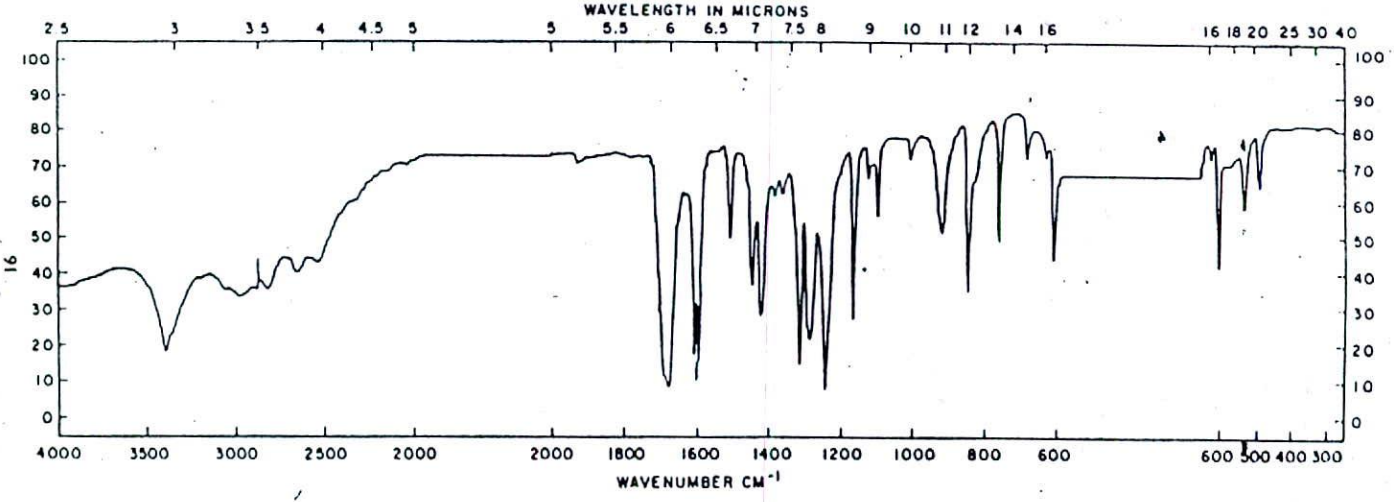


FIGURE 2.18: Infrared spectrum of *p*-hydroxybenzoic acid (KBr disk).

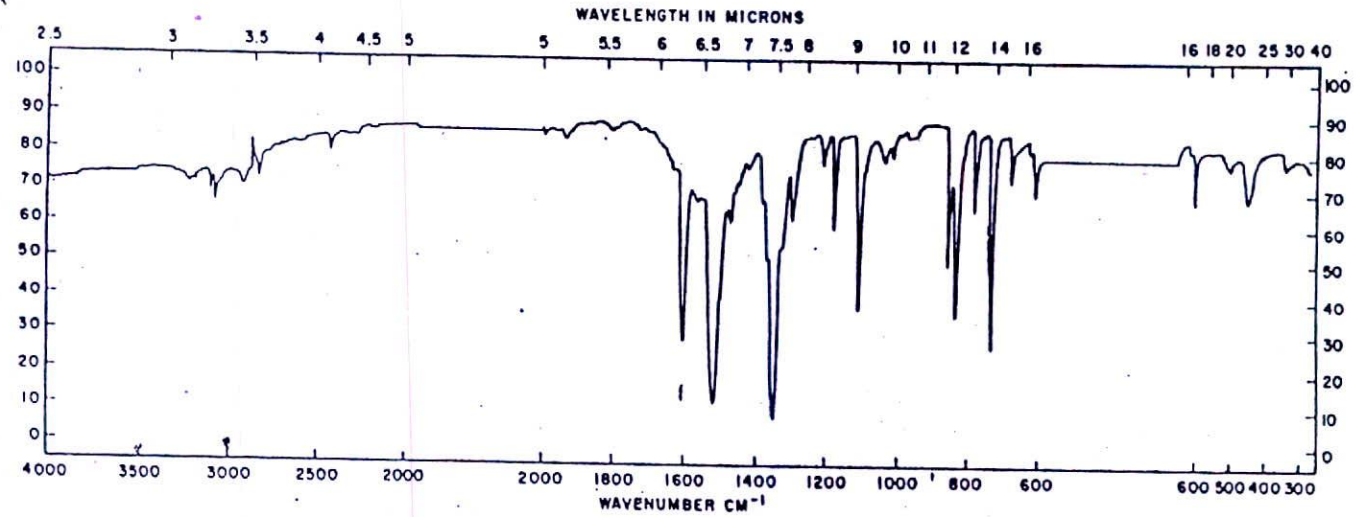


FIGURE 2.19: Infrared spectrum of *p*-nitrotoluene (KBr disk).

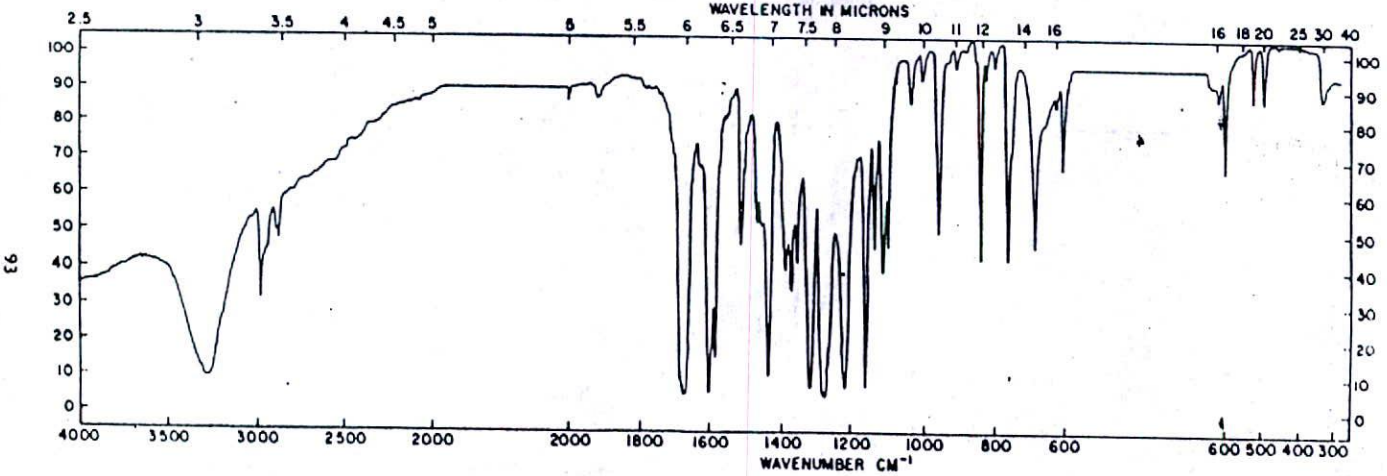


FIGURE 2.20: Infrared spectrum of *n*-propyl *p*-hydroxybenzoate (KBr disk).

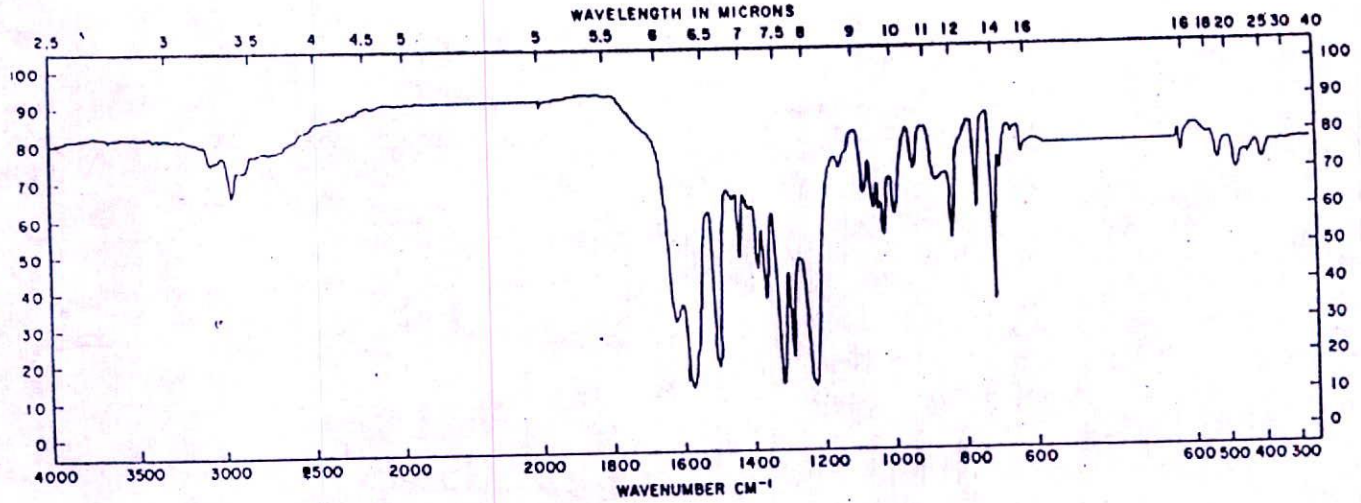
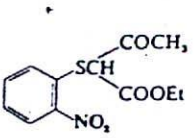
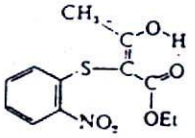


FIGURE 2.21: Infrared spectrum of ethyl (*o*-nitrophenylthio)acetoacetate (K_2Br disk).

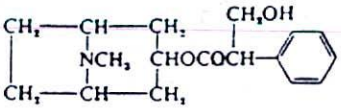
deduced from the usually informative regions, 1670–2000 and 1430–1670 cm^{-1}. In the former region, absorption bands are very weak, and in the latter region, strong bands due to other than C—C stretching occur. The presence of a nitro group is apparent—1512 (s), 1335 (s), 850 (w)—but the absence of absorption bands normally associated with ketone and ester carbonyl groups is noticeable. The reason for this is that this compound is a typical β-ketoester and as such exists mainly in hydrogen-bonded enolic form (VI) rather than as (V). Carbonyl absorption therefore occurs at a longer wavelength than might be expected. Broad weak absorption in the 2600–3000 cm^{-1} region suggests hydrogen bonded O—H stretching.



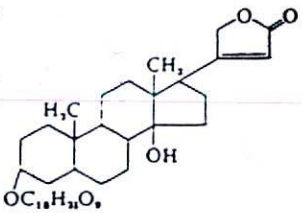
(V)



(VI)

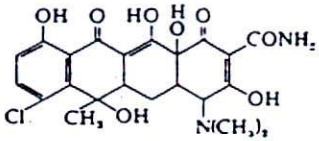


(VII)



(VIII)

(IX)



(X)

The infrared spectrum (Fig. 2.22) of *atropine alkaloid* (VII) is too complex to interpret completely, though certain bands can be identified readily. The strongest absorption peak near 1730 cm^{-1} is due to ester C=O stretching. The OH peak at 3100 cm^{-1} is broad and typical of a chelated compound, and relatively strong absorption in the 2800–3000 cm^{-1} region, due to C—H stretching, would be expected of such a structure. The two bands near 685 and 760 cm^{-1} can be assigned to monosubstituted phenyl C—H deformations; and the substitution pattern in the 1430–1600 cm^{-1} region, though weak, suggests an aromatic compound. The spectrum (Fig. 2.23) of *digoxin* (VIII) is also complex, but once again it is possible to identify some of the prominent bands in the spectrum. A noticeable feature is the strong O—H stretching band near 3440 cm^{-1}. Digoxin is a triglycoside and therefore a large number

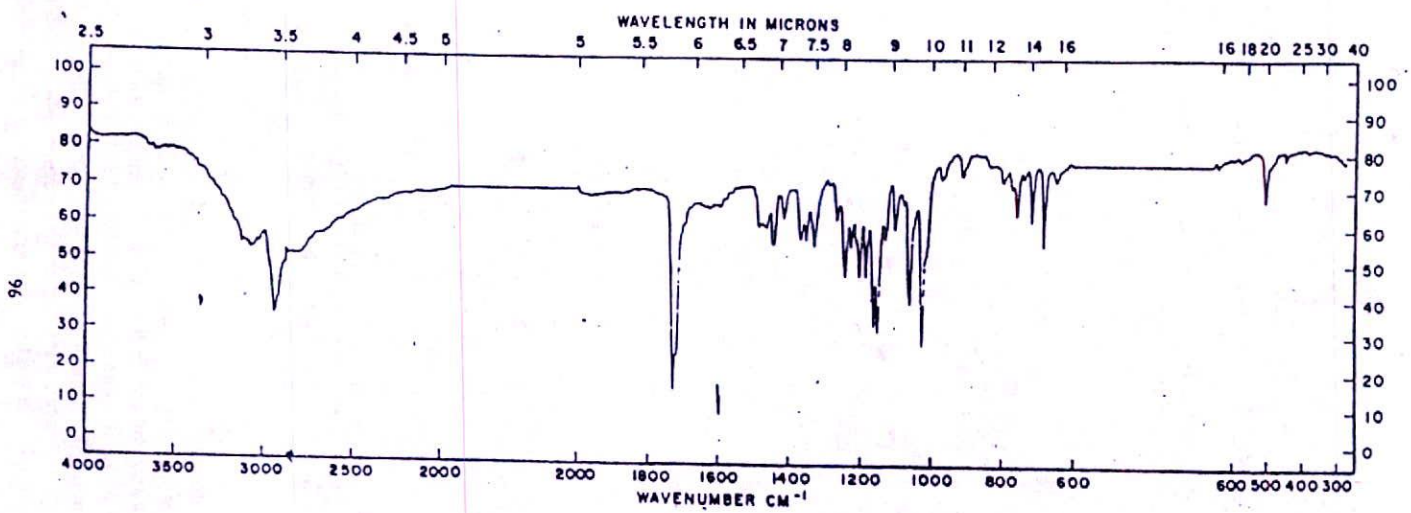


FIGURE 2.22: Infrared spectrum of atropine alkaloid (KBr disk).

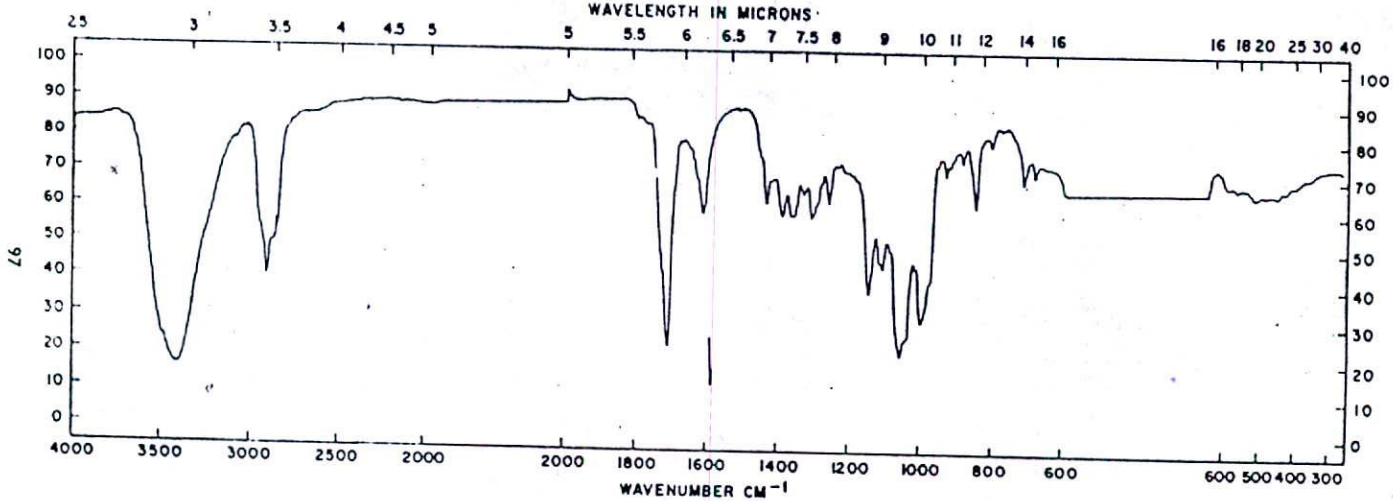


FIGURE 2.23: Infrared spectrum of digoxin (KBr disk).

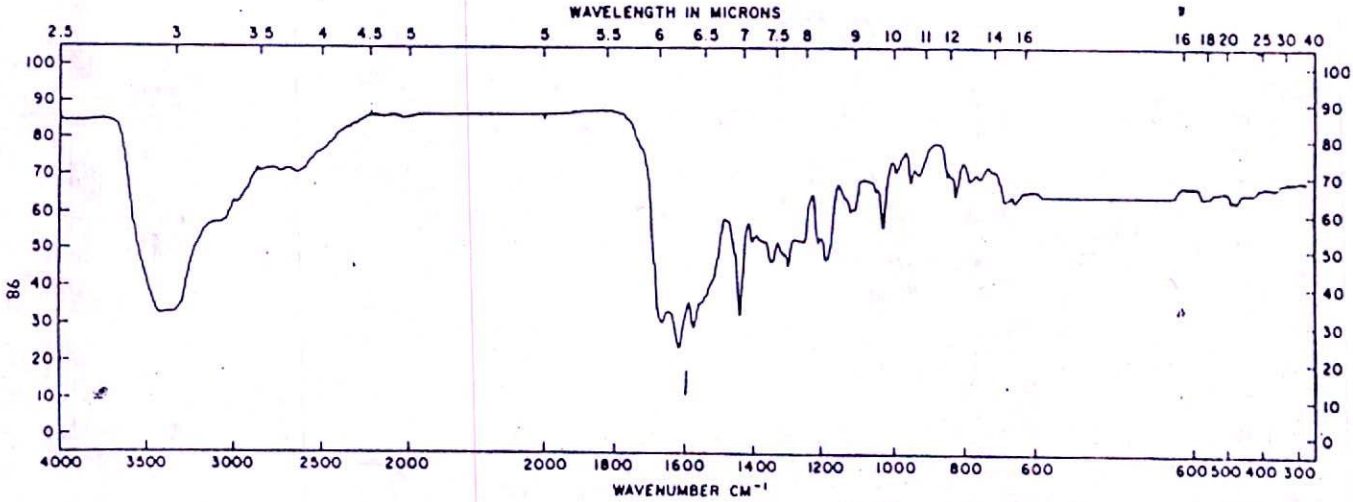


FIGURE 2.24: Infrared spectrum of aureomycin (KBr disk).

of hydroxyl groups are located in the C_3-side chain, in addition to the tertiary OH group at the junction of rings C and D. C—H stretching is present (2850–3000 cm^{-1} region), and the strong band at 1725 cm^{-1} is due to C=O stretching of an α,β-unsaturated lactone. It is difficult to assign a band to C—O stretching. The strong peak near 1065 cm^{-1} is probably due to either alcoholic O—H deformations or ether C—O stretching. The absence of absorption bands in the 1470–1610 cm^{-1} region and the lack of strong bands in the 600–800 cm^{-1} region indicate a nonaromatic compound.

No strong C=O stretching bands are present in the spectrum (Fig. 2.24) of *aureomycin* (X) at wave numbers greater than 1650 cm^{-1}. Carbonyl stretching bands of typical ketones and amides are usually located near 1700 cm^{-1}, but in this instance, all the carbonyl groups are strongly hydrogen-bonded, which results in a shift of the C=O absorption to lower wave numbers. The very broad and strong O—H stretching, centered at 3420 cm^{-1} confirms the presence of hydrogen-bonding. The broad band masks the N—H stretching, and the C—H stretching bands are only barely visible Without the aid of reference compounds, it is difficult to interpret the rest of the spectrum except to conclude that it is suggestive of a conjugated system.

The spectrum (Fig. 2.25) of *ephedrine alkaloid hemihydrate* (IX) was recorded as a 5% solution in chloroform. Four weak bands located between 1700 and 2000 cm^{-1} are readily apparent and are typical of the absorption pattern of a monosubstituted benzene nucleus. The expected strong band near 700 cm^{-1} for such a system is masked by the solvent, but is apparent in the spectrum of a KBr disk of the alkaloid. Absorption bands due to C—H, N—H, and O—H stretching are present in the 2800–3500 cm^{-1} region of the spectrum.

F. INSTRUMENTATION

Single-beam and double-beam infrared spectrophotometers are available. The former type has a limited use in routine qualitative and quantitative studies. Most modern infrared spectrophotometers are therefore of the double-beam type, though some of these can be operated as single-beam instruments. The Beckman IR5A spectrophotometer (Fig. 2.26) is a typical example. The basic components of the double-beam instrument are: radiation source, photometer, monochromator, detector system, and recorder. The optical system of a double-beam infrared spectrophotometer is shown (Fig. 2.27).

1. Radiation Source

Infrared radiation sources are black bodies which, when heated electrically to 1200–1800°C, continuously emit radiation of the desired intensity. Common sources are the Nernst glower and the Globar. The Nernst glower is a small rod composed of oxides of zirconium, thorium, and yttrium, which,

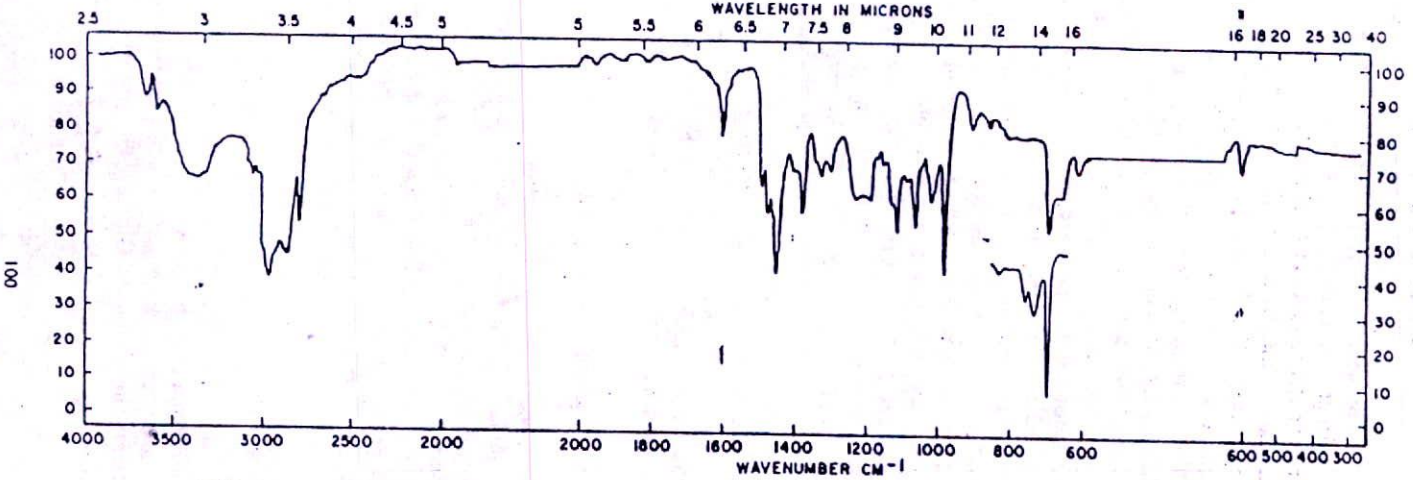


FIGURE 2.25: Infrared spectrum of ephedrine alkaloid hemihydrate (5% w/v in chloroform) (Inset: KBr disk).

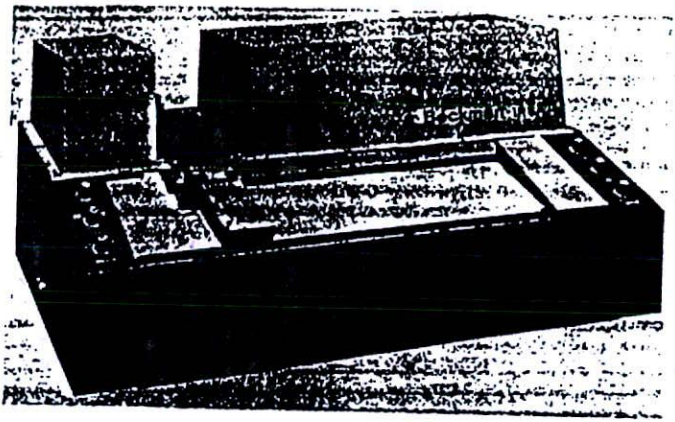


FIGURE 2.26: Beckman model IR5A infrared spectrophotometer. Reproduced with the permission of Beckman Instruments, Inc.

when heated to 1800°C, emits infrared radiation. Secondary electrical heaters are required to start the glower because it is nonconducting when cold. The Globar is a small rod of silicon carbide which is an effective infrared source when heated to 1200°C. Although infrared radiation is emitted continuously by both sources, the radiation energy is not constant, but varies with varying wavelength.

The radiation from the source is split by means of mirrors into two equal beams, one of which passes through the reference cell, while the other passes through the sample cell.

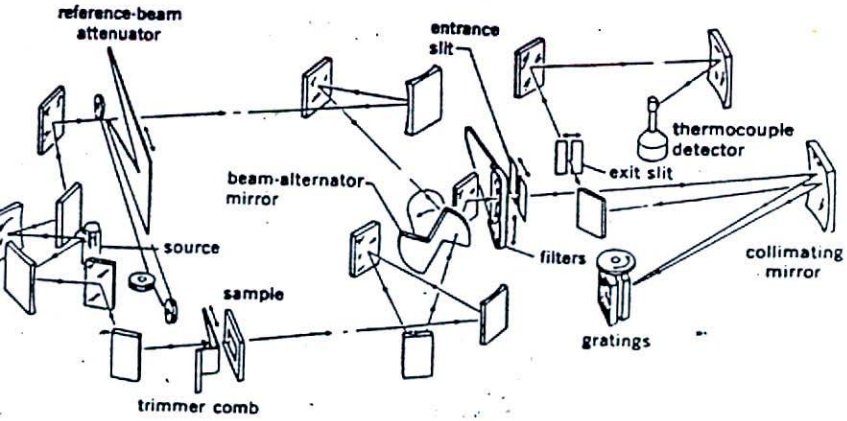


FIGURE 2.27: Optical system of double-beam infrared spectrophotometer.

2. Photometer

The reference and sample beams then enter the photometer area, in which the rotating chopper, i.e., the beam-alternator mirror, alternates the reference and sample beams such that the single beam produced, which passes through the entrance slit into the monochromator, is composed of alternating segments.

3. Monochromator

The beam from the photometer is focused by means of a collimating mirror onto gratings (see Fig. 2.27) or a prism (Fig. 2.28), which disperse the beam

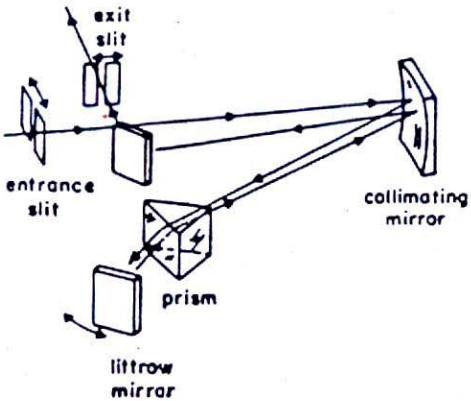


FIGURE 2.28: Prism monochromator.

over a range of frequencies, a small portion of which is reflected back to the collimating mirror. This results in further dispersion. Radiation of a very narrow frequency range emerges into the detector compartment. If the monochromator incorporates a prism, then the frequency of the radiation emerging from the exit slit will be altered by rotating the Littrow mirror. If this mirror is rotated automatically and continuously, the frequency of the radiation reaching the detector will also be continuously changing. Rotating the gratings serves the same purpose. Prisms of calcium fluoride, cesium bromide, and sodium chloride are all employed, but each suffers from the fact that the resolution produced is lower than desirable in certain frequency ranges. The use of gratings permits greater resolution. For this reason, gratings are replacing prisms in all but the cheaper modern instruments.

4. Detector System

Infrared radiation detectors are divided into two categories, thermal detectors and photon detectors. The former category includes thermocouples,

bolometers, and pneumatic (or Golay) detectors. Photon detectors employ semiconductors. Thermocouples are basically two dissimilar strips of metal joined together at each end. One junction is heated by the radiant energy of the alternating signal emerging from the monochromator. An emf is produced which is proportional to the degree of heating. Bolometers are similar. They are resistors with a high temperature coefficient of resistance. Both are operated in a vacuum, which decreases noise and increases sensitivity. Golay detectors contain a nonabsorbing gas, which expands and moves a flexible mirror when heated by the radiant energy. The degree of expansion and hence the motion of the mirror is proportional to the degree of heating. The emf produced, and the motion of the mirror, respectively, are very small and to be meaningful have to be amplified. In photon detectors, radiation excites electrons of a suitable semiconductor (e.g., PbSe) from a nonconducting low-energy state to a higher energy level, which can conduct and produce a signal proportional to the amount of radiation. The signal is actually a measure of a decrease in the resistance of the semiconductor. The major disadvantage of such detectors is that they are effective only over short wavelength ranges. The advantages and limitations of various types of detectors have been discussed in detail by Moss.[17]

When the sample under investigation has absorbed some energy, the sample and reference beams will differ in their radiant energies. Then, the detector system generates a signal which is amplified and fed to a servomotor which moves the attenuator comb, blocking part of the reference beam until the energies of the reference and sample beams are again equal. Beam balance (optical null) is restored. The attenuator comb is connected mechanically to the pen of the recorder so that the transmittance of the sample, as a function of wavelength (i.e., an infrared spectrum) can be recorded (see Fig. 2.29, in which the attenuator comb is diagrammatically represented in the form of a solid wedge).

G. PREPARATION OF SAMPLE

Infrared spectra of gases, liquids, and solids can be obtained. The sample must be dry because water absorbs infrared radiation near 3700 and 1630 cm^{-1} and absorption bands in these regions may be erroneously assigned to the substance being analyzed.

1. Solids

Three different methods of preparing a solid for infrared study are used. The solid may be examined neat, in suspension, or in solution.

The least common of these methods is the first mentioned, which is used occasionally if the solid melts without decomposition. A small quantity is melted and placed between two alkali halide plates (which transmit infrared radiation). They are clamped in a suitable holder. The capillary film which forms is allowed to solidify, then the whole, in a suitable holder, is placed in

the sample beam path. No reference is necessary, i.e., air is the reference. The method most commonly used is the examination of the solid suspended in an inert oil or inert solid. In both instances, the substance must be reduced to very small particles.* The orientation of a crystalline material in the infrared beam affects the intensity of the absorption bands, but this effect is minimized if numerous very small particles are examined. This is an important consideration in quantitative analysis. In the *mull method*, the finely

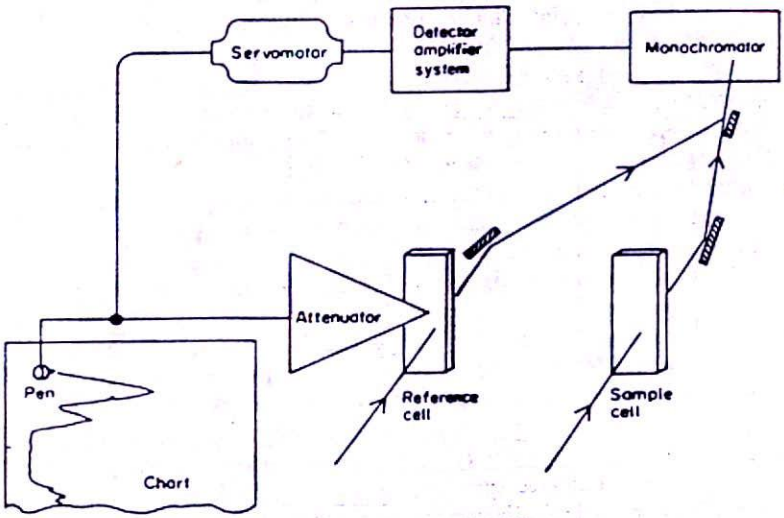


FIGURE 2.29: Beam balance.

powdered sample (ca. 5 mg) is dispersed in a drop of a suitable mulling agent. The use of an agate mortar and pestle or a glass plate and a small, inert spatula are recommended for this purpose. The most popular mulling agent is the mineral oil nujol, a mixture of high molecular weight alkanes. Fluorinated oil, e.g., fluorolube, is also used for this purpose. The disadvantage of using such agents is that they themselves absorb infrared radiation in certain regions and thus obscure some of the absorption bands of the substance being analyzed. Nujol, for example, absorbs strongly near 3000 cm^{-1} (C—H stretching) and less strongly near 1460 and 1375 cm^{-1} (C—H bending). It is transparent in thin layers over the 1375–650 cm^{-1} region. Fluorolube, however, is transparent in thin layers from 1330–5000 cm^{-1}. Thus, two spectra, one in each medium, would yield maximum information. Hexachlorobutadiene is also used as a mulling agent. It has no hydrogen atoms and therefore will be transparent in the regions where nujol absorbs.

* The Wig-L-Bug amalgamator, manufactured by Crescent Dental Manufacturing Company, Chicago, Ill. is useful for this purpose.

The mull is transferred to the surface of a flat alkali halide plate. A second plate is carefully placed on top and the two are manually pressed together, using a rotatory motion to ensure an even spread of the mull. By varying the amount of pressure applied, films of varying thickness can be obtained. The plates are held together in a suitable holder (Fig. 2.30) which is placed in the sample beam path. No reference cell is necessary.

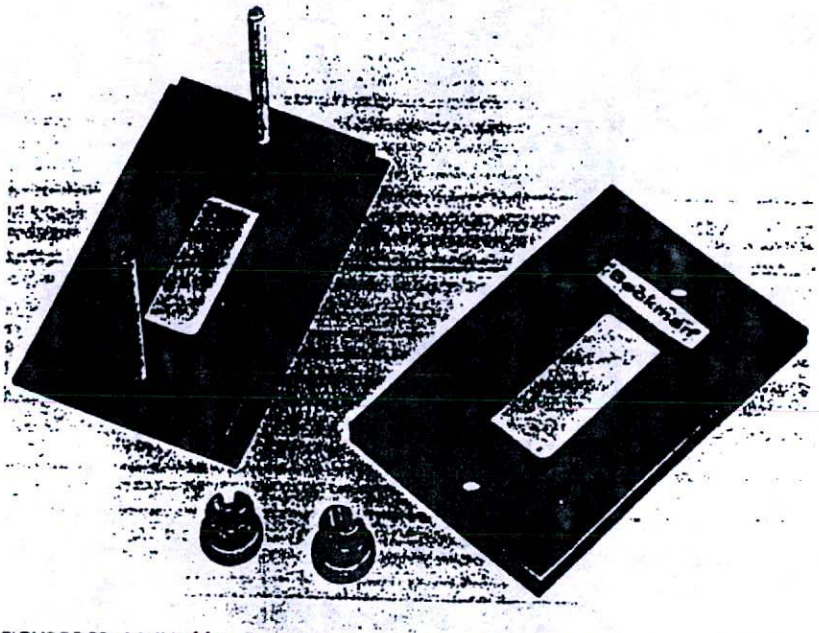


FIGURE 2.30: Mull holder. Reproduced with the permission of Beckman Instruments, Inc.

In the *pressed disk method*, pure, dry, and finely powdered potassium bromide or chloride is intimately mixed with the sample, preferably in a Wig-L-Bug or its equivalent. Generally a concentration of about 1 % w/w is suitable, i.e., 2 mg of the substance is ground with 200–300 mg of KBr. With high molecular weight substances, a somewhat larger concentration is often required. The mixture is compressed in a die under vacuum at room temperature and at high pressure (40,000–50,000 lb/in.^{2}).* This treatment produces a solid transparent disk, which is mounted in a holder and placed in the sample beam path. A reference disk of pure KBr can be used, but as spectral grade KBr is virtually transparent over the 5000–650 cm^{-1} range, it is the common practice to use no reference cell. One criticism of this method

* If the area of the disk is, say, 0.25 in.^{2}, then a pressure at the disk surface of 40,000 lb/in.^{2} is obtained by applying an external pressure of 10,000 lb.

is that during the grinding process, physical and chemical changes can occur and give rise to anomalous spectra (e.g., see Ref. 18).

Solids can also be examined in solution in a suitable solvent. The three most commonly used solvents are carbon tetrachloride, chloroform, and carbon disulfide, each of which has certain disadvantages. They absorb strongly in

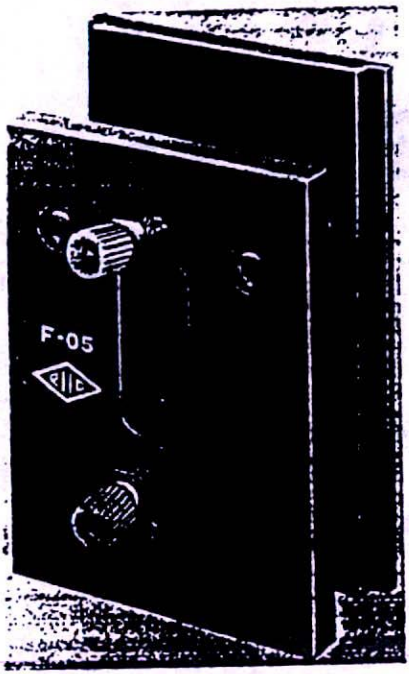


FIGURE 2.31: Solution cell. Reproduced with the permission of Beckman Instruments, Inc.

certain regions (Fig. 2.32) and thus obscure what may be important absorption bands. In addition, many polar substances are not sufficiently soluble in these solvents. The concentration required depends on the cell path length and on the molecular weight of the substance being examined. Generally speaking, a 5% w/v solution of a substance of molecular weight 100 to 500 will usually give a good spectrum using a cell of 0.1 mm-path length; with 1 mm cells, a 1.5% w/v solution is often satisfactory. The prepared solution is transferred to a solution cell (Fig. 2.31) by means of a hypodermic syringe. Solvent evaporation is avoided, especially in quantitative analysis.

Solution cells are of three types. *Demountable cells*, as the name suggests, can be dismantled after use, thoroughly cleaned and polished, and reassembled prior to further use. Spacers of varying thicknesses are available for

such cells with the result that cells of varying path lengths can be constructed. *Sealed cells* of fixed cell path length have the advantage of a cell path length which is accurately and permanently known. They are more difficult to clean than the demountable type. This is achieved by repeated flushings with solvent. The *variable-thickness cell* has a micrometer attachment with which path length can be varied. Cost prohibits the routine use of cells of this type.

When a solution is examined, a compensating reference cell of path length

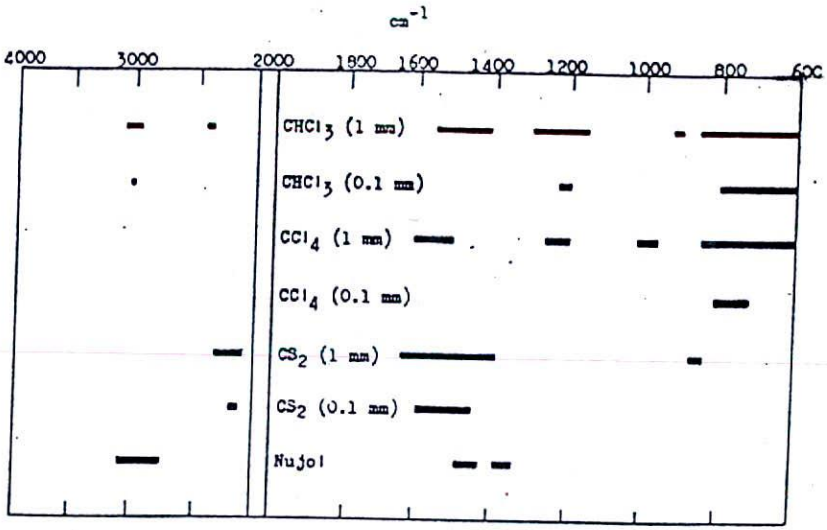


FIGURE 2.32: Strong absorption bands of chloroform, carbon tetrachloride, carbon disulfide, and nujol. (The solvents and the oil absorb strongly in the shaded areas.)

equal to that of the solution cell is necessary. The reference cell contains pure solvent only and is placed in the reference beam. As the path lengths of the sample and reference cells are the same, the recorded spectrum is that of the solute, except in the regions where the solvent absorbs strongly.

Sealed cells of fixed path length are most often employed. A matching set is required. Usually matching pairs of path lengths 0.05, 0.1, 0.5, and 1.0 mm are kept available. Alternatively, one sealed cell of each path length may be available. If this is so, the reference solution is placed in a variable-thickness cell, the thickness of which is adjusted to match that of the sealed cell.

2. Liquids

Liquids are generally examined as thin films to avoid interference of the solvent. They are examined between two alkali halide plates (cf. mulls), and once again control of sample thickness is difficult. Cells with very small path

lengths (0.005 mm and upward) are available for quantitative work when the thickness of the liquid film must be accurately known. Volatile liquids must be examined in a sealed cell of this type. Liquids can also be examined in solution. The same limitations as those described under solutions of solids apply.

3. Gases

Molecules of gases, in contrast with liquids, solids, or solutions, are free to rotate. The result is that with simple gas molecules especially, rotational

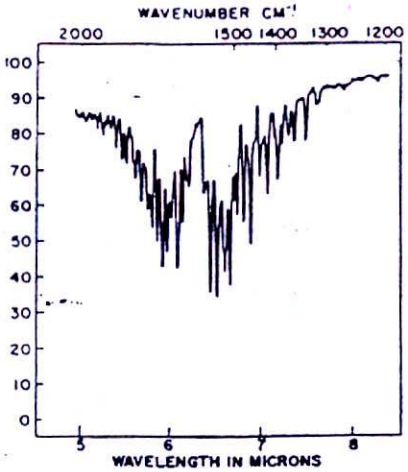


FIGURE 2.33: Atmospheric water measured on a Beckman IR5A spectrophotometer used as a single-beam apparatus.

energy level transitions are observed in their infrared spectra. An abundance of fine structure is obtained corresponding to the rotational energy transitions (Fig. 2.33). The locations of some of these bands are accurately known, and for this reason, various gases are used in the wavelength calibration of a spectrophotometer.

Special techniques are used to weigh very small quantities of gases, and special gas cells with path lengths measured in centimeters (e.g., 10 cm) are employed. Gas cells up to 50 cm in length are used. They are necessary because of the small number of molecules of the gas being examined.

2.3 PHARMACEUTICAL APPLICATIONS OF INFRARED SPECTROSCOPY

Many pharmaceutical substances have been qualitatively identified or quantitatively assayed by means of infrared spectrophotometry. Previous mention has been made to informative reviews in this field of study.[9–11]

A. QUALITATIVE ANALYSIS

In almost every instance, qualitative analysis is performed by comparing the spectrum of the substance with that of an authentic sample. If the spectra of both are identical, then the substance and the authentic sample are the same. There are very few exceptions to this generalization. One of these concerns the spectra of long-chained fatty acids or esters (XI) in which n is large. Such homologs give spectra which are extremely similar.

$$CH_3(CH_2)_nCOOR$$
(XI)

It is well known that when the halide-disk technique is employed, anomalous absorption bands are sometimes observed. These have been summarized by various workers[19–22] who list the reasons as

a. Variations in crystal size and orientation
b. Variations in grinding technique
c. Formation of polymorphic crystalline forms
d. Formation of hydrates
e. Formation of amorphic materials
f. Chemical transformations or combinations.

To identify a compound, it is preferable, therefore, to compare a dilute solution of it with one of an authentic sample. Nevertheless, it is the common practice to perform both qualitative and some quantitative analyses using halide disks. The BP and USP both recognize that spectra vary with the method used to prepare the sample and with the instrument employed, and they require a direct comparison of the spectrum of the substance with that of a similar preparation of an authentic sample. This should not detract from studies such as the one by Warren and co-workers[23] in which the infrared spectra of 23 phenothiazines were recorded and analyzed. Reference to such published spectra enables one to eliminate many possibilities and may even result in a tentative identification which can be confirmed by the USP method. Another example of this approach is the reported study of the infrared spectra of a series of phenols in carbon tetrachloride[24] in which spectra are recorded in a bar-type diagram which, the authors suggest, would be useful in the characterization of unknown phenols.

The presence and identification of impurities in medicinal substances can be confirmed by means of infrared spectrophotometry. The work by Urbanyi, Sloniewsky, and Tishler,[25] to which reference is made in greater detail later, is a good example of this approach.

B. QUANTITATIVE ANALYSIS

Whereas most ultraviolet and visible spectrophotometers provide a chart of absorbance v. wavelength, many infrared spectrophotometers measure, in percentage, the amount of energy transmitted by the substance, i.e., the

transmittance T, as a function of wavelength or frequency of the energy supplied. The transmittance is the ratio of the intensity of the radiation transmitted by the sample I to the intensity of the radiation incident on the sample I_0, i.e.,

$$T = I/I_0$$

All quantitative spectrophotometric measurements are governed by the Beer-Lambert law

$$I = I_0 e^{-kcd}$$

where k is an absorption coefficient characteristic of the substance, c is the concentration of the substance (moles/liter), and d is the thickness of the cell containing a solution of the substance. This expression can be rewritten as

$$\log_{10} \frac{I_0}{I} = \epsilon cd$$

where ϵ is the molecular extinction coefficient (or molar absorptivity) of the substance in units of liters centimeter^{-1} mole^{-1}. The expression

$$\log_{10} \frac{I_0}{I}$$

is termed the absorbance (or extinction coefficient, or optical density) A of the substance. As ϵ is characteristic of the sample, and as d can be controlled, it follows that the absorbance A is directly proportional to the concentration of the substance, i.e.,

$$\log_{10} \frac{I_0}{I} \propto C$$

Transmittance T was defined previously as I/I_0. The following relationships are therefore true:

$$A = \log_{10} \frac{I_0}{I} \qquad A = \log_{10} \frac{1}{T} \qquad A = \log_{10} \frac{100}{\% T}$$

In quantitative intensity measurements, it must always be first confirmed that absorbance is directly proportional to concentration of solute in the concentration range to be used and at the wavelength selected for the assay. This is done conveniently by preparing a series of dilutions of the substance under investigation and recording

$$\log \frac{I_0}{I}$$

or its equivalent, for each at the chosen wavelength. A plot of this v. concentration should give a straight line.

There are two other sources of error in quantitative infrared analysis. Intensity measurements should be in the 20–60% transmission range. A

suitable solution concentration or cell path length should be selected to insure this. Signals can be distorted nearer 0 or nearer 100% transmission.

The second source of error is the uncertainty of the base line I_0. Most organic substances give rise to complex infrared spectra. A suitable band is chosen for the purpose of quantitative analysis. If possible, it should be an isolated symmetrical band because in such an instance (Fig. 2.34a), the value of I_0 can be readily determined. Frequently, bands overlap (Fig. 2.34b), making accurate measurement of I_0 difficult, especially if the neighboring bands arise from impurities or from components of a mixture other than the

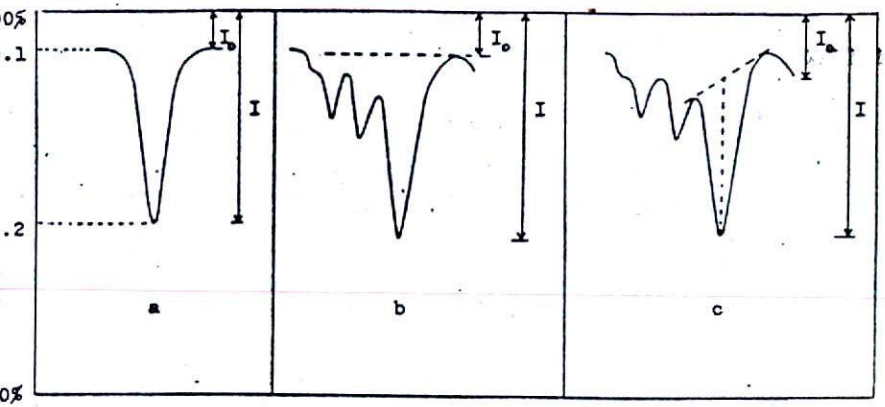


FIGURE 2.34: Selection of base line in quantitative analysis.

substance being determined. In such a situation, the "base line technique" is used—a suitable tangent is drawn to provide the base line.[26] If the neighboring band also arises from the substance being investigated, however, the spectrum will be reproducible, and alternative base lines can be drawn (Fig. 2.34c).

Calculation of the absorbance of a substance (A_s) is illustrated using the ideal situation (Fig. 2.34a). The relationship deduced,

$$A_s = \log_{10} \frac{\% T_0}{\% T}$$

(where T_0 and T are the transmittances of the solvent and the solution of sample, respectively, and correspond to the chosen values of I_0 and I), also holds when the base line technique is employed. From Fig. 2.34a,

$$A_{\text{solvent}} = \log \frac{100}{90.1}$$

$$A_{\text{solvent+sample}} = \log \frac{100}{44.2}$$

Therefore

$$A_s = \log \frac{100}{44.2} - \log \frac{100}{90.1}$$

$$= \log \left(\frac{100}{44.2} \times \frac{90.1}{100} \right)$$

$$= \log \frac{90.1}{44.2} = 0.3093$$

that is,

$$A_s = \log \frac{\% T_0}{\% T}$$

As dispersion of solids in halide disks can give rise to anomalous absorption bands, most quantitative determinations are carried out on solutions of substances. Because of its superior solubilizing properties, chloroform is commonly employed for this purpose; carbon tetrachloride, carbon disulfide, pyridine, and other solvents are also used. All solvents suffer from the disadvantage that they blank out certain areas of the spectrum (see Fig. 2.32). Some substances, however, are not sufficiently soluble in any of the common solvents. In such instances, provided certain precautions are observed, it is possible to perform quantitative analyses using mulls or potassium bromide disks. Hayden and Sammul[27] have shown that reproducible quantitative results can be obtained using the halide-disk technique, provided that the sample and standard disks are prepared under carefully controlled conditions and a modification of the Beer-Lambert law equation is employed for the calculations. These authors used this method for the quantitative analysis of a large number of pharmaceutical substances. The mull technique has also been adapted to quantitative infrared analysis.[20]

1. Reference Standard

The substance used in the preparation of the standard solution or disk, in both quantitative and qualitative analysis, is almost invariably a pure sample of the substance under investigation. Comer and Ribley,[28] however, used a solution of benzoic acid in chloroform to establish absorbance ratios for several drug substances and found that the accuracy of this technique was equal to the usual method of using the drug substance as a reference standard. Similarly, Hayden[29] has developed a method for the determination of glyceryl trinitrate by infrared analysis using benzoic acid as a secondary standard in place of the normal reference standard (an adsorbate of glyceryl trinitrate on lactose). From these results and others, it would seem that, in future, secondary standards will be employed to a greater extent. Their use would eliminate the need for storing large numbers of different primary reference standard compounds.

C. PRACTICAL INFRARED ANALYSIS

Exercises

E2.1. Identification of Selected Medicinal Compounds. Four single substances chosen from Tables 2.1 and 2.2 will be supplied, as well as authentic pure samples (reference standards) of these substances. Prepare each sample and reference standard in the manner suggested in the USP or BP, obtain the infrared spectrum of each, and compare each pair.

The four substances will be so chosen to require the student to record the spectrum of a thin film, the spectrum of a solution, the spectrum of a dispersion in KBr, and the spectrum of a suspension in mineral oil. If the spectra of the specimens, obtained when KBr or mineral oil is used, differ from those of the reference standards, this may be due to polymorphism (the existence of a substance in more than one crystalline form) or due to other reasons (see p. 109). The procedure suggested by the USP XVII, p. 809, should be adopted, i.e., prepare solutions of both the reference standard and the specimen under test in a suitable solvent, remove the solvent by evaporation, and prepare a new dispersion or suspension of each residue for a second examination. If infrared spectral differences are still observed, it can be concluded that the specimen and reference standard are not identical.

E2.2. Identification of the Medicinal Component of a Tablet. The tablets supplied will contain the salt of an organic base (see examples marked with a superscript *b* in Table 2.1). The appropriate reference standard will also be supplied. Confirm the identity of the medicinal component using the USP test entitled "Identification—Organic Nitrogenous Bases" (USP XVII, p. 908).

E2.3. Infrared Spectra of Primary, Secondary, and Tertiary Amine Salts. Three unnamed pharmaceutically active amine salts, such as those mentioned here, will be supplied. Record their infrared spectra as suspensions in mineral oil, and by reference to these spectra and to the literature[13] decide which one is

(+)-amphetamine sulfate (a primary amine salt)
ephedrine sulfate (a secondary amine salt)
quinine hydrochloride (a tertiary amine salt)

Explain the reasons for your conclusions.
(*Note:* Amine salts other than the ones named may be supplied).

E2.4. Identification of Pharmaceutically Useful Phenothiazines. A medicinally active phenothiazine base or salt will be supplied. (If the latter, dissolve it in a small volume of water, basify the solution with dilute sodium hydroxide solution, and extract the liberated base with ether. Wash the ether solution with water, dry it over sodium sulfate, filter, and remove the ether from the

filtrate). Record the spectrum of the base as a thin film. Compare your spectrum with those published in the literature[23] and make a tentative identification.

E2.5. A Study of Complex Formation by Means of Infrared Spectroscopy. Quinine and phenobarbital are known to react to form a 1:1 complex,[18] the formation of which can be confirmed by means of infrared spectroscopy. Prepare the complex by dissolving 1 mmole (324 mg) of quinine alkaloid and 1 mmole (232 mg) of phenobarbital in hot absolute ethanol (5 ml) and cooling the solution to 0°. The crystalline complex will separate within 2 hr. Collect the precipitate and wash it with small quantities of cold ethanol. It should melt at 185°.

Prepare a pure sample of phenobarbital by crystallizing a commercial sample from aqueous ethanol. The mp should be 174°.

Record the mp of a good commercial sample of quinine base. A sample, mp 177°, is suitable for this study without purification.

Prepare a physical mixture of phenobarbital (23 mg) and quinine (32 mg) on a powder paper using a spatula to mix the powders.

Prepare potassium bromide disks of all four specimens, by shaking a mixture of 1 mg of each with 300 mg quantities of potassium bromide in a Wig-L-Bug amalgamator for 5 sec, then pressing the disks at a pressure of 40,000 psi.* Record the infrared spectrum of each over the range 2000–1400 cm^{-1} (5–7 μ). The spectrum of the physical mixture should be an additive spectrum of the individual components. The spectrum of the complex should differ from that of either component.

E2.6. Infrared Absorption Vibrations of Functional Groups. Three, four, or five chemically related compounds will be supplied in unmarked sample tubes. The chemical identity of one will be revealed. Record the infrared spectra of all the compounds, using as many different ways of preparing the samples as possible.

Identify the characteristic functional-group absorption bands in each spectrum, and suggest structures for each compound. Confirm your identification using other simple physical or chemical tests (e.g., bp; mp; refractive index; BP or USP identity tests) or by comparison with spectra of authentic samples which will be supplied on request.

(Typical example: *p*-nitrophenol; *p*-aminophenol; *p*-phenetidin; phenacetin.)

E2.7. Infrared Spectra of Steroids. A selection of the steroids listed in Tables I and II will be available. Each student (or group of students) will record the spectra of two of them as dispersions in KBr, retain a copy of each

* These conditions for the preparation of the disk differ from those suggested in the original publication. A pressure of 40,000 psi (i.e., 8000 lb pressure was used to prepare a 0.2-in.^{2} disk) was best when the equipment available to the author was used.

for his own use (see later), and deposit a copy of each with the instructor. In this way, a library of spectra will be compiled. When the library is complete, the student will record the spectrum of another steroid and identify it from the compiled library of spectra, all of which will have been recorded on the same instrument.

On the spectra retained by the student (previous instructions), identify as many as possible of the stretching and deformation vibrations.

E2.8. Qualitative Reaction Sequence Study. The interaction of *o*-chloronitrobenzene and α-mercaptoacetic acid in the presence of sodium bicarbonate, followed by acidification, gave compound (I), $C_8H_7NO_4S$, which was converted to (II), $C_9H_9NO_4S$, when heated with methanol containing 10% concentrated sulfuric acid. Compound (II) was oxidized with potassium permanganate and glacial acetic acid, which gave (III), $C_9H_9NO_6S$. Hydrolysis of (III) produced compound (IV) which had a molecular formula $C_7H_7NO_4S$. Reduction of (IV) by means of zinc and ammonium chloride gave (V), $C_7H_9NO_3S$, whereas reduction with stannous chloride and hydrochloric acid gave compound (VI), $C_7H_9NO_2S$.[30,31]

The infrared spectra of compounds (I–VI) are reproduced here (Figs. 2.35–2.40, respectively). All were recorded using the KBr disk technique. By means of these spectra, identify the six compounds and suggest the origin of each of the major vibration bands.

E2.9. (a) Confirmation of the Validity of the Beer-Lambert Law Using Solutions of Caffeine in Chloroform, and (b) Determination of the Caffeine Concentration in a Chloroform Solution of the Drug. The band near 10.26 μ is suitable for the quantitative determination of caffeine in chloroform.[32]

Prepare a solution of caffeine in chloroform by dissolving approximately 1 g, accurately weighed, in chloroform in a 25-ml volumetric flask. Make up to volume and mix. This stock solution contains approximately 40 mg/ml. From it, using a 5-ml narrow bore burette and volumetric flasks, make the following dilutions with chloroform:

Stock solution, ml	Final volume, ml	Approximate concentration, mg/ml	Solution identity
5	10	20	A
4	10	16	B
2.5	10	10	C
1	10	4	D

Record the spectra of solutions A–D in 0.5 mm cells, with chloroform in the reference cell. Calculate the absorbance of the band at 10.26 μ for each, using the base line technique and record a plot of absorbance vs. concentration. If the Beer-Lambert law is obeyed over this concentration range, a straight line will be obtained.

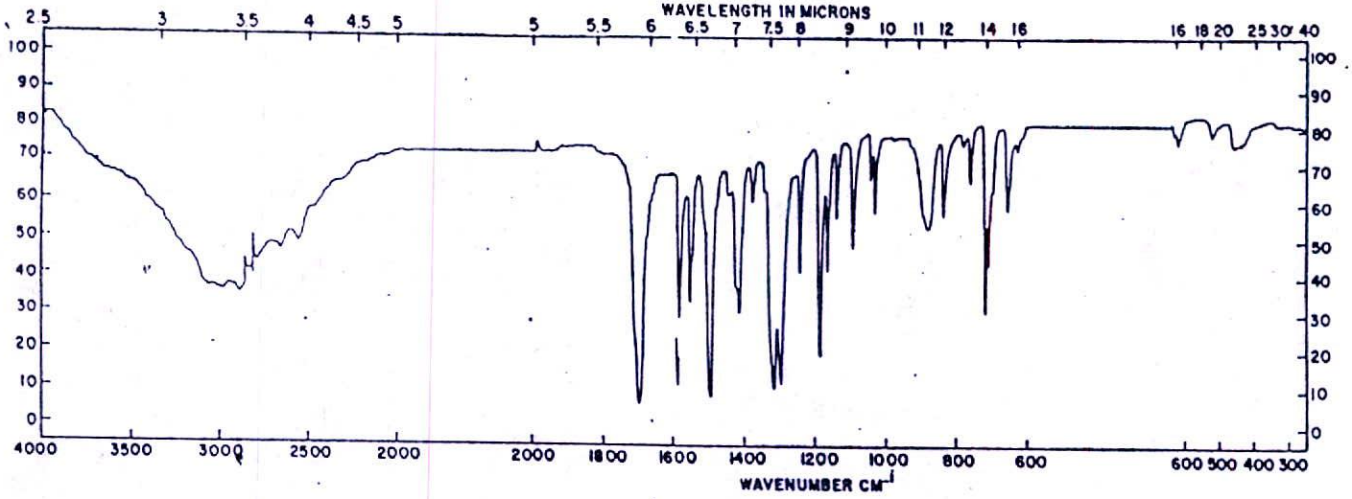


FIGURE 2.35: Infrared spectrum of compound (I) (Exercise 2.8) (KBr disk).

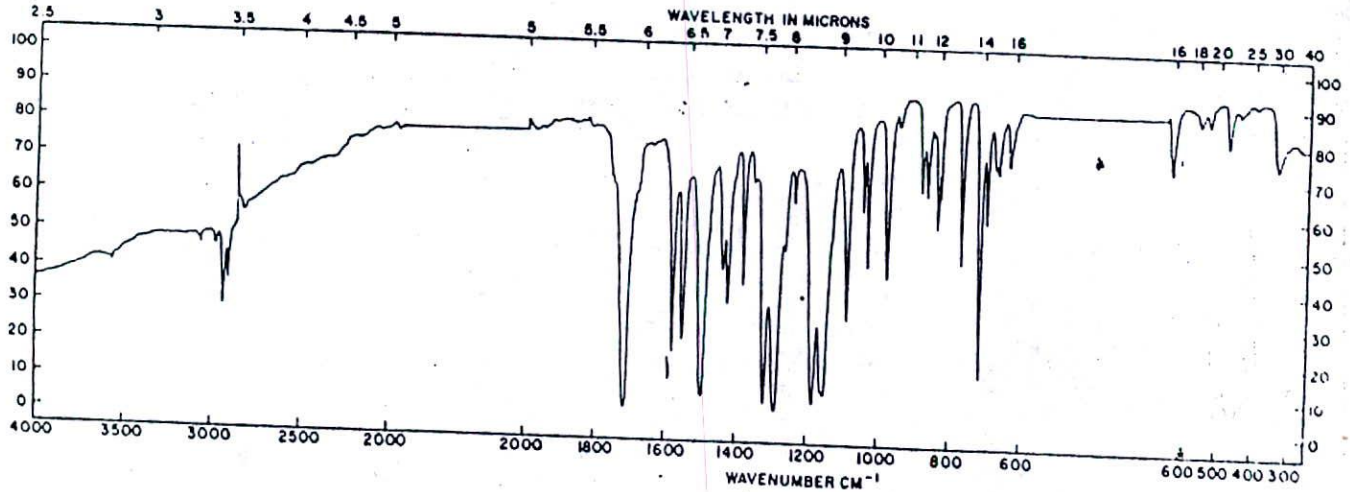


FIGURE 2.36: Infrared spectrum of compound (II) (Exercise 2.8) (KBr disk).

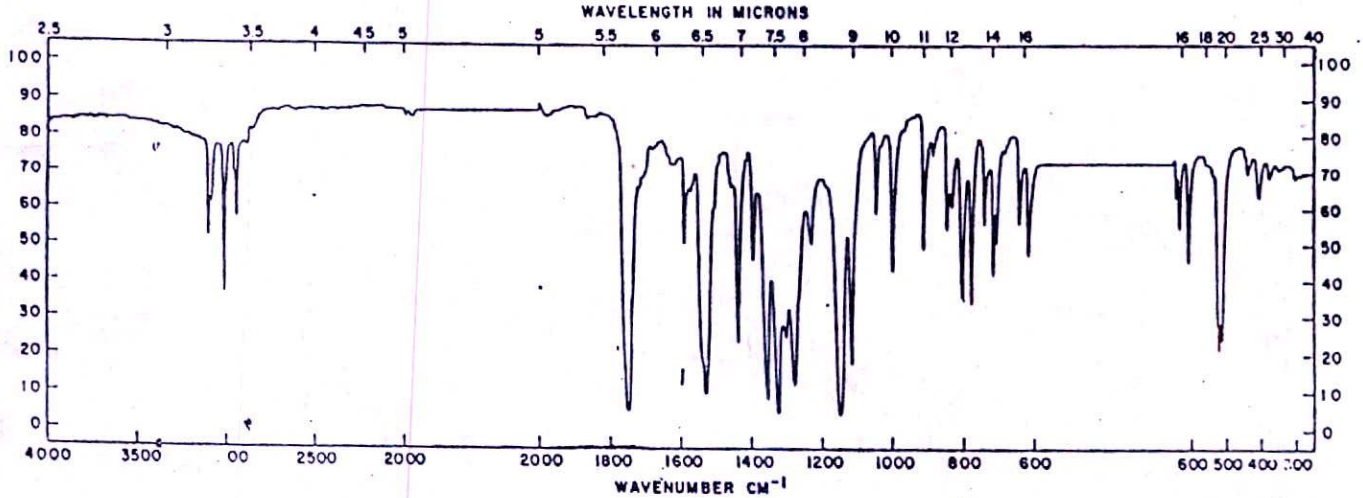


FIGURE 2.37: Infrared spectrum of compound (III) (Exercise 2.8) (KBr disk).

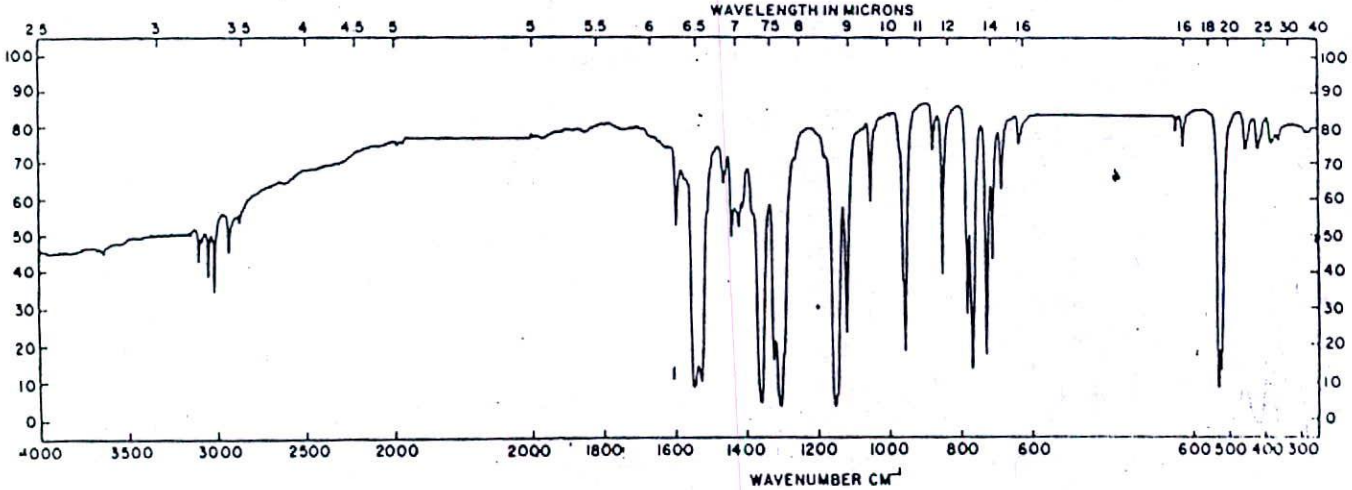


FIGURE 2.38: Infrared spectrum of compound (IV) (Exercise 2.8) (KBr disk).

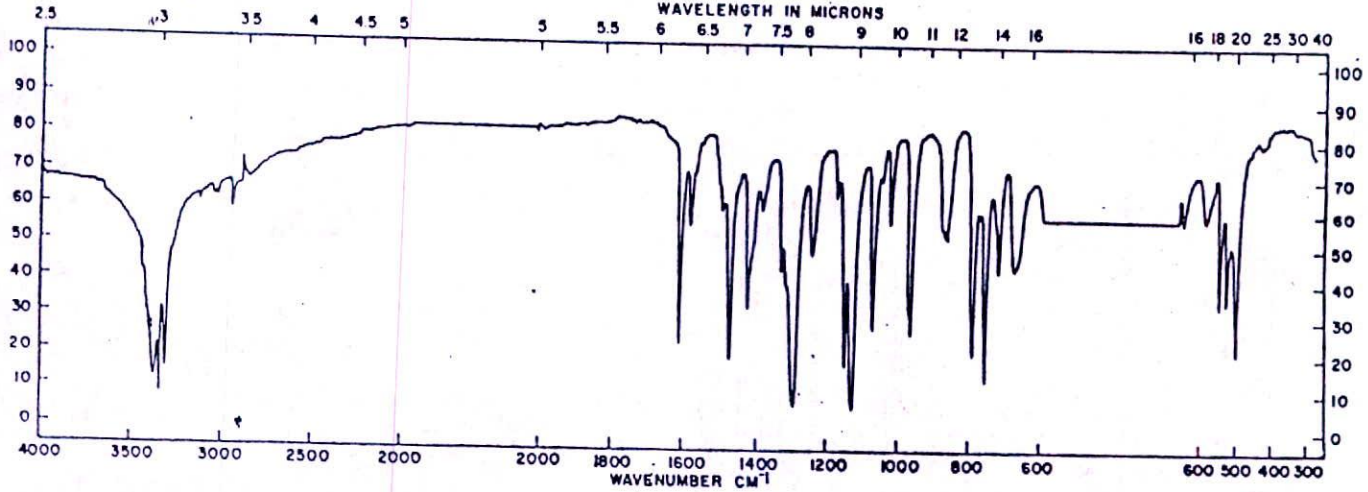


FIGURE 2.39: Infrared spectrum of compound (V) (Exercise 2.8) (KBr disk).

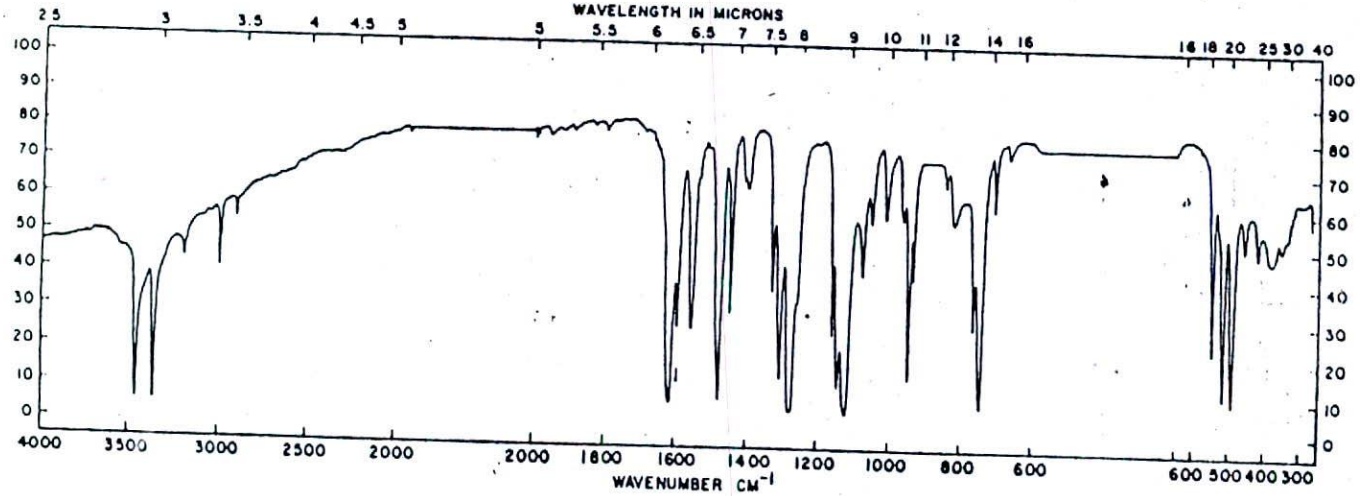
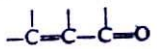


FIGURE 2.40: Infrared spectrum of compound (VI) (Exercise 2.8) (KBr disk).

A chloroform solution containing approximately 8 mg/ml of caffeine will then be supplied. Determine the exact concentration.

E2.10. Official Infrared Spectrophotometric Assay. A sample of one of the compounds or preparations listed in Table 2.3 will be supplied, as well as the appropriate reference standard. Carry out the assay process described in the USP.

E2.11. Assay of Ethinyl Testosterone Tablets using the Potassium Bromide Disk Technique. The absorption band at 6.03 μ, due to the



chromophore, was used.[22]

Chromatograph a weighed amount of ethinyl testosterone tablet mixture, equivalent to 25 mg of active ingredient on a celite (10 g)–water (5 ml) column. Wash the column with isooctane (50 ml) and by means of gentle air pressure, blow out the excess isooctane. Elute the ethinyl testosterone with chloroform (150 ml), then evaporate the eluate to dryness in vacuo at less than 50°. Dissolve the residue in absolute methanol and dilute to volume in a 25-ml volumetric flask.

Prepare a reference solution containing 1 mg pure ethinyl testosterone per milliliter.

Dry some spectroscopic grade potassium bromide at 105° for at least 16 hr and use the dried material to prepare sample and reference disks. Mix aliquots (0.10 ml) of the methanolic solutions of the sample and of the reference standard with 200 mg quantities of KBr by hand-grinding (10 min) or vibrator-grinding (6 min) procedures. Press the mixtures at an evacuation of < 1 mm Hg, and at a force of 20,000 lb for 1 min, then determine the average thickness of each disk, in millimeters, in the area struck by the infrared light. Clear, uniform disks are desirable, but cloudy disks can be used.

Determine the absorption of each disk at 6.03 μ using the base line technique.

Calculate the *absorptivity coefficient* K_s for the standard from the equation

$$K_s = \frac{A_s}{CL}$$

where A_s is the absorbance of standard, C is the concentration (% w/w), and L is the average thickness of the disk (millimeters).

Calculate the amount of active ingredient in the sample tablet using the following equation:

$$\text{Amount of ethinyl testosterone (milligrams) per tablet} = \frac{A_u}{K_s \times L_u} \times \text{wt. (mg) KBr mixture} \times \frac{\text{total vol.}}{\text{aliquot vol.}} \times \frac{\text{average wt. per tablet}}{\text{wt. of sample}}$$

where A_u is the absorbance of the sample (base line technique) and L_u is the average thickness of sample disk (millimeters).

E2.12. Quantitative Determination of Phenacetin by Infrared Spectrophotometry Using Benzoic Acid as Reference Standard. In an assay of this type, the absorptivity ratio (pure phenacetin vs. pure benzoic acid) must first be determined. This ratio is constant for a particular infrared spectrophotometer over a narrow concentration range, and once it has been determined, the assay of phenacetin no longer requires a phenacetin reference standard. Henceforth, pure benzoic acid can be used as a secondary reference standard.[28]

Determination of Absorptivity Ratio. Prepare a solution of pure benzoic acid by dissolving approximately 1 g, accurately weighed, in chloroform, in a 100-ml volumetric flask. Make up to volume with chloroform and mix. Record the infrared spectrum, using solution cells of path length 0.1 mm, with chloroform in the reference cell. Determine the absorbance of the band near 5.91 μ, and calculate the absorptivity a_b from the expression

$$a_b = \frac{A_b}{C_b \times L}$$

in which a is the absorptivity, A is the absorbance, C is the concentration (grams per liter), L is the absorption path length (centimeters), and subscript b indicates benzoic acid.

Prepare a similar solution of pure phenacetin, using approximately 1 g, accurately weighed, and chloroform to 100 ml, and determine its absorbance at the maximum near 6.61 μ. Calculate the absorptivity from the expression

$$a_p = \frac{A_p}{C_p \times L}$$

in which the symbols are the same as before and the subscript p indicates phenacetin.

Determine the absorptivity ratio, a_p/a_b, which is reduced to

$$a_p/a_b = \frac{A_p \times C_b}{C_p \times A_b}$$

if the benzoic acid and the phenacetin solutions are both examined in the same cell.

Assay of a Sample of Phenacetin, Using Benzoic Acid as Secondary Standard. Prepare a new chloroform solution of pure benzoic acid containing an accurately known quantity of benzoic acid (0.8–1.0% w/v). Prepare a chloroform solution of the phenacetin of unknown purity, containing an accurately known quantity of the drug (0.8–1.0% w/v).

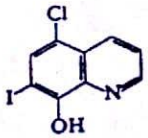
Determine the absorbance of the former solution at the maximum near 5.91 μ, and the absorbance of the latter solution at the maximum near 6.61 μ,

and calculate the concentration of $C_{10}H_{13}NO_2$ (pure phenacetin) in the latter solution using the following expression:

$$\text{conc. (g/liter)}C_{10}H_{13}NO_2 = \frac{A_p \times C_b}{A_b} \times \frac{1}{a_p/a_b}$$

From this result, determine the per cent $C_{10}H_{13}NO_2$ in the sample of phenacetin.

E2.13. Quantitative Determination of 5-Chloro-7-Iodo-8-hydroxyquinoline, and Qualitative Determination of Common Impurities (5,7-diiodo-8-hxdroxyquinoline; and 5,7-dichloro-8-hydroxyquinoline) in a Commercial Sample of Iodochlorhydroxyquin USP by Infrared Analysis.



C_9H_5ClINO 5-chloro-7-iodo-8-hydroxyquinoline

The USP method for the determination of iodochlorhydroxyquin is based on the compound's halogen content. Its disadvantage is that the method does not distinguish between the pure compound and its intermediates, which may be present as impurities in the synthesized product. The result is that samples which contain less than 60% of 5-chloro-7-iodo-8-hydroxyquinoline can pass the USP tests and could be considered as being of USP quality.[25] A more specific assay procedure is obviously necessary. The reference describes a suitable one, based on the fact that the intensity of the absorption band at 14.4 μ was proportional to the amount of 5-chloro-7-iodo-8-hydroxyquinoline in the sample. A plot of the ratio $\log_{10}(T_{14.9\mu}/T_{14.4\mu})$ vs. concentration was linear over the range investigated (2–10 mg iodochlorhydroxyquin/ml CS_2).

The spectrum of 5-chloro-7-iodo-8-hydroxyquinoline does not have absorption bands at 10.95 μ, 12.15 μ, or 13.45 μ. These bands, if present, are due to 5,7-diiodo-8-hydroxyquinoline (10.95 μ), 5-chloro-8-hydroxyquinoline (12.15 μ), and 5,7-dichloro-8-hydroxyquinoline (13.45 μ) and can be used for quantitative determinations of these impurities.

Method. Dissolve about 50 mg accurately weighed, of iodochlorhydroxyquin USP in carbon disulfide in a 10-ml volumetric flask, add solvent to volume, and mix. Record the complete spectrum vs. a carbon disulfide blank using 3-mm cells. Determine the ratio

$$\log \frac{\%T_{14.9\mu}}{\%T_{14.4\mu}}$$

This is the absorbance A_u of the sample.

In the same way, prepare a solution of pure 5-chloro-7-iodo-8-hydroxyquinoline (approximately 50 mg, accurately weighed) in carbon disulfide (10 ml) and record its spectrum, using 3-mm cells. Determine the ratio

$$\log \frac{\%T_{14.9\mu}}{\%T_{14.1\mu}}$$

This is the absorbance A_s of the reference standard.

Calculate the quantity in mg of C_9H_5ClINO in the sample of iodochlorhydroxyquin by means of the formula $10C\,(A_u/A_s)$, where C is the concentration in milligrams per milliliter of 5-chloro-7-iodo-8-hydroxyquinoline in the standard solution. From this, determine the per cent w/w C_9H_5ClINO in the sample of iodochlorhydroxyquin USP.

Examine the spectrum of iodochlorhydroxyquin and determine qualitatively which, if any, of the common impurities are present in the sample.

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