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# INTRODUCTION

The final result of drug analysis will be obtaining an infrared (IR) spectrum. Obtaining a spectrum may not be possible in every case but this is what the analyst should strive for. IR is used in the laboratory almost exclusively for qualitative analysis. However it is possible to use this method for quantitative analysis also. The value of this technique is the widespread application to drug analysis and the strength and validity it gives to conclusions rendered in casework.

The following articles and references will give the analyst background in the theory. Again, be sure to acquaint yourself with manuals for the instrument and any of its accessories.

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# Infrared Spectrometry

## I. INTRODUCTION

(1)

Infrared radiation refers broadly to that part of the electromagnetic spectrum between the visible and microwave regions. Of greatest practical use to the organic chemist is the limited portion between 4000 cm$^{-1}$ and 666 cm$^{-1}$ (2.5–15.0 $\mu$). Recently there has been increasing interest in the near infrared region, 16,668–4000 cm$^{-1}$ (0.6–2.5 $\mu$), and the far infrared region, 700–200 cm$^{-1}$ (14.3–50 $\mu$).

(2)

The study of infrared spectroscopy should include not only the practical aspects of qualitative and quantitative analysis but also its theoretical basis and consideration of such phenomena as inversion doubling, Fermi resonance, etc. This text attempts to present both the practical and theoretical aspects of infrared spectroscopy in such a way as to provide the inexperienced worker in the field with the necessary background to use it as a tool, either in an industrial laboratory concerned primarily with analysis or for research in any other type of laboratory.

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### 1.1. FREQUENCY, WAVELENGTH, AND ENERGY OF ELECTROMAGNETIC RADIATION

In order to describe various regions of the electromagnetic spectrum, it is first necessary to present the relations that exist between the wavelength, frequency, and energy of electromagnetic waves. The relationship between *frequency* and *wavelength* of electromagnetic radiation is given by

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

where

$\lambda$ = wavelength

$\nu$ = frequency

$c$ = velocity of light

The relationship between *frequency* and *energy* is given by

$$E = h\nu \qquad (1\text{-}2)$$

where

$E$ = energy

$h$ = Planck's constant

Let us consider specific examples of how these equations are used. Wavelength can be expressed in a variety of units, all of which can be referred to the basic unit, the *centimeter* (cm). The units most commonly employed in the ultraviolet region are the *angstrom* and the *millimicron*, while in the infrared region the *micron* is usually used. These units are defined as follows:

$$1 \text{ angstrom (Å)} = 10^{-8} \text{ cm}$$

$$1 \text{ micron } (\mu) = 10^{-4} \text{ cm}$$

$$1 \text{ millimicron } (m\mu) = 10^{-7} \text{ cm}$$

Let us consider an example of how frequency is calculated from the wavelength of radiation.

*Example 1*

Calculate the frequency associated with a wavelength of $3\,\mu$. The velocity of light $c$ is given as

$$c = 3 \times 10^{10} \text{ cm/sec}$$

Since $\lambda\nu = c$,

$$\nu = \frac{c}{\lambda} = \frac{3 \times 10^{10} \text{ cm/sec}}{3 \times 10^{-4} \text{ cm}} = 1 \times 10^{14} \text{ sec}^{-1}$$

An alternate way of describing the wavelength of radiation is in terms of its *wavenumber*, which is defined by the following equation:

$$\text{Wavenumber } [\text{cm}^{-1}] = \frac{1}{\text{Wavelength [cm]}} \qquad (1\text{-}3)$$

The units of wavenumber are reciprocal centimeters. The following calculation will illustrate the conversion of wavelength in centimeters to the wavenumber unit. We shall employ the symbol $\bar{\nu}$ to designate wavenumber for the present, although in the current literature the symbol $\nu$ is customarily used for frequency in $\text{sec}^{-1}$ as well as wavenumber in $\text{cm}^{-1}$.

*Example 2*

Calculate the wavenumber equivalent to a wavelength of $3\,\mu$.

$$\bar{\nu} = \frac{1}{\lambda}$$

$$= \frac{1}{3 \times 10^{-4} \text{ cm}}$$

$$= 3333.33 \text{ cm}^{-1}$$

To calculate the energy associated with a given frequency or wavelength, use is made of equation (1-2).

*Example 3*

Calculate the energy associated with the frequency calculated in Example 1. The value of Planck's constant is $6.67 \times 10^{-27}$ erg-sec.

$$E = h\nu$$

$$= 6.67 \times 10^{-27} \text{ erg-sec} \times 10^{14} \text{ sec}^{-1}$$

$$= 6.67 \times 10^{-13} \text{ erg}$$

## 1.2. SPECTRAL REGIONS OF ELECTROMAGNETIC RADIATION

While we shall be primarily concerned with the infrared region of the electromagnetic spectrum in this text, let us first consider the electromagnetic spectrum in general. Figure 1-1 is a schematic

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representation of the regions of the electromagnetic spectrum that are of interest in analysis. These may be divided into the far-infrared, infrared, near-infrared, visible, ultraviolet, and X-ray regions, using wavelength as the criterion defining the boundaries between them. This classification is somewhat arbitrary, and the student should keep in mind that in practice the boundaries are by no means well defined and application of various analytical techniques may overlap somewhat from far-infrared spectroscopy at the long-wavelength end of the spectrum to X-ray spectroscopy at the short-wavelength end.

In the short-wavelength region waves have high energy, and when a molecule absorbs radiation of these short wavelengths the energy changes occurring in it are large. X-ray spectroscopy detects the electronic transitions of inner-shell electrons that occur when this high energy is absorbed. In the ultraviolet region electronic transitions of outer-shell electrons occur, and spectra measured in this region are often referred to as *electronic spectra*.

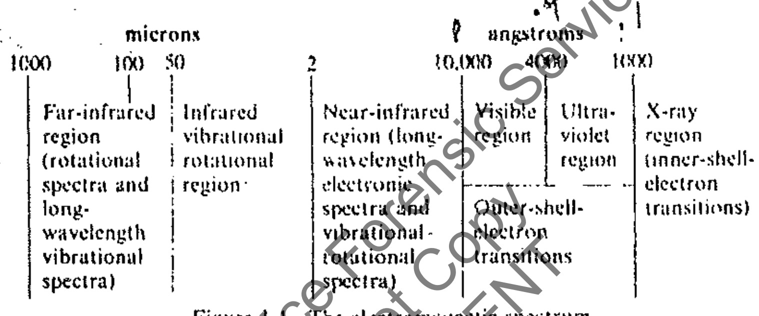

Figure 1-1. The electromagnetic spectrum.

Energy transitions in the visible and near-infrared spectral regions can be either electronic or *vibrational–rotational* in nature. For most materials, the visible spectra are representative of electronic transitions while the near-infrared spectra are representative of vibrational–rotational energy changes.

The infrared region, which can somewhat arbitrarily be defined as the region from 2 to 50 $\mu$, is representative of transitions in vibrational and rotational energy. It is this region which will concern us in this text.

The far-infrared region (at long wavelengths) is characterized by rotational energy transitions, although some vibrational transitions of low frequency may also occur in this region.

In a spectroscopy laboratory one may find instruments designed to operate in the various regions of the spectrum and, accordingly, called ultraviolet, visible, near-infrared, infrared, and far-infrared spectrophotometers. While in some respects the data that may be obtained with each of these instruments are related to data obtainable with others, our primary concern here will be with infrared spectra as measured on infrared spectrophotometers.

(1)

From the brief theoretical discussion which follows, it is clear that even a very simple molecule can give an extremely complex spectrum. The organic chemist takes advantage of this complexity when he matches the spectrum of an unknown compound against that of an authentic sample. A peak-by-peak correlation is excellent evidence for identity. It is unlikely that any two compounds, except optical enantiomorphs, give the same infrared spectrum.

Although the infrared spectrum is characteristic of the entire molecule, it turns out that certain groups of

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atoms give rise to bands at or near the same frequency regardless of the structure of the rest of the molecule. It is the persistence of these characteristic bands that permits the chemist to obtain useful structural information by simple inspection and reference to generalized charts of characteristic group frequencies.

## II. THEORY

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

Infrared radiation in the range from about 10,000–100 cm$^{-1}$ (1–100 $\mu$) is absorbed and converted by an organic molecule into energy of molecular vibration. This absorption is also quantized, but vibrational spectra appear as bands rather than lines because a single vibrational energy change is accompanied by a number of rotational energy changes. It is with these vibrational-rotational bands, particularly those occurring between 4000 cm$^{-1}$ and 666 cm$^{-1}$ (2.5–15 $\mu$), that we shall be concerned. The frequency or wavelength of absorption depends on the relative masses of the atoms, the force constants of the bonds, and the geometry of the atoms.

Band positions in infrared spectra are presented either as wavelengths or frequencies. The common unit of wavelength in infrared spectrometry is the micron ($\mu$), equal to $10^{-3}$ mm. Frequencies are usually expressed in terms of wavenumbers ($\bar{\nu}$) whose unit is the reciprocal centimeter (cm$^{-1}$). In terms of this unit, the wavenumber is the reciprocal of the wavelength in centimeters. Or, when the wavelength is in microns, the wavenumber is $\frac{1}{\mu} \times 10^4$. The energy of radiation is directly proportional to the frequency.

Band intensities are expressed either as transmittance ($T$) or absorbance ($A$). Transmittance is the ratio of the radiant power transmitted by a sample to the radiant power incident on the sample. Absorbance is the logarithm, to the base 10, of the reciprocal of the transmittance, $A = \log_{10} (1/T)$. A concise compilation of approved spectrometry nomenclature has recently been published.[39]

There are two types of molecular vibrations: stretching and bending. A stretching vibration is a rhythmical movement along the bond axis such that the interatomic distance is increasing or decreasing. A bending vibration may consist of a change in bond angles between bonds with a common atom, or the movement of a group of atoms with respect to the remainder of the molecule without movement of the atoms in the group with respect to one another. For example, twisting, rocking, and torsional vibrations involve a change in bond angles with reference to a set of coordinates arbitrarily set up within the molecule.

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

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

The three fundamental vibrations of the nonlinear, triatomic water molecule can be depicted as shown at top of page 5

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

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# BASIC OPTICS

CLAUDE W. COOK

## INTRODUCTION

As the title implies, this article is designed to present some very basic information concerning the field of optics. Information given here will serve as a foundation of sorts, should the reader plan to delve more deeply into any of the various aspects of this field. By the same token, sufficient information is given that the reader will be enabled to explain some of the whys-and-wherefores, either in response to questions posed in court, by members of the lay public, or in the course of giving instruction on this subject.

The article will be divided into five parts. This will permit a separation of the various facets of the topic under discussion, and also provide a point for separation -- should a space problem exist which would prohibit complete publication in one issue.

The field of OPTICS deals with vision and/or the various lenses, mirrors, and prisms which comprise an optical system. Light and lighting techniques (to include some of the properties of light) must also be included in a general coverage of the topic, for without light there would be no vision and, hence, no need for us to even consider the subject.

That the field of optics is an extremely complicated one, goes without saying; therefore, the coverage given here will be quite basic and abbreviated (after all, this is an article -- not a text or treatise on the subject). We shall endeavor to give coverage to this complex and complicated field in as uncomplicated a manner as possible, holding the use of mathematics and mathematical formulas to a bare minimum. Hopefully, however, our coverage will be such that those areas of the subject that affect us in our day-to-day dealings with optical equipment will at least be touched upon.

Electrical, electronic, and mechanical matters used to control stage or instrument movement will not be considered here, nor will automatic tracking systems used in the study of astronomy. We should be aware of these matters, along with rack-and-pinion and worm-gear movement, but let's not let this become more complicated than it already is.

Should the reader wish to delve more deeply into the subject, or some particular phase or facet of it, ones local library might be a good place to start. Quite a number of good books are available which deal with lenses, microscopes and microscope techniques, telescopes,

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photography (both from the standpoint of the camera and camera techniques and darkroom photography), and other areas you may wish to explore. Should some specific text not be available at your local library, they may be able to obtain a copy on loan from another library having a more complete reference section covering this area. Texts, such as I am alluding to, tend to be rather expensive, and one should refrain from purchasing a given volume until assured that it, in fact, contains the specific material in which he may be interested. You might with to buy the book at some later date, but check it out at the library first -- it might prevent a needless expenditure of hard-earned-cash.

## BASIC OPTICS - PART I (VISION AND THE HUMAN EYE)

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Have you ever known a dealer in hearing aids who spoke in a voice more than two decibels above a whisper, or have you wondered why the print on an optometrist's business card appeared to have been designed by the same person whose hobby it is to engrave the Lord's Prayer on the heads of pins? It may be a perfectly innocent coincidence, but one could get the impression that it is all a sales tactic designed to convince prospective clients that they are in dire need of either a hearing aid or eyeglasses (depending on which specialist you see).

It might also have occurred to you (as it has to me), that modern society has somehow been hoodwinked into siding with the faction who originated this conspiracy. While young children are normally blessed with the eyesight of young eagles, the school texts and storybooks provided them have print with letters and figures about half an inch in height, whereas, the printed matter routinely received by our older citizens appears to have been typeset by the same person who designs those optometrists' business cards. If one were somewhat cynically inclined, he might easily get the impression that in the near future our older citizens might be expected to be able to read microfiche with the unaided eye -- in fact, with medical science making constant progress in increasing the human lifespan, the microscope may soon be considered an unnecessary hindrance for aging laboratory personnel.

While the above seems a bit far afield from the topic of sight and the human eye, it was just my way of getting myself in the mood to write on the subject. Optics (according to dictionary definition), has to do with the eye, vision, and/or the various lenses, prisms, or mirrors which comprise an optical system -- it also deals with the properties of light and the proper illumination of objects or specimens, for without light there would be no vision and, hence, no need for the rest of the optical paraphernalia just alluded to.

While I have a natural tendency to put the cart before the horse, I shall endeavor (insofar as it is within my capability to do so) to keep this writing in some semblance of logical order. We shall, therefore, proceed with a basic coverage of human vision before becoming involved with matters pertaining to optical systems and their components.

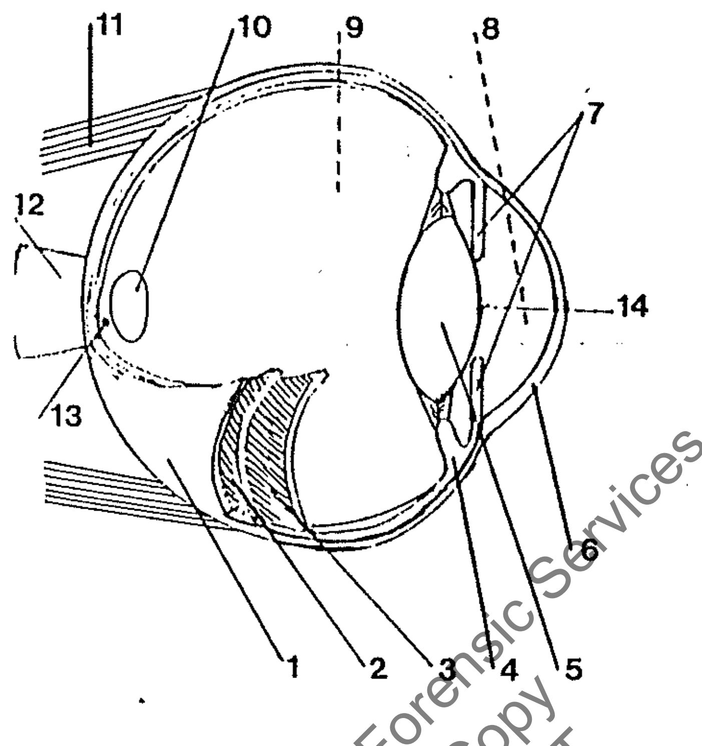


FIGURE 1-1

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This is a cut-away drawing of the human eye, with the various parts being numbered for identification.

1. Sclera
2. Choroid
3. Retina
4. Ciliary body
5. Lens
6. Cornea
7. Iris
8. The anterior chamber, which is filled with the aqueous humor. *
9. Posterior segment of the eye, which contains the vitreous humor.
10. "Blind spot" where the optic nerve exits the eye.
11. Ocular muscles
12. Optic nerve
13. Fovea
14. Pupil, the opening in the center of the iris.

The anterior and posterior chambers more correctly refer to those chambers of the eye located in the anterior segment. The anterior segment is that segment located forward of the lens, the anterior chamber being separated from the posterior by the lens and iris, however, both are filled with the aqueous humor.

The posterior segment of the eye (shown at 9) is comprised of all of the interior of the eye from the lens on back. It is this segment that is filled with the vitreous humor.

## LIGHT TRANSFORMED TO VISION

The eye is roughly spherical in shape, having an opening located at the front (the pupil), through which light may enter. Were you to look through this opening -- which the Opthalmologist does by use of an opthalmoscope, an instrument consisting of a centrally perforated mirror with a light source, designed for viewing the interior of the eye -- especially, the retina -- you would be able to visually examine the retina. On the retina are located all of the light reception cells which make sight possible. (This would be roughly analogous to looking through the lens of a camera and seeing the film at the rear. Actually, since the eye is spherical in shape, it might be more like being able to examine the inner surface of a ball by looking through a small hole perforating the outer shell.)

### The Rods and Cones

The rods and cones are the light receptors of the eye, and are designated by these names because of their appearance (as seen under a high degree of magnification). The cones are concentrated in a very small area of the retina, called the fovea (estimates are that there are a bit less than 10 million cones on the retina of each eye, with perhaps 10 times this number of rods).

With the normal eye (one which is focusing properly), the light rays from outside the eye are in sharpest focus on the fovea. The area which immediately surrounds the fovea is called the macula lutea (a Latin terms, meaning "yellow spot", because of its coloration). The circular area, which comprises the combination of the fovea and macula lutea, is not much greater in diameter than the head of a pin.

The cones of the fovea and macula lutea make possible all of our ability to see colors and fine detail. Beyond the limits of the macula lutea (outside the yellowish area), fewer and fewer cones exist, with rods becoming the predominant receptors. While the areas having highly concentrated numbers of cones enable us to observe color and see fine detail (to include reading), the cones are practically useless in seeing objects even a bit out of our direct line of sight (things seen from the corner of the eye). Additionally, the cones function only in good lighting conditions or in response to relatively bright light sources.

The rods work to take up the slack, taking over completely in reduced light, and they also give us our peripheral vision. The rods, however, are not color sensitive, hence, our night vision is almost completely limited to black-and-white. On entering a dimly lit area from bright sunlight, the cones become non-functional. You may note a short period of time when, in fact, you may not be able to see anything at all.

### Visual Purple

In the brief interval between the time that the cones stop functioning and when the rods take over, a pigment called visual purple becomes important. Visual purple is being constantly produced by the rods, and must be present if the rods are to respond to dim light, however, it

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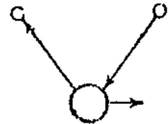
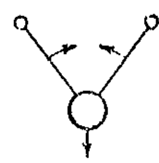
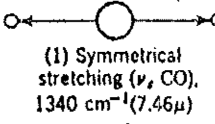
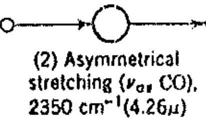
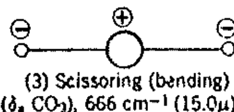


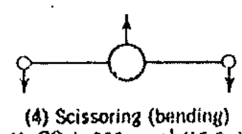




The $CO_2$ molecule is linear and contains three atoms; therefore it has four fundamental vibrations ((3 × 3) − 5).




⊕ and ⊖ indicate movement perpendicular to the plane of the page.

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

The various stretching and bending modes for an $AX_2$ group appearing as a portion of a molecule, e.g., the $CH_2$ group in a hydrocarbon molecule, are shown in Figure 1. The $3n-6$ rule does not apply since the $CH_2$ represents only a portion of a molecule.

The theoretical number of fundamental vibrations (absorption frequencies) will seldom be observed because overtones and combination tones increase the number of bands, whereas other phenomena reduce the number of bands. The following will reduce the theoretical number of bands.

1. Fundamental frequencies that fall outside of the 2.5–15 μ region.
2. Fundamental bands that are too weak to be observed.
3. Fundamental vibrations that are so close that they coalesce.
4. The occurrence of a degenerate band from several absorptions of the same frequency in highly symmetrical molecules.
5. The failure of certain fundamental vibrations to appear in the infrared because of lack of required change in dipole character of the molecule.

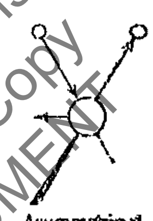
Assignments for stretching frequencies can be approximated by the application of Hooke's law. In the application of the law, two atoms and their connecting bond are treated as a simple harmonic oscillator composed of two masses joined by a spring. Equation 1, derived from Hooke's law, states the relationship between frequency of oscillation, atomic masses, and the force constant of the bond.

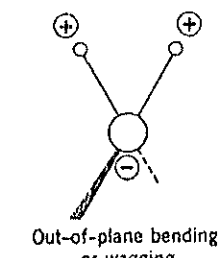
$$\nu = \frac{1}{2\pi c}\left(\frac{f}{\dfrac{MxMy}{Mx + My}}\right)^{1/2}$$

where $\nu$ = the vibrational frequency (cm$^{-1}$)
$c$ = velocity of light (cm/sec)
$f$ = force constant of bond (dynes/cm)
$Mx$ and $My$ = mass of atom $x$ and atom $y$, respectively (g).

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

Application of the formula to C—H stretching, using



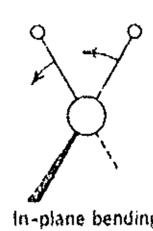




Fig. 1. Vibrational modes for a $CH_2$ group. (+ and − indicate movement perpendicular to the plane of the page.)

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$19.8 \times 10^{-24}$ g and $1.64 \times 10^{-24}$ g as mass values for C and H, respectively, places the frequency of the C—H bond vibration at 3040 cm$^{-1}$ (3.30 $\mu$). Actually, C—H stretching vibrations, associated with methyl and methylene groups, are generally observed in the region between 2960–2850 cm$^{-1}$ (3.38–3.51 $\mu$). The calculation is not precise because effects arising from the environment of the C—H within a molecule have been ignored. The frequency of infrared absorption is commonly used to calculate the force constants of bonds.

The shift in absorption frequency following deuteration is often employed in the assignment of C—H stretching frequencies. If the hydrogen in an X—H group is replaced by deuterium, equation 1 indicates that the ratio of the C—H to C—D stretching frequencies should equal $\sqrt{2}$. If the ratio of the frequencies, following deuteration, is much less than $\sqrt{2}$ we can not assume that the vibration is simply a C—H stretching vibration, but rather a mixed vibration involving interaction or coupling with another vibration. The actual mode of vibration is a combination of both.

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

| | |
|---|---|
| C—C, C—O, C—N | 1300–800 cm$^{-1}$ (7.7–12.5 $\mu$) |
| C═C, C═O, C═N, N═O | 1900–1500 cm$^{-1}$ (5.3–6.7 $\mu$) |
| C≡C, C≡N | 2300–2000 cm$^{-1}$ (4.4–5.0 $\mu$) |

To approximate the vibrational frequencies of bond stretching by Hooke's law, the relative contributions of bond strengths and atomic masses must be considered. For example, a superficial comparison of the C—H group with the F—H group, on the basis of atomic masses, might lead to the conclusion that the stretching frequency of the F—H bond should occur at a lower frequency than that for the C—H bond. However, the increase in the force constant from left to right across the first two rows of the periodic table has a greater effect than the mass increase. Thus the F—H group absorbs at a higher frequency (4138 cm$^{-1}$, 2.42 $\mu$) than the C—H group (3040 cm$^{-1}$, 3.30 $\mu$).

INTERACTION. When two bond oscillators share a common atom they seldom behave as individual oscillators unless the individual oscillation frequencies are widely different. This is because there is mechanical coupling or interaction between the oscillators. For example, the carbon dioxide molecule, which consists of two C═O bonds with a common carbon atom, has two fundamental stretching vibrations: an asymmetrical and a symmetrical stretching mode. The symmetrical stretching mode consists of an in-phase stretching or contracting of the C to O bonds, and absorption occurs at a wavelength longer than that observed for the carbonyl group in an aliphatic ketone. The symmetrical stretching mode produces no change in the dipole moment of the molecule and is therefore "inactive" in the infrared, but is easily observed in the Raman spectrum near 1340 cm$^{-1}$ (7.46 $\mu$). In the asymmetrical stretching mode, the two C to O bonds stretch out of phase; one C—O bond stretches as the other contracts. The asymmetrical stretching mode, since it produces a change in the dipole moment, is infrared active; the absorption (2350 cm$^{-1}$, 4.26 $\mu$) is at a shorter wavelength (higher frequency) than observed for a carbonyl group in aliphatic ketones.

This difference in carbonyl absorption frequencies, displayed by the carbon dioxide molecule results from strong mechanical coupling or interaction. In contrast, two ketonic carbonyl groups separated by one or more carbon atoms, show normal carbonyl absorption near 1715 cm$^{-1}$ (5.83 $\mu$) because appreciable coupling is prevented by the intervening carbon atom or atoms.

Coupling accounts for the two N—H stretching bands in the 3497–3077 cm$^{-1}$ (2.86–3.25 $\mu$) region in primary amine and primary amide spectra, for the two C═O stretching bands in the 1818–1720 cm$^{-1}$ (5.50–5.81 $\mu$) region in carboxylic anhydrides and imide spectra, and for the two C—H stretching bands in the 3000–2760 cm$^{-1}$ (3.33–3.62 $\mu$) region for both methylene and methyl groups.

Useful characteristic group frequency bands often involve coupled vibrations. The spectra of alcohols have a strong band in the region between 1212 and 1000 cm$^{-1}$ (8.25–10.00 $\mu$) which is usually designated as the "C—O stretching band." In the spectrum of methanol this band is at 1034 cm$^{-1}$ (9.67 $\mu$); in the spectrum of ethanol it occurs at 1053 cm$^{-1}$ (9.50 $\mu$). Branching and unsaturation produce absorption characteristic of these structures[40] (see discussion of alcohols). It is evident that we are not dealing with an isolated C—O stretching vibration, but rather a coupled asymmetric vibration involving C—C—O stretching.

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

Interaction arising from coupling of stretching and bending vibrations is illustrated by the absorption of secondary acyclic amides. Secondary acyclic amides, which exist predominantly in the *trans* configuration, show strong absorption in the 1563–1515 cm$^{-1}$ (6.40–6.60 $\mu$) region; this absorption involves coupling of the N—H bending and C—N stretching vibrations.

The requirements for effective interaction may be summarized as follows:

1. The vibrations must be of the same symmetry species if interaction is to occur.

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2. Strong coupling between stretching vibrations requires a common atom between the groups.
3. Interaction is greatest when the coupled groups absorb, individually, near the same frequency.
4. Coupling between bending and stretching vibrations can occur if the stretching bond forms one side of the changing angle.
5. A common bond is required for coupling of bending vibrations.
6. Little or no interaction occurs between groups separated by two or more bonds.

HYDROGEN BONDING. Hydrogen bonding can occur in any system containing a proton donor group (X—H) and a proton acceptor Y if the s orbital of the proton can effectively overlap the $p$ or $\pi$ orbital of the acceptor group. Atoms X and Y are electronegative with Y possessing lone pair electrons. The common proton donor groups in organic molecules are carboxyl, hydroxyl, amine, or amide groups. Common proton acceptor atoms are oxygen, nitrogen, and the halogens. Unsaturated groups, such as the ethylenic linkage, can also act as proton acceptors.

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

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

*Inter*molecular hydrogen bonding involves association of two or more molecules of the same or different compounds. Intermolecular bonding may result in dimer molecules (as observed for carboxylic acids) or in polymer molecules, which exist in neat samples or concentrated solutions of monohydric alcohols. *Intra*molecular hydrogen bonds are formed when the proton donor and acceptor are present in a single molecule under spacial conditions that allow the required overlap of orbitals, for example, the formation of a 5- or 6-membered ring. The extent of both inter- and intramolecular bonding is temperature dependent. The effect of concentration on intermolecular and intramolecular hydrogen bonding is markedly different. The bands that result from intermolecular bonding generally disappear at low concentrations (less than about 0.01 *M* in nonpolar solvents). Intramolecular hydrogen bonding is an internal effect and persists at very low concentrations.

The change in frequency between "free" OH absorption and bonded OH absorption is a measure of the strength of the hydrogen bond. Ring strain, molecular geometry, and the relative acidity and basicity of the proton donor and acceptor groups affect the strength of bonding. Intramolecular bonding involving the same bonding groups is stronger when a 6-membered ring is formed than when a smaller ring results from bonding. Hydrogen bonding is strongest when the bonded structure is stabilized by resonance.

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

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

FERMI RESONANCE. As we have seen in our discussion of interaction, coupling of two fundamental vibrational modes will produce two new modes of vibration, with frequencies higher and lower than that observed when interaction is absent. Interaction can also occur between fundamental vibrations and overtones or combination-tone vibrations. Such interaction is known as Fermi resonance. One example of Fermi resonance is afforded by the absorption pattern of carbon dioxide. In our discussion of interaction, we indicated that the symmetrical stretching band of $CO_2$ appears in the Raman spectrum near 1340 $cm^{-1}$ (7.46 $\mu$). Actually two bands are observed: one at 1286 $cm^{-1}$ (7.78 $\mu$), one at 1388 $cm^{-1}$ (7.20 $\mu$). The splitting results from coupling between the fundamental C=O stretching vibration, near 1340 $cm^{-1}$ (7.46 $\mu$), and the first overtone of the bending vibration. The fundamental bending vibration occurs near 666 $cm^{-1}$ (15.00 $\mu$), the first overtone near 1334 $cm^{-1}$ (7.55 $\mu$).

Fermi resonance is a common phenomenon in infrared and Raman spectra. It requires that the vibrational levels be of the same symmetry species and that the interacting groups be located in the molecule so that mechanical coupling is appreciable.

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## THE INFRARED SPECTRUM OF A COMPOUND

Generally, infrared spectra are recorded on chart paper and presented in graphic form. A number of different ways of presenting a spectrum are possible; however, they are sufficiently similar in their essential characteristics to permit us to select one example--a spectrum recorded on a commercial spectrophotometer using the standard chart paper supplied by the manufacturer, shown in Figure 1-2—for detailed examination.

Along the abscissa of spectral chart paper is plotted the wavelength, or some related function such as wavenumber. In the spectrum shown in Figure 1-2 both wavelength in microns and wavenumber in $cm^{-1}$ are shown. The ordinate of the spectrum is some function of the amount of radiation absorbed (or transmitted) by the sample at each wavelength. A spectrum is thus a graphical presentation of the amount of radiation absorbed as a function of wavelength. Commercial spectrophotometers generally scan the pertinent wavelength region and make a tracing of the radiation transmitted. A region in the spectrum where radiation is absorbed is called an absorption band. In Figure 1-2 absorption bands are seen as valleys in the spectral trace. For example, a very deep valley may be seen at 13 $\mu$, which indicates a strong absorption band at that point. By choosing another method of presenting the ordinate of the chart paper it is possible to have absorption bands appear as peaks rather than as valleys, and spectra presented in this manner will quite often be found in the literature.

It will be seen that in Figure 1-2 the abscissa is plotted linear with respect to wavelength. It would also be possible to plot it linear with respect to wavenumber. Spectra of both types can be found. While spectra with linear wavelength plots are still quite common, with the advent of grating instruments many workers have begun to feel that plotting wavenumbers linearly is the better method of presentation. Commercial spectrophotometers can be adjusted to record spectra either way. A spectrum recorded linear with respect to wavelength appears to have different band widths when compared to the same spectrum recorded linear with respect to wavenumber when the same length of chart paper is used in each case. This is so because at short wavelengths the wavenumber intervals are closer together than at long wavelengths. For example, the wavelength interval from 2 $\mu$ to 5 $\mu$ corresponds to the wavenumber interval 5000 $cm^{-1}$ to 2000 $cm^{-1}$ while the interval from 5 $\mu$ to 10 $\mu$ corresponds to the wavenumber interval from 2000 $cm^{-1}$ to 1000 $cm^{-1}$. The net effect of this inverse relationship between wavelength and wavenumber is to give a crowding of the absorption bands at low wavelengths if the abscissa is a linear wavelength function. Since adjustable scan speeds and variable chart paper speeds are available on many spectrophotometers, this crowding of absorption bands can usually be eliminated. In early instruments, which used NaCl prisms as monochromators, since the resolution of NaCl is low at the short wavelengths (2-6 $\mu$), the spectral charts were presented linear with respect to wavelength to avoid crowding of absorption bands in the more useful region where resolution was good.

The variable plotted along the ordinate of the infrared spectrogram is a function of the amount of radiation of given wavelength absorbed by the sample. The amount of radiation of one wavelength incident on the sample ($P_0$) can be related to the amount transmitted

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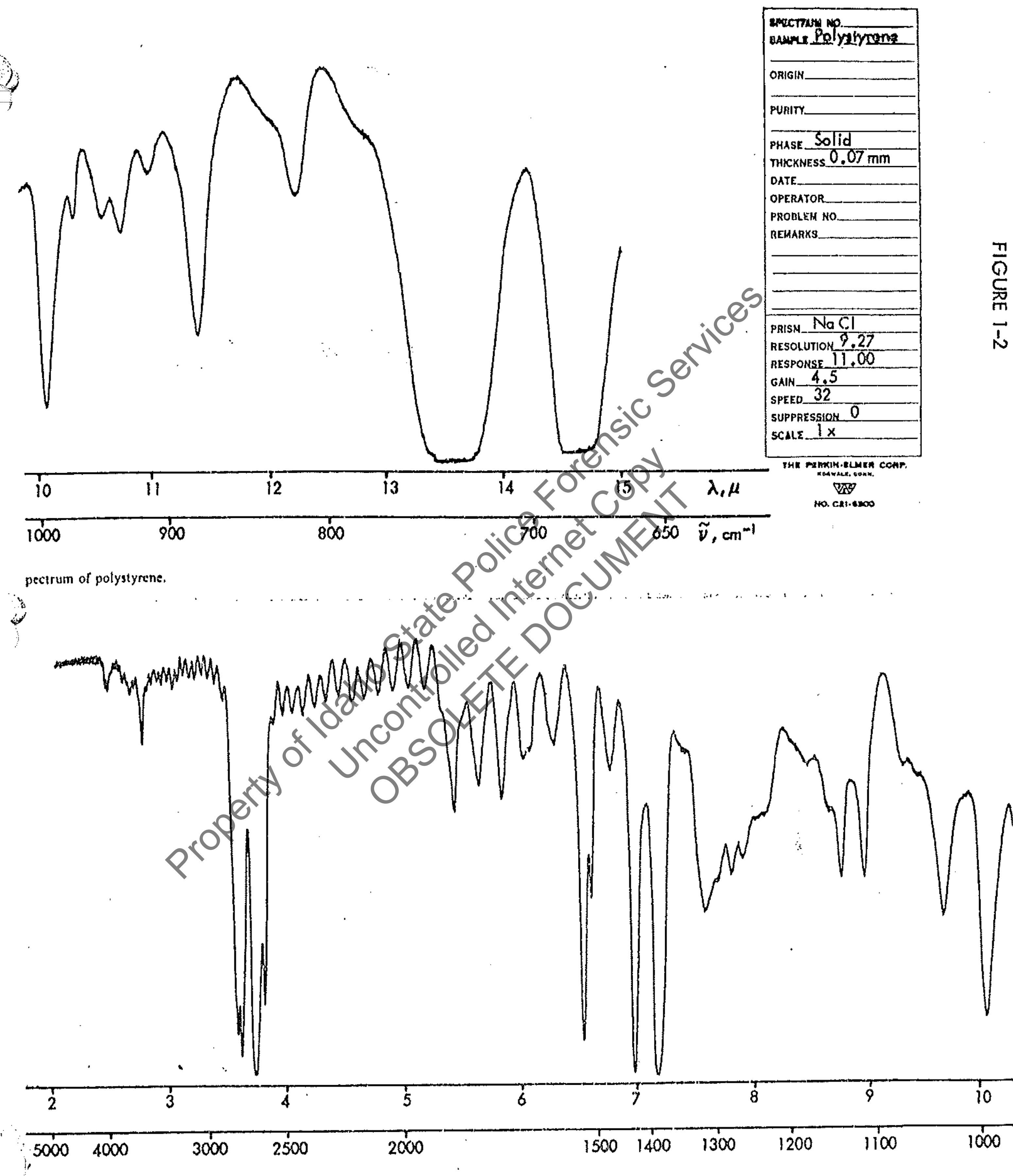

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pectrum of polystyrene.

Figure 1-2. Infrared spectrum

by this sample ($P$), to the path length in the sample ($b$), and to the number of particles or the concentration of the sample ($c$) by the equation

$$\ln \frac{P_0}{P} = abc$$

where $a$ is a constant for any given material at a given wavelength. This equation is called *Beer's law* (also the Beer-Lambert or Beer-Bouguer law), and the relationship that it presents between the amount of radiation absorbed and the concentration is used in quantitative analysis. Chapter 6 will discuss this law in greater detail. For the present discussion of the infrared spectrogram it is necessary to define two terms which may appear in different formulations of Beer's law. One, the *transmittance T*, is given by

$$T = \frac{P}{P_0}$$

The second, the *absorbance A*, is given by

$$A = \log \frac{1}{T} = \log \frac{P_0}{P}$$

Either of these terms can be used as the ordinate in an infrared spectrum since both are related to the amount of light absorbed by the sample. Thus, an infrared spectrum can be a plot of absorbance or transmittance *versus* wavelength or wavenumber, in any desired combination. In the United States, the ordinate scale is usually so arranged that, regardless whether transmittance or absorbance is used as the ordinate, absorption peaks appear as valleys in the trace. However, tracings with the scale arranged differently, and which, of course, will appear upside down compared to the customary plots, are still found in the literature, and the student should become as familiar with them as with the others.

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# Instruments

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The schematic optical diagram in Figure 2-1 applies to one particular double-beam infrared spectrophotometer. However, it demonstrates the salient features of this kind of instrument, which is the type found in most analytical laboratories. Details and information relative to other types of instrument systems will be presented in Section 2.3.

The energy radiated by the source *SO* is split into sample and reference beams by the plane mirror *M1*. Mirror *M2* focuses the sample beam on a comb-shaped device used to adjust the 100% level, and *M3* focuses the reference beam on the optical wedge, whose function is explained below.

The sample and reference beams are recombined at the rotating sector mirror *C* into a single beam consisting of alternate pulses of reference and sample beam radiation. This section of the instrument is called the photometer; here the pulsating radiation may be used to indicate the difference in energy between the sample and reference beams. In addition, the photometer contains the means of

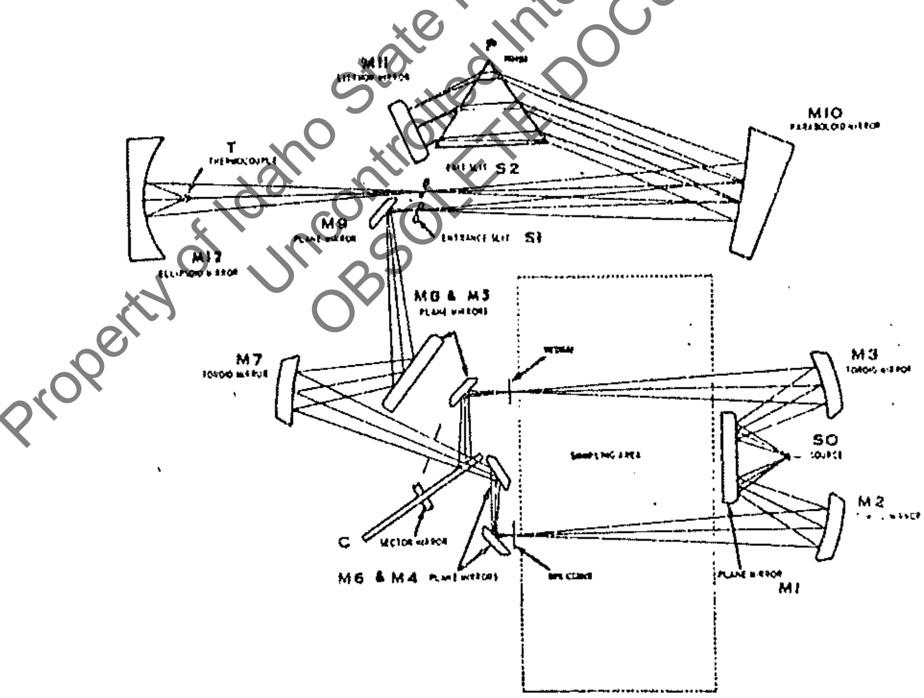


Figure 2-1. Schematic optical diagram of a double-beam infrared spectrophotometer. (Courtesy of Perkin-Elmer Corporation.)

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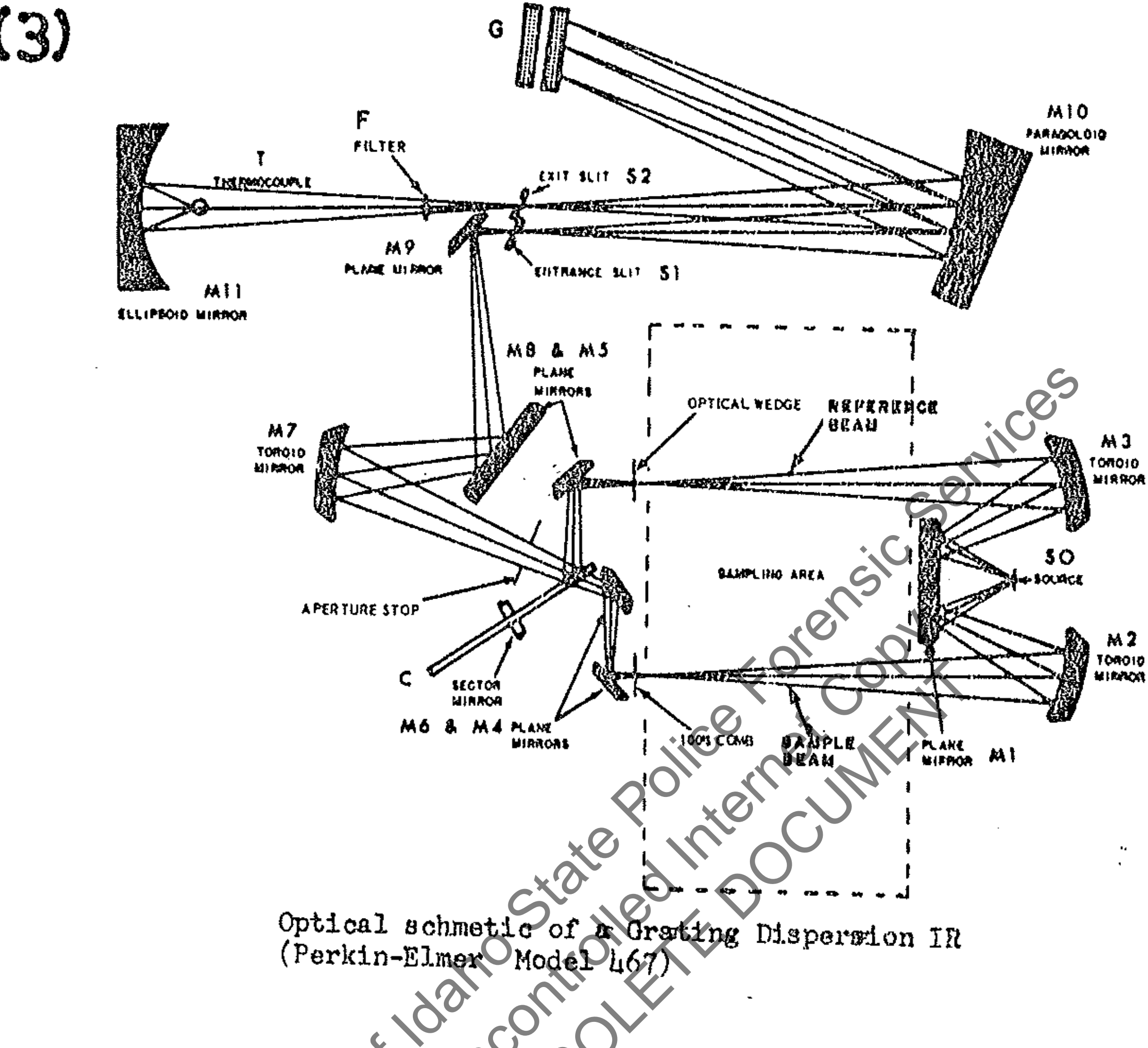


Optical schmetic of a Grating Dispersion IR (Perkin-Elmer Model 467)

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equalizing the energy in the two beams in any wavelength interval isolated by the monochromator, which follows in the optical system and is described below. The means of obtaining optical null is an optical-wedge type of attenuator actuated by the servo system of the instrument so as to adjust its position in the reference beam to equalize the energy in the two beams. The position of the recorder pen in the instrument at all times indicates the position of the wedge. It therefore indicates the transmission of the sample at the wavelength for which the instrument is set.

Mirror *M7* refocuses the alternate pulses of reference and sample beam energy on the entrance slit *S1* of the monochromator. The monochromator performs three functions basic to the operation of an infrared spectrophotometer:

1. It disperses the radiation into its component wavelengths.
2. It selects the particular wavelength of radiation to be transmitted to the detector.
3. It maintains approximately constant energy at the detector at all wavelengths when no sample is in the instrument.

The energy which passes through the entrance slit is collimated (all rays made parallel to each other) by mirror $M10$. The collimated beam proceeds to the dispersing element, which in this case is the prism $P$. The mirror $M11$ returns the beam through $P$ to $M10$, which refocuses the beam on the exit slit $S2$. This monochromator system is called a Littrow configuration and the mirror $M11$ is referred to as a Littrow mirror. The angle of the Littrow mirror relative to the prism determines the wavelength of the radiation which passes through $S2$. In scanning, the Littrow mirror is rotated by a cam so as to move the spectral band across the exit slit at a predetermined rate. Thus the energy reaching the detector changes in sequence from one wavelength to the next.

The width of the slit openings determines both the width of the spectral interval and the amount of energy which passes through the exit slit. The slit width is varied by a second cam coupled to the wavelength cam to maintain constant energy level as the wavelength is scanned with no sample in either beam. This energy level, therefore, determines the resolution of the instrument at each wavelength. The spectral interval passed by a given exit slit width is called the *spectral slit width*.

The energy which passes through the exit slit is then concentrated onto the detector $T$ by the mirror $M12$.

(3) In the grating instrument

The radiation impinges on the grating, which disperses the radiation into its component wavelengths. Depending on the position of the grating and its resolving power, a certain narrow bundle of frequencies will be directed toward the exit slits to the detector. The actual range of frequencies exiting from the monochromator will also be determined by the mechanical slit width of the exit slits.

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## COMPONENTS OF INFRARED SPECTROPHOTOMETERS

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### Sources

Absorption spectrophotometers require the use of continuous sources of radiation—that is, sources which radiate energy over the entire band of spectral interest, without sharp discontinuities created by emission lines or self-absorption bands. In the infrared portion of the spectrum, all of the commonly used sources are incandescent solids. The useful sources all approximate the theoretical performance of a blackbody radiator; it is of course impossible for any to exceed it. The radiation efficiency of any real object compared to an ideal blackbody is called *emissivity*.

*Blackbody Radiators.* According to the well-known Planck distribution law, the spectral distribution of energy emitted by an ideal blackbody is determined solely by the temperature of the radiating element. Figure 2-2 shows this distribution for a source at 1500°K, which is the range in which commonly used sources are generally operated.

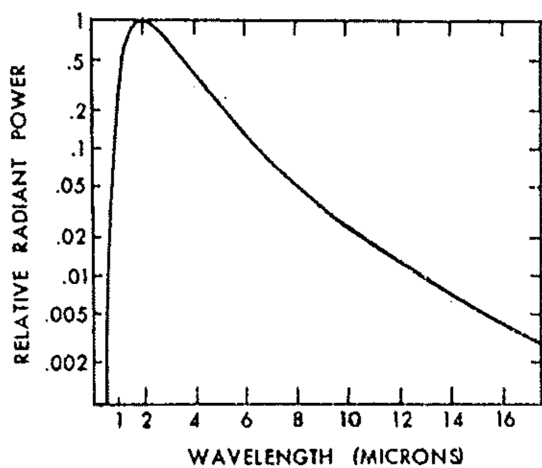


Figure 2-2. Spectral distribution of energy emitted by a 1500°K blackbody source.

Certain key properties of this figure should be noted. With an energy peak at 1.85 μ, the energy distribution drops off more sharply on the short-wavelength side than on the long-wavelength side. Even with this slower drop-off at longer infrared wavelengths, the radiant power at 15 μ, for example, is only 0.55% of the peak. The slit width of the spectrophotometer must be adjusted to compensate for this wide variation of available radiant power. In most of the spectrophotometers used by analytical chemists the slit widths are automatically programmed to compensate for this as well as other factors, so as to maintain a constant reference energy level over the entire spectral range of the instrument.

In view of the fact that the distribution curve in Figure 2-2 is affected only by temperature, the effect of varying the temperature should be considered next. First, if the temperature is raised, the peak shifts toward shorter wavelengths, thus putting a greater percentage of the radiated energy in the visible region, which is not of interest in an infrared instrument. Of more direct interest is how the radiant power at any given infrared wavelength varies with temperature. For wavelengths that are long compared to the peak wavelength, the radiant intensity is very nearly proportional to the absolute temperature. Therefore, the potential gain of utilizing sources significantly hotter than 1500°K is meager relative to the accompanying disadvantages of more complex control systems, shorter source life, and similar factors.

It should be noted that all bodies radiate to all others. Thus, any warm object within an infrared spectrophotometer may become a source radiating more energy than it receives from its surroundings. The optical path in an instrument is always carefully shielded from radiation from hot electronic components. Potentially troublesome, however, are warm samples, which of necessity, are directly in the optical path. For example, a sample at 77°C (350°K, 171°F) exhibits a radiation peak at 8.4 μ whose intensity is 1.7% of that due to a 1500°K source at the same wavelength. Because of the slower decrease with wavelength of the 350°K source compared to the 1500°K source, the former radiates about 6% as much energy as the latter at 15 μ.

***Sources Commonly Used.*** Analytical instruments most commonly use either a Nernst glower, a Globar, or a wire coil source. In the near infrared, tungsten lamps such as the type used for projectors are frequently adapted.

The *Nernst glower* is made from rare earth oxides. It is generally shaped as a cylinder up to a few millimeters in diameter and a few centimeters long, and is fitted with platinum leads. Thus, it is conveniently shaped for focusing efficiently on a monochromator en-

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trance slit. It generally operates in the 1400 to 1600°K range. Except for a deficiency in its emissivity below 5 $\mu$, which is partly compensated by the proximity to its peak radiance, the Nernst glower is an efficient radiator. In utilizing a Nernst glower one must take into account its large negative temperature coefficient of resistance. At room temperature its resistance is so high that it is not feasible to heat it by passing a current through it. Instead, instruments employing Nernst glowers provide an indirect means of preheating the glower to a dull red temperature, after which the direct heating takes over. Nernst glowers do not deteriorate on exposure to the atmosphere but are subject to mechanical distortion, which is their most severe practical limitation.

A *Globar* is made from silicon carbide or carborundum. It operates generally in a slightly lower temperature range than a Nernst glower. For wavelengths shorter than about 7 $\mu$ it has a significantly higher emissivity than a Nernst glower. Beyond this the two are comparable except for a drop in the Globar emissivity between 10 and 14 $\mu$. Globars are not troubled by the negative temperature coefficient and the susceptibility to mechanical distortion of the Nernst glower. The most serious problem in working with Globars is that large thermal gradients are produced around the electrical contacts, which frequently necessitates water cooling to avoid arcing problems. In some cases Globars have been made with a large diameter in the vicinity of the contacts and a smaller cross section in the area radiating to the slit. This minimizes the problem and in some cases reduces the requirement for water cooling to more convenient air cooling. In addition, it should be noted that Globar rods are generally of larger diameter than Nernst glowers, thus requiring a larger electrical power input for a given radiant energy through the entrance slit. However, the larger diameter of a Globar makes it easier to illuminate wider slits uniformly with a Globar than with a Nernst glower.

Different types of *incandescent wire sources* have been incorporated in analytical infrared spectrophotometers. Their greatest limitation is reaching a sufficiently high temperature without excessively shortening their usable lifetime. Nichrome coils have been utilized up to a temperature of about 1100°K without unduly shortening their life. Another variation of a coil source is a rhodium or platinum coil packed in a sealed ceramic tube. This type of source operates at about 1500°K, with a usable life of approximately 1000 hours.

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***Single-Beam Systems.*** The earliest work in infrared spectroscopy utilized single-beam systems. Although still prevalent for some unique applications, primarily related to infrared physical measurements, these systems play only an infrequent role in analytical chemistry.

***Double-Beam Optical Null Systems.*** Virtually all instruments used for analytical chemical applications utilize a double-beam optical null photometric system. In these instruments an electro-optical servo system continually attenuates the energy in the reference beam so that there is no net signal difference between the reference beam and the sample beam. The recording pen indicates the position of the reference beam attenuator and therefore the relative transmittance of the sample.

Because the servo system always drives to a null signal, the spectrum is basically independent of time or spatial changes in source intensity, amplifier drift, and, most important of all, atmospheric absorption bands. The optical systems for this method of photometry are designed to insure symmetry of the reference and sample beams with respect to path length, number of reflections, etc. However, the operator of such an instrument must keep in mind the fact that insertion of a sample cell may introduce a sufficient asymmetry between the reference and sample beams so that atmospheric absorption bands may appear in the spectrum.

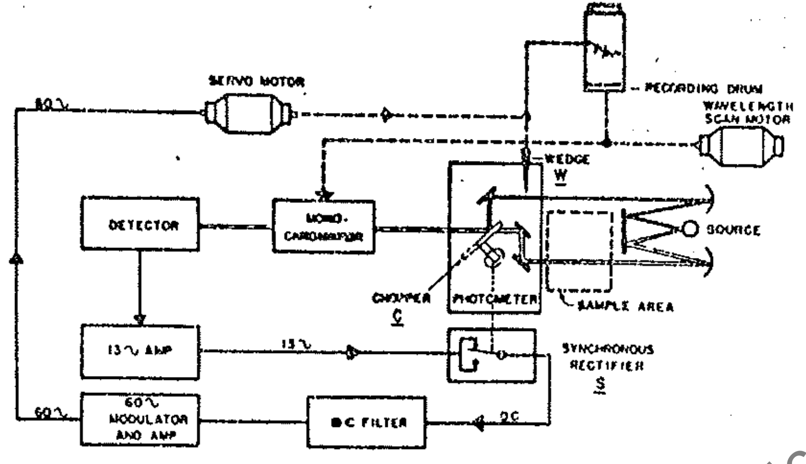


**Figure 2-4. Schematic diagram of an optical null double-beam system. (Courtesy of Perkin-Elmer Corporation.)**

Figure 2-4 shows a schematic diagram of an optical null system. Some of the key components in this system may now be described.

The *sector mirror C* alternately reflects reference beam energy and transmits sample beam energy through the remainder of the system. The rate at which a spectrophotometer can be scanned is essentially limited by the speed of this sector, which in turn is determined by the speed of response of the detector. If the latter is a thermocouple or a metal bolometer, the most commonly used detectors in analytical instrumentation, the chopping frequency is generally in the 10 to 13 cycles/sec range.

The *synchronous rectifier S* is mechanically or electrically coupled to the sector mirror. It converts the amplified low-frequency output of the detector to direct current. The rectifier is phased with the optical chopper mirror so that the polarity of the rectified output indicates the condition of unbalance of the optical null system. That is, one polarity indicates more energy in the reference beam than in the sample beam, and the opposite polarity indicates more energy in the sample beam than in the reference beam. A balanced null signal, of course, indicates equal energy in the two beams. The polarity of the synchronously rectified voltage determines the direction in which the servo motor drives the optical attenuator.

The *reference beam attenuator* or *wedge W* is driven by the servo system so as to maintain a null energy balance between the reference and sample beams. The attenuator itself is generally a device with an open area which varies linearly with position. For example, it commonly resembles a thin comb with a small number of very long V-shaped teeth. The openings are precision etched in a very thin metal sheet to insure the shape of the opening and the ability to position it in a uniform portion of the beam. These two—i.e., the uniform variation of the open area with position and the constancy of the energy distribution over the open area of the attenuator—are the most important factors influencing the photometric accuracy of the system. The attenuator usually consists of three to five open V's, so as to average over small nonuniformities in the cross section of the image on it or in the local sensitivity of the detector surface. The fact that the thickness of the attenuator may be only 0.003 in. makes it a delicate component, highly susceptible to damage by pencils, etc.

Having examined the working basis and requirements of a double-beam optical null system, we shall next examine some of the properties of such systems of which the analyst should be aware. The accuracy of calibration of these systems is generally quite constant until the open area of the attenuator becomes very small.

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For very small openings it is virtually impossible to insure precise calibration. The principal cause for this is the effect of slit width. If the slit is very narrow, the zero position of the attenuator is sharply defined. As the slit widens, the attenuator will have to move farther to stop the energy and produce zero signal. There is an analogous effect near 100%.

When a spectrum is recorded, quantitative results can be derived from it only if the zero and 100% levels are established. We must first define what is meant by these terms before the method of measurement becomes clear.

The *100% level* is the pen position corresponding to zero absorption in the sample at the wavelength being analyzed. Note that this is not the level indicated in a record made with no sample or sample cell in either beam. The latter is frequently referred to as the $P_0$ line and is characterized by smooth, small variations throughout the working region of a properly operating instrument. However, the $P_0$ line neglects factors such as reflection losses at cell windows and absorption at the wavelength under study in the cell window, in the solvent, or in any material in the beam other than the sample being analyzed.

One technique commonly used to determine the true 100% level at the position of a particular band is to interpolate this level from the level of the spectrum in nonabsorbent regions close to this analytical wavelength. To insure accurate interpretation of the data it is necessary to record a preliminary curve of everything involved in the spectrum except the sample itself, preferably recording this on the same chart as the spectrum of the sample. For a solid sample this preliminary curve is the same as the $P_0$ line. If a KBr pressed disk is being used, more accurate results would be obtained if the preliminary curve were recorded with a pure KBr disk without sample mounted in the same holder as that to be used with the KBr disk with sample. For a liquid sample a run with the same cell empty, or filled with the solvent, if any, would satisfy the requirement. The same holds true for a gas or vapor, where a carrier gas, if any, should be taken into account for completeness.

A second technique for determining the 100% line is differential analysis. If this is adopted, a cell (or disk) devoid of sample but otherwise identical with that being inserted in the sample beam, including solvent or carrier gas, if any, is placed in the reference beam. Thus, the only difference between the two beams is the sample material itself. While it should be noted that a liquid cell with sample cannot be accurately compared with a liquid cell containing no sample at all because of the difference in reflection losses within the two cells, the resultant differential spectrum will generally be adequate for an accurate determination of the true 100% level at any wavelength. This method has the advantage of closely approximating the symmetry condition, which is so important to double-beam optical null systems. However, if the solvent has bands of high absorbance itself, the reference cell may remove excessive amounts of energy from the system in these regions of the spectrum and cause fallacious results due to sluggish operation of the null system. This may be handled with greater confidence if a system employing automatic gain control (see Section 2.4D) is utilized.

Measurement of the *zero level* is not as simple as one might expect. The complication arises from the inherent lack of energy of a double-beam optical null system at zero transmittance, where both beams are blocked. Under these conditions it is possible for the optical attenuator to drift or coast below its zero position, since the system has no means of returning the errant attenuator to the true zero. In a well-operating instrument there are three potential causes for fallacious zero reading: a too rapid approach to zero, an improper electrical balance, and scattered radiation.

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*Too Rapid Approach to Zero.* When the attenuator reaches zero too rapidly, the momentum of the system causes the attenuator to move below zero. There are well-defined techniques to overcome this difficulty. One is to block both beams under conditions where the attenuator is transmitting a significant amount of energy and then unblock the reference beam a very small amount in order to introduce a creeping down-scale motion of the attenuator. When the attenuator comes to rest near zero, the reference beam must be completely unblocked. Another is to scan the absorption edge of an optical material slowly. If the material is sufficiently thick, the zero level will be well defined and the approach to zero under normal scan conditions will be slow enough to avoid the drift-below-zero problem.

*Improper Electrical Balance.* The electrical balance control in an optical null system equalizes the reference and sample signal phases, including the effects of spurious pickup signals within the instrument. A negative unbalance will drive the attenuator below zero when both beams are blocked. A positive unbalance will tend to stop the attenuator before it reaches the true zero. The balance control is set for zero drift under zero energy conditions, i.e., with both beams blocked. Therefore, patience must be exercised to avoid the problem of momentum drift discussed above.

*Scattered or Unwanted Radiation.* This is energy of wavelengths different from the small spectral interval under observation which nevertheless reaches the detector. Thus, even when there is total attenuation at the wavelength of interest, the scattered or unwanted radiation will cause a residual signal which prevents the attenuator from indicating true zero. Consequently, the zero level of the system should be determined under conditions where the percentage of scattered radiation is known to be trivial. The sources of scattered radiation are discussed in Section 2.5, and the effects of scattered radiation in quantitative work are considered in Chapter 6.

### 2.3C. Dispersing Elements

Up to a few years ago prisms were the usual dispersing elements of infrared instruments. Now grating instruments have reached a state of development where they have become commonplace. With the large variety of instrumentation available to the analyst today, a wise selection of the best instrument for a particular problem or combination of problems depends particularly on a familiarity with the properties of the dispersing elements.

The dispersing element spreads out the spectral continuum of energy radiated by the source so that only narrow regions of the spectrum pass through the exit slit to the detector. The dispersing element determines both the resolution of the instrument through its angular dispersion characteristics and the spectral range of the instrument by virtue of its inherent physical properties. It is the object of this section to summarize the properties of the dispersing elements in most common use in infrared spectrophotometers.

*Prisms.* A prism placed in a light beam refracts (i.e., bends) the light which is incident at an angle different from the normal. The index of refraction $n$, and the angle of incidence determine the amount of bending. The use of a prism as a dispersing element depends on the fact that the refractive index changes with wavelength so that different wavelengths are refracted by different amounts. Also, the variation of $n$ with $\lambda$ is a single-valued, unique function for the material of the prism.

The two prime requisites of a dispersing prism are that it have a high transmission in the applicable spectral region and that its angular dispersion be sufficient. As indicated in Section 2.2A, both

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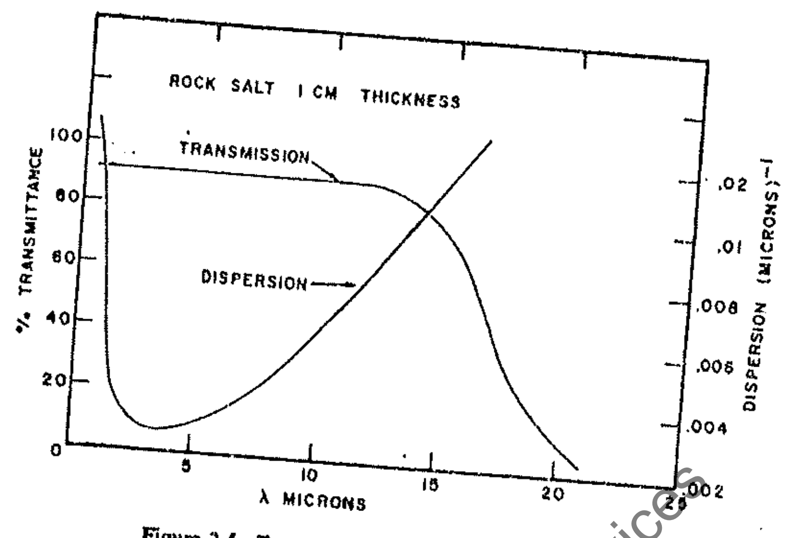


**Figure 2-5.** Transmission and dispersion of NaCl.

the transmission efficiency and the angular dispersion directly affect the signal-to-noise ratio for a given resolution. However, these requisites are not independent of each other. Angular dispersion depends directly on *dn/dλ*, i.e., the variation of the index of refraction with wavelength. This derivative is called the *dispersion*. The nature of the physical mechanism of the transmission of radiation is such that the dispersion is greatest near a region of absorption. In Figure 2-5, where the transmission and dispersion of rock salt (NaCl) are shown, the interdependence of these variables may be noted in the vicinity of 15 μ. From the figure it is apparent why NaCl is efficiently usable as a prism material in the infrared only between roughly 5 and 15 μ, even though the material is quite transparent at wavelengths much shorter than 5 μ.

Rock salt has proved to be the most generally useful prism material in infrared analytical instrumentation. Its dispersion is high in a spectral region particularly rich in absorption bands -5 to 15 μ

**TABLE 2-1. Properties of Infrared Optical Materials**

| Material | Wavelength Limits, μ | Refractive Index | Cold Water Solubility g/100 g $H_2O$ |
|---|---|---|---|
| Fused silica ($SiO_2$) | 0.16—4.0 | 1.45 at 1.0 μ | 0 |
| Artificial sapphire ($Al_2O_3$) | 0.17—6.5 | 1.76 at 1.0 μ | $9.8 \times 10^{-5}$ |
| Lithium fluoride (LiF) | 0.12—8 | 1.38 at 2.0 μ | 0.27 |
| Calcium fluoride ($CaF_2$) | 0.13—11 | 1.42 at 2.0 μ | $1.7 \times 10^{-3}$ |
| Barium fluoride ($BaF_2$) | 0.15—13 | 1.46 at 2.0 μ | 0.17 |
| Silicon (Si) | 1.2—15 | 3.4 at 10 μ | 0 |
| Sodium chloride (NaCl) | 0.20—22 | 1.50 at 9.0 μ | 35.7 at 0°C |
| Germanium (Ge) | 1.8—23 | 4 at 2 μ | 0 |
| Silver chloride (AgCl) | 0.4—25 | 1.98 at 10 μ | 0 |
| Potassium chloride (KCl) | 0.21—26 | 1.46 at 10 μ | 34.7 |
| KRS-6 (TlBr—TlCl) | 0.21—30 | 2.18 at 10 μ | 0.32 |
| Potassium bromide (KBr) | 0.2—33 | 1.53 at 10 μ | 54 |
| Potassium iodide (KI) | 0.2—40 | 1.62 at 10 μ | 127 |
| KRS-5 (TlBr—TlI) | 0.5—40 | 2.37 at 10 μ | 0.05 |
| Cesium bromide (CsBr) | 0.2—42 | 1.66 at 10 μ | 124 |
| Cesium iodide (CsI) | 0.24—[illegible]5 | 1.74 at 10 μ | 44 |

(2000 to 667 cm⁻¹)—and continues to be adequate to supply additional information to even shorter wavelengths. Some instruments with rock salt prisms scan as far as 1 μ (10,000 cm⁻¹). More commonly though 2.5 μ (4000 cm⁻¹) is the short-wavelength limit of such instruments because of a change in sample cell requirements



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in that region.

The most versatile prism instruments provide for relatively simple interchange of prism material, both to optimize the performance in a given range and to extend the efficient performance range of the instrument to shorter and longer wavelengths. Table 2-1 lists the important properties of the prism and window materials commonly used in analytical instrumentation. Most of these materials must be artificially grown crystals to be adequately pure and homogeneous for optical use. They are also generally notable for varying degrees of undesirable properties, such as sensitivity to moisture and to scratching. $CaF_2$, which is widely used, forms one exception to this: it is insoluble in water and is very hard. Because most of the prisms are very soluble in water and will tend to fog (lose their polish) if subjected to condensation, infrared spectrophotometers generally operate with the prism at a temperature higher than its surroundings. With even the most exacting care, moisture-sensitive prisms will always tend to fog with time. While they can be repolished, the techniques involved are such that the task should generally not be undertaken in a chemical laboratory. Another physical property requiring attention is the variation of the refractive index with temperature. Errors in wavelength calibration due to this effect may be avoided within reasonable temperature limits by use of a bimetallically controlled mechanism, which is incorporated in most prism instruments.

***Gratings.*** Gratings used in the infrared are of the plane reflection type, which consists generally of a glass blank with an aluminum coating containing many closely and precisely spaced parallel grooves. The theory of light dispersion by a grating may be pursued in any good textbook on optics. Suffice it to say here that the incident light is dispersed by the phenomenon of diffraction, which is fundamentally a wave interference effect among the light rays from the long series of grooves.

If one is to take full advantage of a grating spectrophotometer, it is desirable to become familiar with the fundamental properties of radiation diffracted from a grating. First of all, in contrast to a prism, (a) the spectral distribution of radiation from a grating follows simple trigonometric laws, and (b) the energy diffracted at a given angle is not of a single wavelength but consists of a multiplicity of wavelengths. Both of these unique properties are demonstrated in the grating equation, which for a reflection grating may be stated as

$$mN\lambda = 2\sin\theta\cdot\cos\delta \qquad \text{or} \qquad \frac{\nu}{mN} = \frac{\csc\theta}{2\cos\delta} \qquad (2\text{-}2)$$

Figure 2-7 sketches the geometry involved: $\theta$ is the mean of the angles of incidence and diffraction, measured relative to the grating

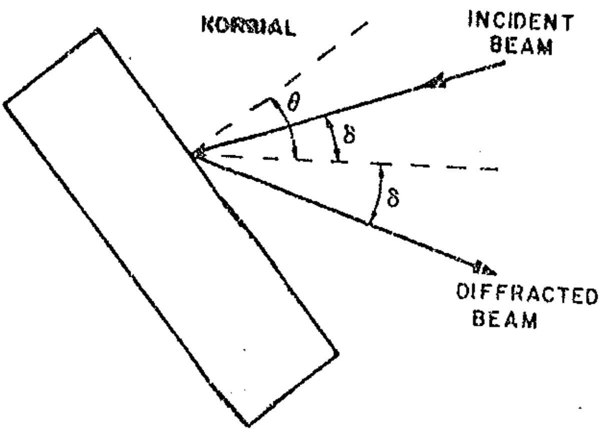


Figure 2-7. Diffraction at the surface of a reflection grating.

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normal, $\delta$ is the difference between the incident or the diffracted angle and the mean angle $\theta$, $m$ is the number of grooves per unit length, $\lambda$ is the wavelength, $\nu$ is the wavenumber, and $N$ is the order number. The first unique property mentioned above follows from the fact that for each given order $N$, $\lambda$ is proportional to sin $\theta$ and $\nu$ is proportional to csc $\theta$, since the other quantities in the equation are all constants of the grating and the optical system. The order number is the term used to describe the second property indicated above. By virtue of the theory of diffraction, $N$ is any integer. Thus, if a continuum of radiation is incident on a grating, the diffracted energy at a specific angle consists of a series of integrally related wavelengths. For example, if one sets a 100 groove/mm grating at an angle which allows 12 $\mu$ first-order radiation to reach the exit slit, one will also detect second-order 6 $\mu$ energy, third-order 4 $\mu$ energy, fourth-order 3 $\mu$ energy, fifth-order 2.4 $\mu$ energy, sixth-order 2 $\mu$ energy, etc. In order to obtain monochromatic radiation from a grating it is essential to provide a means of eliminating all but one order from this series of integrally related wavelengths.

There are two standard methods for sorting orders in an infrared spectrophotometer. The classical technique is to use a second, prism monochromator, generally placed in front of the grating monochromator. The fore-monochromator allows only a limited spectral band to enter the grating monochromator, so that only a single order remains to be diffracted at any given grating angle. Scanning requires the two monochromators to track together - i.e., keep in coincident calibration.

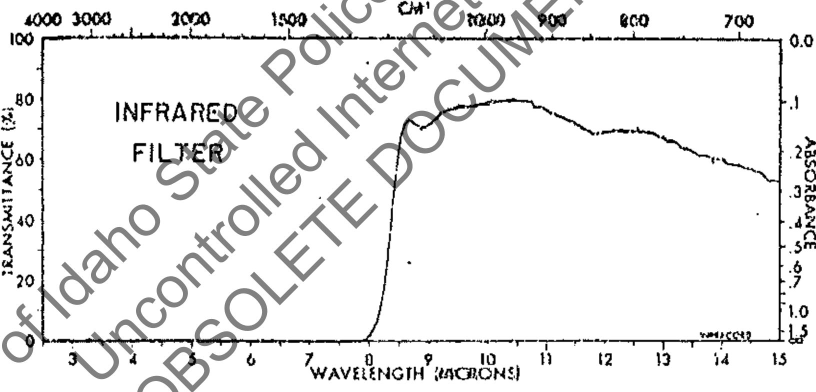


Figure 2-8. Characteristics of a typical long-wave-pass interference filter.

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The second method for order elimination is the use of filters. Before 1959 this method was limited to the far infrared, where no prism material was available, and to the near infrared, where simple filters were available. However, partly in fulfillment of the requirements of military applications of infrared, multilayer interference filters with suitable characteristics for order sorting in the infrared region have now been developed. Up to 1963, filter grating systems utilizing interference filters had been developed for operation to 40 $\mu$. In most applications of filter grating systems, the gratings are used in first order only, primarily because of the simplicity of filter requirements. First-order operation calls for long-wave-pass filters--i.e., filters which transmit at wavelengths longer than a sharp cutoff and which thoroughly reject radiation of shorter wavelengths or higher orders. The characteristics of a typical long-wave-pass filter are shown in Figure 2-8. Filters of this type reject at least 99.95% of the energy in the dead region. The filters are relatively insensitive to moisture and are durable in normal instrument environments. They transmit an average of 80% of the incident energy, which puts their inherent losses in the same class as the reflection losses of

prisms.

Filter grating systems have not been available long enough in analytical instrumentation to evaluate their ultimate usefulness compared to fore-prism grating systems. However, the filter grating systems have demonstrated many advantages which have clearly established their place in analytical instrumentation. Most of the pertinent advantages result from the use of a filter in place of a fore-prism monochromator and from the versatility of the filter itself.

For example, the optical path length through a filter grating system is shorter by the path length of a monochromator, which decreases the effect of atmospheric absorption within the instrument. This decreases the need for removing $H_2O$ and $CO_2$ from the spectrophotometer. The effect of these absorption bands is too frequently overlooked, particularly in high-resolution spectroscopy. Because of the shorter optical path length, the filter grating instrument is also more compact, thus occupying less laboratory bench space.

In addition, the environmental problems generally associated with infrared materials—moisture sensitivity and the requirement of temperature compensation—are eliminated, and the artificial wavelength range restrictions imposed by the prism materials are lifted. The useful short-wavelength region for a given prism material is determined by the decreasing dispersion and the long-wavelength limit is determined by absorption.

Filter grating systems are free from the slit width effects which are occasionally troublesome in fore-prism grating systems. These effects arise from the fact that the slit width of the prism monochromator must be large enough to cause its pertinent band of dispersed energy to fill the entrance slit of the grating monochromator. When operating in higher orders of the grating, the angular dispersion is quite high. Under these conditions the slit width of the grating monochromator, and therefore also that of the fore-prism monochromator, is generally quite large in comparison to a normal slit width for a prism monochromator. Particularly if the system is being operated with a high-energy, wide-slit program, there will be a tendency to experience order interference when operating in the higher orders. In a filter grating system the effectiveness of the filter is independent of the slit width, since the band of energy entering the grating monochromator does not change with slit width.

Another valuable property of grating spectrophotometers which relates to the angular dispersion properties of gratings is the nearly constant spectral slit width in wavenumbers which accompanies a constant-energy slit program. This contrasts to prism instruments, where spectral slit width variations in frequency units of as much as 10 to 1 will occur. These variations are limited roughly to 2 to 1 for a grating instrument. Since band widths and separations are related to energy factors and therefore to frequency differences, the above property of grating instruments, combined with an overall higher resolution, makes grating spectrophotometers exceptionally valuable in the interpretative applications of infrared spectroscopy, such as structure determination.

***Grating Efficiency and Polarization.*** In connection with grating spectrophotometers it is common practice to specify the "blaze" angle and wavelength of the gratings employed. It is, therefore, pertinent to discuss the meanings and implications of these terms to avoid the pitfalls of over- or under-emphasis of their significance.

Blazing a grating increases its energy efficiency over a wide range of angles on both sides of the blaze angle, which is the angle at which the diffracted energy is maximized. The blaze angle depends on the shape of the grooves, which is determined by the shape of the ruling diamond and by its angle of contact with the surface being ruled. Figure 2-9 is a diagrammatic representation of the shape of the rulings of a blazed grating. The blaze angle $\theta_0$ is the grating



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angle between the normal to the broader face of the groove and the grating normal. There also exists a secondary angle of peak efficiency near the "antiblaze," normal to the narrower face of the groove.

Modern blazed gratings are capable of diffracting up to as much as 85% of the incident energy of a given wavelength at angles near the blaze. The efficiency of a grating in first order holds up quite well over a very wide angular range. For example, a grating blazed

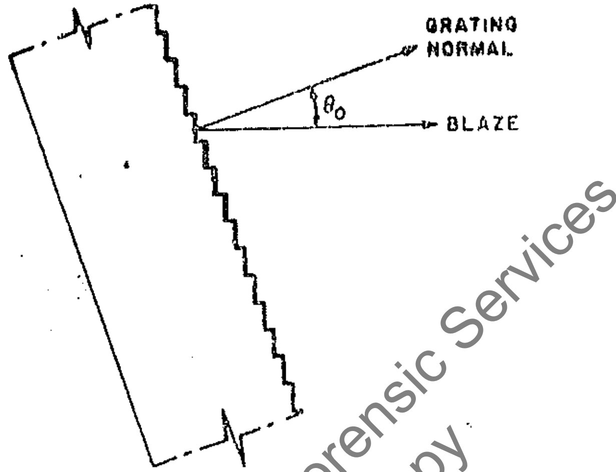


Figure 2-9. Groove shape of a blazed grating.

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at about 27° is usable with no less than half its peak efficiency between approximately 17° and 45°. This angular range becomes much smaller in the higher orders.

Although the efficiency curve proves to be asymmetrical when plotted against angle, as well as against wavelength, the curve is nearly symmetric when plotted against frequency. The following are useful guide rules with respect to grating efficiency:

1. A grating operates with 50% or more of its peak efficiency in the first order from $\frac{1}{2}$ its blaze frequency to $1\frac{1}{2}$ times its blaze frequency—i.e., from $\nu_B/2$ to $3\nu_B/2$, or from $2/3\lambda_B$ to $2\lambda_B$. This is a factor of 3 in frequency or wavelength.
2. In higher orders, the width of the 50% or greater relative efficiency range is the same as in first order *on a frequency scale*. Higher orders correspond to higher frequency, which leads to the conclusion that the same frequency interval corresponds to a smaller usable wavelength range $\Delta\lambda$ in higher orders. That is, in higher orders the grating efficiency remains high over smaller wavelength and angular ranges near the blaze.

### 2.3D. Detectors

The function of the detector is to convert the infrared radiation into an electrical signal. In view of the following two facts, this task should not be taken lightly:

1. The energy of an infrared photon is low. For example, a 3-$\mu$ photon has an energy of roughly 1.0 electron volt and a 30-$\mu$ photon about 0.1 electron volt. From these energies one may expect that the applicability of infrared solid state detectors, which depend on the activation of some photoelectronic phenomenon within the solid by the photon energy, is limited.
2. Analytical applications almost always require information over a broad spectral range, which necessitates use of a nonselective detector.

(3) SUMMARY OF INFRARED DETECTORS

a. Thermal detectors: Most widely used; reliable, but not particularly fast response, nor sensitive. Thermocouples, thermopiles.

b. Resistance detectors: Resistance within detector changes with changes in temperature. Bolometers.

c. Pneumatic detectors: Depend on gas expansion and contraction inside sealed chamber. Usually slow and very fragile. Golay detector.

d. Pyroelectric detectors: Majority operate best at low temperatures, 30° K and up.

| Alloys | Coolants |
|---|---|
| In-Sb | Nitrogen |
| Cd-Hg-Te | Helium |
| Cu-Ge | Hydrogen |
| Hg-Ge | |
| TGS Bolometer | |

Best practical pyroelectric detector available presently is the Triglycine sulfate (TGS) pyroelectric bolometer. Operates at room temperature, has broadest range of detection, and highest sensitivity together with fastest response. Currently used in far infrared version of Perkin-Elmer Model 180.

(4) **Heterodyne Detection of Laser Radiation.** Heterodyne detection has been employed to detect weak infrared signals (L11, L19, L27). The radiation to be measured is mixed with that of a local oscillator whose frequency is chosen to produce a microwave difference frequency which can be detected electrically. Such detectors are far more sensitive and faster than conventional infrared detectors. When it is possible to use such a detector with a laser spectrometer, S/N will be dramatically improved over that of either grating or Fourier spectrometers.

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(18)

# Optimizing the Operating Parameters of Infrared Spectrometers

W. J. Potts, Jr. and A. Lee Smith

In order to achieve best results with modern ir spectrometers, users must take considerable care to optimize the setting of the slit program, response time, gain, and scan time. In this paper, explicit recommendations are given for carrying out such adjustments in a logical and self-consistent manner. First, a set of conditions is derived for the recording of general purpose spectra. From this starting point, settings may be modified to record spectra under the following special conditions: low noise level, limited optical energy, high resolution, and rapid scanning. Proper use of the scan speed suppression control is discussed.

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## Introduction

The versatile, highly sophisticated ir spectrometers currently available present their users with a frustrating dilemma: either become expert in the theory and dynamics of ir spectrometers, or accept inferior performance from an improperly adjusted instrument. For users who are reluctant to accept either alternative, we have attempted to develop systematic procedures for optimizing spectrometer settings, and to present these procedures in such a way that they are easily understandable and widely applicable, in the hope that these instruments will be used to produce the high performance of which they are capable.

Spectroscopy in the ir region of the spectrum is energy limited in the sense that ultimate performance is limited by detection of the signal rather than by optical diffraction limits. This situation arises principally from the poor sensitivity and high noise level of ir detectors (as compared with photomultipliers employed in other spectral regions), combined with the intrinsically low energy of ir sources. Thus, until a significant improvement in detectors or sources occurs, we must recognize that in recording a spectrum we are unable to achieve simultaneously the ultimate in resolution, the smoothest trace, and the fastest recording times; in practice, some compromise must be accepted in one, or more usually all, of these variables. (Some progress has been made in detector development; it is possible that within the next few years the difficulties discussed here may be largely eliminated.)

As a consequence, the spectrum produced on the spectrometer chart is not the true absorption spectrum of the substance being sampled; it shows distortions introduced by the spectrometer itself.[1,2] However, these distortions may be reduced significantly by proper choice of instrument scanning parameters. The nature of these distortions and procedures to minimize them are the subjects of this article.

W. J. Potts is with The Dow Chemical Company, and A. L. Smith is with the Dow Corning Corporation, both of Midland, Michigan 48640.

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## Significant Parameters

### Finite Slit Width

The basic design of dispersive type monochromators is well known.[3,4] For our purposes, it is important to recognize one fundamental limitation: the spectrometer tries to make monochromatic radiation out of a continuous energy spectrum, and never quite succeeds. The spectral slit width, or frequency spread at half-maximum of the radiation passing the exit slit, is proportional to the mechanical slit width for a well adjusted spectrometer, or

$$\Delta\nu_{1/2} \propto s. \quad (1)$$

Other contributions to the breadth of $\Delta\nu_{1/2}$ (diffraction limits and optical aberrations) are small compared with the slit term in an energy limited spectrometer during usual operation. The energy distribution in the frequency bundle falling on the detector surface is known as the slit function of the monochromator (Fig. 1). For a well-adjusted instrument this distribution is usually quasi-triangular.[5] The combination of the slit function and the true shape of an absorption band gives (for an infinitely slow scan) the observed line shape (Fig. 2). Clearly, the distortion introduced into the spectrum depends strongly on the slit function of the monochromator. It is also obvious that the narrower the slit, the smaller the net effect of both slit width and slit function on the recorded spectrum. The intensity of a band as a function of spectral slit with $\Delta\nu_{1/2}$ has been studied by Ramsay[6] and others.[7-10] As a rule of

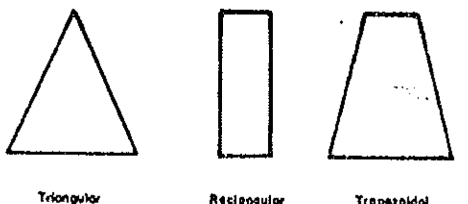


Fig. 1. Some hypothetical slit functions of monochromators.

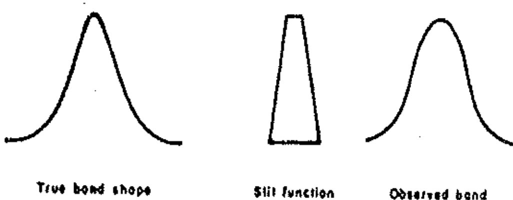


Fig. 2. Effects of slit function on line shape.

thumb, it has been suggested that $\Delta\nu_{1/2}$ should be less than one-fifth the bandwidth. (Bandwidth is defined here as the width of the band envelope at half its maximum absorbance). This requirement implies a spectral slit width of less than 0.8 $cm^{-1}$ for the narrower bands in condensed phases—indeed a stringent requirement for present spectrometers. An alternative approach for measuring true band shapes and intensities, which has obvious limitation, is to use a deconvolution program to calculate undistorted spectra.[11]

Resolution, which may be defined loosely as the ability of the spectrometer to separate two adjacent frequencies, is inversely proportional to slit width. In practice, it is usually determined by scanning pairs of lines whose separation is known from previous high resolution studies[12,13] Such a measurement may not, however, be completely reliable because of spurious resolution effects.[14] Generally, one uses the highest practical resolution in order to achieve more accurate band shapes and intensities consistent with other spectrometer limitations.[15]

## Servo Energy

Modern spectrometers use a chopping or beam alternating system to achieve stable operation. Because of the slow response to thermoelectric detectors, the chopping speed is low—usually 5–20 c/s. In the double-beam optical null system[16,17] an alternating signal at the detector indicates unbalance between the sample and the reference beam. The instrument responds by moving the optical attenuator into (or out of) the reference beam in such a way as to attempt to match exactly the absorption in the sample beam. Thus, the movement of the optical wedge more or less faithfully traces the absorption spectrum of the sample, and, indeed, it is the motion of the attenuator which is actually recorded; however, the fact that a finite signal exists at the detector indicates that the recording pen always lags behind the true sample transmission.

Thus, the system: radiation-detector-amplifier-servo motor-optical wedge, forms a closed loop which is the primary servo loop of the spectrometer. It is imperative for proper spectrometer operation that the energy of this loop be optimized: if it is too low, the attenuator motion will be sluggish and response will be incomplete (Fig. 3); if it is too high, the system may overshoot badly on rapidly changing signals and may, in fact, break into oscillation about the equilibrium point. Amplification of the detector signal in the servo loop is controlled by the gain setting. The *energy* available from the detector for activating the loop varies with the brightness of the source, the efficiency of the detector, and the slit width of the monochromator. Expressed in quantitative terms, the servo energy is proportional to the gain and to the *square* of the mechanical slit width, or

$$\text{servo energy} \propto s^2 g. \qquad (2)$$

(This relationship is true because the total energy passed by the monochromator is proportional to the product of the fraction of total energy passed by each of the two slits. If the slit openings are maintained equal, as is usually the case, the energy falling on the detector is proportional to the square of the slit width. For a more complete discussion, see Ref. 3.)

These variables must be adjusted initially to give the proper servo energy, and subsequently maintained in proper balance by compensating adjustments of gain and slit settings.

Ratio-recording spectrometers[18] do not use an optical attenuator; nevertheless, these spectrometers are still

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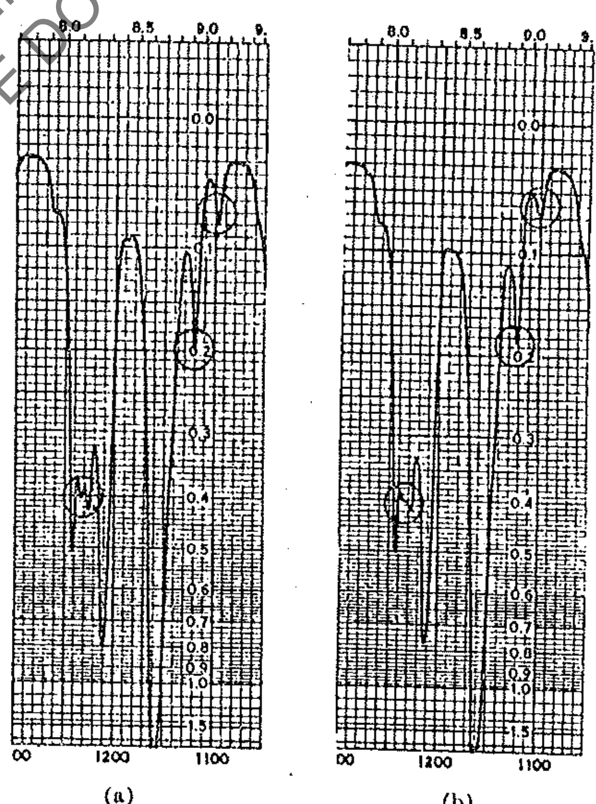


Fig. 3. (a) Scan with normal servo energy. (b) Scan with one-third normal servo energy.

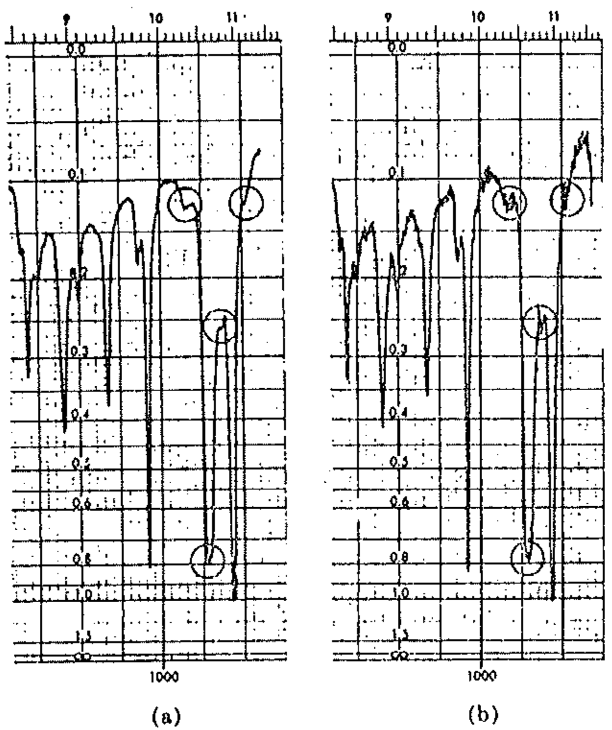

Fig. 4. (a) Normal scan. (b) Same conditions, except one-half normal slit width and gain increased to restore servo energy.

subject to the same basic limitation of slit width, noise, response time, and scan time.

## Noise

Noise, or unwanted random fluctuations of the recording pen, usually originates in the detector and first amplifier stage. The noise level sets a practical limit to the gain or degree of amplification of the detector signal. Noise is directly proportional to the gain, or

$$N \propto g. \tag{3}$$

Thermoelectric detectors are characteristically inefficient, and the signal-to-noise is therefore low compared to that of a photomultiplier, for example. The principal sources of noise are thermal agitation of electrons in the detector and input transformer (Johnson noise), and tube noise in the first stage of amplification. Noise is detrimental, since it introduces into the spectrometer record an uncertainty which produces disporportionately large errors in absorbance. For example, 1% noise superimposed on a band showing 20% transmission may give as much as 2.6% error in absorbance; irregularities in the background trace contribute an additional 0.6% for a total error of 3.2%.[19] These errors are greatly magnified for measurements near zero or 100% transmission.[19–21] From a qualitative standpoint, noise is undesirable because bands are easily lost in noise (Fig. 4), and, further, the unmeasurable but real confidence factor becomes low for a noisy spectrum. Finally, excessive noise interferes with the proper functioning of the speed suppression control (see below). A realistic noise level for general spectrophotometer work is 0.5% $T$ average peak to peak; reasons for this suggestion will become apparent.

## Response Time

Noise in the spectrometer can be minimized by (a) tuning the ac amplifier to the chopping frequency, a procedure which rejects signals of other frequencies (noise); (b) using an RC network to filter the dc signal resulting from synchronous demodulation of the amplified ac signal, or (c) incorporating inertia or changing the mechanical advantage in the mechanical part of the servo system. All these devices have the effect of increasing the time required for a signal to reach its steady state value, this time being the *response time* or *response period* of the servo loop.

The relationship between the Johnson noise voltage $E_J$ and the servo system bandpass $\Delta f$ is given by the Nyquist equation:

$$E_J^2 = 4kTR(\Delta f) \times 10^7, \tag{4}$$

where $k$ is Boltzmann's constant, $T$ is the absolute temperature, and $R$ is the detector resistance. The bandpass is inversely proportional to the time constant of the servo loop $\tau$, or

$$\Delta f \propto \tau^{-1}. \tag{5}$$

(Time constant for a critically damped system is defined as the time required for the servo response to reach within $1/e$ of its steady state value. Response time, or response period, as used here, is equivalent to about four

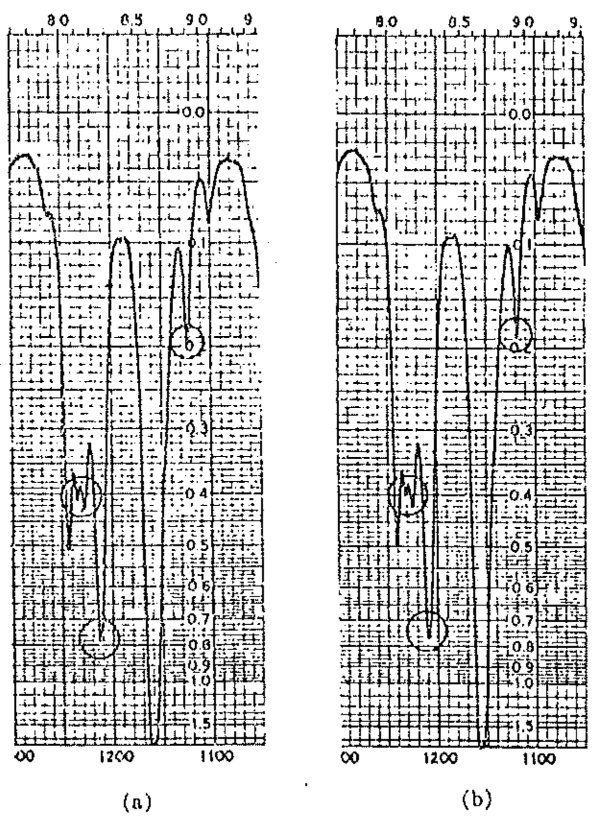

Fig. 5. (a) Normal scan with 0.5 sec response time. (b) Same conditions, except 2.0 sec response time.

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time constants.) As a result of these relations, and Eq. (3), we find that

$$N \propto g\tau^{-1/2}. \tag{6}$$

The scanning speed of the spectrometer must be consistent with the response time in order for the recorder to follow accurately the detector signal. If the scan rate is too high, band shapes and intensities will be distorted, and weak bands may be missed completely (Fig. 5).[1,22] It has been suggested[23] that the speed should not exceed 0.4 bandwidths per response period if band intensities are to suffer no more than 2% error from dynamic distortion. We use this criterion in our optimizing procedure. Bandwidths in condensed phases range from 2–20 $cm^{-1}$ or more, with the largest number falling in the range 4–7 $cm^{-1}$.[24]

The spectrometer response time is adjusted in some spectrometers by switching filter networks in or out of the servo amplifier (period adjustment) or, in others, by changing the attenuator drive speed, or by some combination of these manipulations. Regardless of the mechanism, any system which adds electrical or mechanical inertia to the servo loop effectively increases its response time. The response time can be determined by measuring with a stopwatch the time required for full-scale deflection of the pen when the sample beam shutter is closed or opened (that is, equilibrium 100% transmission to equilibrium zero % transmission averaged with the corresponding time for the reverse deflection). This method is an approximation, and is useful only in an underdamped servo system; in our discussion we deal, for the most part, with underdamped systems.

## Relationship Between the Variables

We have noted that the energy passed by the monochromator is proportional to the square of the slit width at any wavelength. If we eliminate the spectrometer gain $g$ between Eqs. 2 and 6, and impose the condition that servo energy should be constant, we have the relation

$$N \propto s^{-2}\tau^{-1/2}. \tag{7}$$

This important equation tells us that the noise decreases as the *square* of the slit opening, but only as the *square root* of the response time. An equivalent relationship has been given by Luft.[25] Thus, to halve the noise, we may either open the slits by a factor of the square root of two, or increase the response time (and scan time) by four. Usually the former choice will be more expedient.

It is the experience of the authors that trading a low noise level for higher resolution is usually not profitable; any slight advantage in real resolution is more than cancelled by the increased uncertainty in the recording (Fig. 4). High resolution conditions should only be employed in the few cases in which they are mandatory, such as resolving bands from vapor phase spectra.

## Speed Suppression

Modern optical null spectrometers incorporate special circuitry which causes the wavelength scan motor to run at a speed that is an inverse function of the error signal in the attenuator servo loop. As a result, the spectrometer scan rate decreases when a region of absorption is encountered, the amount of decrease depending on the suppression setting. The slow scan rate persists a second or two after the attenuator comes into balance, whereupon normal scan speed is resumed. This device, if properly used, allows rapid scanning over empty spectral regions and slower scanning in populated regions.

Since proper adjustment of the suppression control can increase the productivity of a spectrometer by a factor of two or three, proper manipulation of the system is important. In fact, it might be said that this control makes the attainment of dynamically reproducible spectra a practical procedure.

Obviously, a high noise level precludes the use of realistic suppression values; the system will suppress on noise and nothing will be gained. This is another reason why the noise level should not exceed 0.5% $T$ peak to peak.

## Optimizing the Spectrometer Settings

We have now summarized the physical principles involved in spectrometer dynamics. Before proceeding further it is important to define the specific purpose for which the spectrometer will be used, for this purpose has considerable bearing on just how the spectrometer parameters are to be set. Following is a list of general applications; a systematic method of achieving optimum spectrometer settings for each of these situations is discussed in detail below.

(1) *General purpose spectra.* A standard setup should be employed for most qualitative and quantitative analyses. These conditions should be determined carefully, and once established, should be adhered to except for special cases as noted. Presumably, all reference spectra will be obtained under these conditions, as will the great majority of spectra used in chemical applications. The advantages of doing qualitative and semiprecision quantitative analyses under a set of fixed conditions are well known.[2] Since a dynamically accurate spectrum is vastly more useful than a carelessly run one—even for rough survey work—it is a matter of real importance to establish carefully the parameters used for general purpose spectra.

(2) *Low noise level spectra.* Where best precision quantitative analyses are being performed, especially when ordinate expansion is employed, a low noise level is desirable. This condition usually requires some sacrifice of resolution and increase in scan time.

(3) *Limited optical energy spectra.* Many spectroscopic applications cause loss of transmission throughout the entire spectrum; common examples are the use of beam condensers, ATR units, and samples which scatter radiation. In these applications an $I_0$ line may result which is so low that interpreting the spectrum is difficult. The $I_0$ trace can be returned to normal by placing screens or shutters in the reference beam of a double-beam spectrometer, but, as a result, the spectrometer must operate with a fraction of its normal energy. Unless the operating parameters are adjusted

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to compensate, the spectrum so produced will be of poor quality. Some further sacrifice in noise level and resolution is usually necessary for limited energy operation.

(4) *High resolution spectra.* For applications where best resolving power is required (such as the study of band contours in vapor phase spectra), the slit width must be decreased. Unless changes are made in the other operating parameters, the spectrum will be most unreliable. An increase in both noise level and time of scan usually will be required.

(5) *Fast scans.* In order to observe transient phenomena, or obtain spectra of unstable materials, the scan time must be decreased. As a result of this and other necessary changes, lower resolution and probably some distortion of the spectrum will result.

## General Purpose

Once general purpose (or standard) conditions are established, other operating conditions are easily derived. We therefore start with a discussion of a systematic method whereby such conditions can be established.

It must be understood that there are limits to what can be attained in operation of a spectrometer, and our general purpose conditions are a compromise between resolution, noise level, and time spent in obtaining a spectrum. A noise level of 0.5% $T$ or lower is strongly recommended, for reasons cited above, especially the fact that full advantage cannot be taken of a speed suppression device unless this condition is met. The user must decide for himself what time limits and resolution limits he will live within; we strongly suggest, however, that a scan time of 20–30 min and a resolution of $\Delta\nu_{1/2}$ = 1–3 cm$^{-1}$ are practical and realistic limits for general purpose conditions with the spectrometers currently available.

The procedure for establishing these conditions, starting from scratch, is a bit tedious. As is obvious from the preceding discussion, the operating parameters are highly interrelated; meaningful measurements of the effect of one parameter on the quality of the spectrum cannot be made if other parameters have unrealistic values. Thus, establishing the values of operating parameters is largely a cyclical method of successive approximations. The following systematic approach seems to be, in the experience of the authors, satisfactory.

*Step 1.* Choose (arbitrarily) a slit width program. Considerable time will be saved in the following procedure if the slit width chosen at this point is wider than one would consider ideal. As Eq. (7) suggests, high resolution is difficult to achieve; furthermore, most ir spectroscopic applications to materials in condensed phases do not require the ultimate in resolution. The standard slit program suggested by the spectrometer manufacturer may be unrealistic for practical chemical work; a better initial choice is about one and a half times this setting.

*Step 2.* Establish an approximate scan time. For the first trial, 1 h to scan the entire spectrum is about right. (This interval can be reduced by about a factor of three when speed suppression is added further in our procedure.)

*Step 3.* Pick a servo response time consistent with this time of scan. From Stewart's[24] criterion of 0.4 bandwidths scanned per response period, from the fact that relatively few condensed state absorptions are narrower than ≈3 cm$^{-1}$ and given a 1-h scan time, the response period will fall in the approximate range 0.5–2 sec. Although lower noise will result from choice of a longer response period, the effect on band intensities of an unrealistically long period will be evident when dynamic response checks are made (see below). At this point the choice should be toward a shorter response period.

*Step 4.* Adjust the gain control for correct servo energy. The servo loop has too much energy if it tends to oscillate, and in this situation the gain should be reduced. Reduction of the gain control in this case must be carried to a point somewhat below cessation of oscillation; if operation of the spectrometer is attempted too close to this point, the servo system may break into oscillation at a later time; also, overshoot may be excessive (see further discussion under step 7). On the other hand, too little servo energy will cause the servo system to be sluggish. An improperly tracked, or dead, spectrum will result. For routine spectrometer operation, the energy of the servo system should be such that the pen overshoots 2–4% in response to a sudden partial blocking or uncovering of the sample beam (assuming an underdamped servo system as usually employed with ir spectrometers). For situations in which a long response time is used (see below) and the pen motion is slow, the dead spot test is useful; even for situations of short response time the dead spot criterion must be met. The test is carried out as follows: with the $I_0$ trimmer adjustment set to give double-beam balance near the midpoint of the chart, place an object (such as a hand) in the sample beam, and slowly withdraw it, marking the exact point on the recorder chart at which the pen comes to balance. Repeat this test in the reference beam, again noting the final pen position. These two points should coincide (*within the limits of the noise level to be chosen*); if they are separated significantly further than this, more servo energy is required, obtained by increasing the gain control. These tests should be carried out in a region where atmospheric absorption is absent, e.g., at 1000 cm$^{-1}$.

*Step 5.* Check the noise level. The noise level is conveniently measured by scanning a short region of the spectrum with nothing in either beam, using a region free from atmospheric absorption bands—the region from 900–1100 cm$^{-1}$ is suitable. For most practical chemical work, an average peak-to-peak noise level of 0.5% $T$ or less is highly desirable. This level allows detection of weak bands (which would otherwise be lost in the noise) and is mandatory if speed suppression is to be used to its greatest advantage.

If the noise level is higher than 0.5% $T$, the slits will have to be widened, and steps 4 and 5 repeated. The amount of noise will indicate the factor of slit width in-

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crease necessary: to reduce noise by a factor of two, for example, widen the slits by a factor of the square root of two, and decrease the gain by a factor of two (see Eqs. 2 and 3). Decreasing noise by increasing the response time should be avoided because it will lead to dynamic distortion, as revealed subsequently, or force a commensurate increase in scan time. If the noise level is quite undetectable, it may indicate that the criterion of correct servo energy has not been met (repeat steps 4 and 5); if repetition of step 4 shows correct servo energy, and noise is still not detectable, the slit width can be decreased to provide better resolution (repeat steps 1, 4, and 5).

While ~0.5% $T$ noise is tolerable, it is best to have a noise level somewhat lower than this to allow for the eventual, slow deterioration of the spectrometer. Slight decrease in spectrometer performance can be corrected by small upward adjustment of the gain control, if a conservative noise level has been chosen.

*Step 6.* Adjust spectrometer drift (balance). When both beams are completely blocked, the pen should show essentially no motion; if the pen moves under these conditions, the servo is said to drift or be out of balance. This imbalance will lead to a net displacement of the pen from its true equilibrium position under normal operating conditions: an up-scale drift with both beams blocked will cause the pen to read too high a transmission value at each point in a scanned spectrum; a down-scale drift results in low transmission values. Therefore, drift must be kept to a minimum. The balance control is set so that, with both beams completely blocked, the pen motion is as small as possible. (Some operators prefer a *small* amount of up-scale drift in order partly to compensate for difficulties in defining 0% $T$ in an optical-null double-beam spectrometer. See Ref. 3.)

*Step 7.* Automatic speed suppression is now added. The basic scan rate can then be increased by about a factor of four. This rate will be attenuated appropriately by the speed suppression device when absorption bands are being scanned. The noise level is rechecked to ensure that it is ≈0.5% $T$ average peak to peak. The scan rate, with the suppression control off, is determined with a stopwatch; an error signal is then introduced by turning the pen balance (or drift) control, with the beam shutters open, in order to displace the pen by about 2% from its equilibrium position. The suppression setting is then advanced until the scan speed has been slowed by a factor of three or four. This test should be repeated by displacing the pen in the opposite direction to that used previously. After rebalancing the beams, the operator should recheck the scan rate to ensure that the spectrometer is not suppressing on noise. If it is, either the noise level or the suppression setting must be reduced. Occasionally, stray signal pickup will cause a reduction in the scan rate at high suppression settings. Such pickup can be detected by turning the amplifier gain to zero (no noise) and comparing the scan rate at different suppression settings; the scan rate should remain the same.

*Step 8.* A test spectrum is run. Some standard material, having a variety of sharp and broad bands (such as indene, which is also useful as a wavenumber calibration standard[16]), should be used. Then the spectrum is rescanned at one-fourth the normal rate. Unless the two spectra have essentially identical absorption band ratios (using pairs of bands in which a sharp band is compared with a broad one), the servo system is not dynamically correct.

Probably most common is the condition in which the more slowly scanned spectrum shows higher absorption intensity for the sharper bands, i.e., the sharp bands are too weak under standard conditions. This condition will result from either too little servo energy or too long a servo response time with respect to scan rate chosen. If settings must be revised, servo energy should be rechecked and corrected by widening the slits. (Servo energy can be increased by increasing gain, but this will result in greater noise, whose tolerable limit we are presumably already near.) If the servo energy is correct, the response time must be decreased. This adjustment will increase noise, which must be reduced by decreasing servo gain; this, in turn, leads to low servo energy that must be corrected by widening the slits. If the response time cannot be decreased further, a slower scan rate or more speed suppression must be employed. After appropriate adjustments are made, the test spectrum is rescanned at both the normal rate and slow (one-fourth normal) scan rate. Now these spectra should be essentially identical. (If the slit width has been changed in this process, the ratio of sharp band intensity to broad band intensity will have changed, but these new ratios are now fixed by the conditions under which the spectrometer must be operated to have a dynamically correct system.)

On the other hand, the original test spectra may have shown the absorption intensity to be *lower* for sharp bands in the more slowly scanned spectrum, i.e., the pen is overshooting the sharp bands under standard conditions. This condition will result from a servo system that is underdamped too far, usually caused by too much servo energy. (Other adjustments in the electronics of the spectrometer will also affect such things as damping and overshoot. In our discussion we have assumed that such adjustments have been properly made by the manufacturers.) If this is the case, servo energy is rechecked with the dead-spot test (described in step 4); the spectrometer slit width may be decreased to decrease servo energy, as long as the magnitude of the dead spot is within the noise level. After appropriate adjustments are made, the test spectrum is rescanned at normal and slow (one fourth normal) scan rate. Now these spectra should be essentially identical.

The conditions established by these procedures should be regarded as standard and should form the basis for all other spectrometer setups. It is important to ensure that the slit program as established above is *strictly reproducible*; once established, it should not be changed, except for the special conditions discussed below.

Spectrometer performance is best checked by running test spectra frequently—at least once a day. The day-to-day tune-up of the spectrometer should be accom-

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plished by varying only the servo gain control, the amount of speed suppression used, the setting of the balance control, the source current, and definition of chart 0% $T$. Attempts to tune up the spectrometer by varying slit width and response time should not be made; such adjustments are no substitute for maintenance repair work on the spectrometer.

## Low Noise Level

In the general purpose procedure above, the noise level will be just detectable in a recorded spectrum. However, if scale expansion is employed, the noise will now be distinctly noticeable, and if ultimate precision in quantitative analysis or ultimate sensitivity to small absorption bands is desired, noise should be reduced. With scale expansion at the desired level (for instance, 4×), a short portion of the $I_0$ line is scanned; noise level should now be 2% $T$ (assuming that 0.5% $T$ was the noise level under general purpose conditions). Presumably, if 4× scale expansion is being employed, reduction of noise level to 0.5% $T$ on the expanded scale is desirable, that is, noise should be reduced by a factor of four. This can be achieved by—(a) increasing the response time by a factor of sixteen, and decreasing the rate of scan by the same factor. This approach is impractical for scans involving a sizeable fraction of the complete spectrum, but is reasonable if only a short region of the spectrum is required, as for a quantitative analysis involving one or two absorption bands; (b) widening slits by a factor of two (thereby increasing servo energy by a factor of four), which now allows the gain to be decreased by a factor of four. The attendant loss of resolution by a factor of two is usually not significant in quantitative analysis. (See Ref. 3 for an extended discussion of this point.) Some combination of these two methods can, of course, be used. For example, increasing slit width by a factor of the square root of two allows the gain to be reduced by a factor of two; the other factor of two in noise reduction can be accomplished by increasing the response time by a factor of four (and increasing scan time by the same factor).

## Limited Optical Energy

Let us suppose that some special device (for example, a beam condensing apparatus) removes 75% of the energy at all wavelengths, thus resulting in an $I_0$ line near 25% $T$. In order to have a full scale spectrum, i.e., an $I_0$ line near 100%, a beam attenuator that removes 75% of the energy is placed in the reference beam. Under these conditions, the servo system will be operating with only 25% of its normal power, and the resulting spectrum will be distorted to some extent. For purposes of simple identity of a sample, this distortion may or may not be tolerable; in any case, a spectrum that is distorted will furnish less than the best obtainable information; if it is necessary to attenuate the reference beam to allow for even greater servo energy loss, the distortion will be severe. In order to ensure an undistorted spectrum (assuming, for illustration, that 25% of normal energy is available), the following approaches can be taken—(1) The spectrometer slits can be widened by a factor of two, thereby increasing servo energy by a factor of four. This will restore full servo energy. This approach is usually best, even though resolution is halved. (2) The gain may be increased by a factor of four to restore full servo energy. This process results in a factor of four increase in noise, i.e., to ≈2% average peak to peak. If only strong bands are being investigated, this approach is satisfactory; but weak bands, conceivably important, would be hidden in the noise. (3) The gain may be increased by a factor of four, and the noise increase may be compensated by increasing the response time and scan time by a factor of sixteen. This approach is practical only if a fairly narrow region of the spectrum is under investigation. (4) A combination of these approaches can be used so that only a small decrease in resolution, a small increase in noise, and a small increase in time constant and scan time result; further compromise may be made by arranging those parameters so that the servo operates with, for instance, 50% of normal energy, which will cause only a relatively small amount of distortion of the spectrum.

## High Resolution

In order to obtain a spectral slit width of, for example, one-fourth the normal value, the spectrometer slit width program must be decreased by a factor of four. This will reduce the servo energy by a factor of sixteen; and unless much of this energy is recovered by increasing the gain, the spectrometer will be so dead that no useful information can be obtained. To recover full servo energy, the gain would have to be increased by a factor of sixteen, which would result in an average peak-to-peak noise of ≈8%. To restore a ≈0.5 % peak-to-peak level, the response time and time of scan would have to be increased by the factor 256, which may be beyond the capabilities of the spectrometer and the patience of the operator. Therefore, obtaining a factor of four better resolution is not possible without some sacrifice in the other operating conditions.

Fortunately, applications requiring high resolution usually do not require the high demands of servo following fidelity and low noise level that we have set for general purpose operating conditions. The principal application of high resolution is investigation of rotation-vibration band ensembles of molecules in the vapor phase, where purely qualitative data (with respect to the ordinate axis) are sought. Cell lengths and pressures will usually be chosen so the bands being investigated will be fairly intense, so that considerable noise—perhaps as much as 2% or more average peak to peak—can be tolerated. Also, some decrease in servo following fidelity can be tolerated, and the spectrometer can be operated with ≈0.5 of normal servo energy. This compromise should not be used if weaker bands are to be investigated. Therefore, if resolution of, e.g., four times normal is mandatory, the gain increase could be as low as a factor of eight which, without change in time constant, would result in an average peak-to-peak noise of 4%; if now the time constant and scan time are increased by a factor of four, the resultant noise will be 2%, and the spectrum can be obtained in a reasonable

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**Table I. Summary of Procedure for Establishing Operating Parameters for General Purpose Spectra**

1. Choose slit setting
2. Choose scan time
3. Pick consistent response time
4. Adjust gain for proper servo energy
5. Check noise
6. Adjust balance
7. Add speed suppression and reduce scan time appropriately
8. Run test spectra

**Table II. Summary of Procedures for Establishing Operating Parameters for Special Purpose Spectra**

A. *Low noise*
   1. Establish general purpose conditions
   2. Determine noise level reduction factor
   3. Widen slits by $(\text{factor})^{1/2}$ and reduce gain by (factor); or, increase response and scan time by $(\text{factor})^2$
   4. Run test spectrum

B. *Limited energy*
   1. Establish general purpose conditions
   2. Determine energy attenuation factor
   3. Widen slits by $(\text{factor})^{1/2}$; or increase gain by (factor) and increase response time and scan time by $(\text{factor})^2$
   4. Run test spectrum.

C. *High resolution*
   1. Establish general purpose conditions
   2. Determine slit reduction factor
   3. Restore servo energy by increasing gain
   4. Increase response time and scan time to obtain tolerable noise level
   5. Run test spectrum

time. However, the best practice in high resolution applications is to employ the maximum increase possible in time constant and scan time, and tolerate as little decrease in servo energy as possible. (Often the practical upper limit to scan time is set by the condition that at slow scans the long residence time of the recorder pen at one spot on the chart paper will lead to recorder ink saturating the paper and rendering the spectrum unreadable. If long scan times are contemplated, a modified recorder pen should be employed.)

At noise levels as great as 2%, the speed suppression device will suppress on noise, and becomes effectively a constant speed attenuating device. In fact, speed suppression can be used in this way as a means of slowing the scan rate by a large factor so gear changes are not often necessary.

## Fast Scan

In order to obtain useful data in a time significantly less than normal scan time (roughly 100 $cm^{-1}$/min), the scan rate must be increased appropriately. The difficulty here is twofold: first, the scanning speed per band width may become comparable to the chopping frequency for narrow (or moderately narrow) bands, so that an entire absorption band may be traversed during a single cycle of the chopper; second, most commercial spectrometers do not permit reduction of the servo response time (or the scan time) to the extent required for truly rapid scans. Nevertheless, if some distortion can be accepted in the spectrum, the spectrometer may be programmed as follows. First, the required scan speed is determined. If, for example, we wish to use a 5-min scan, and our standard scan is 30 min, we should first reduce the response time by a factor of six. If this is not possible, the response time should be reduced as far as possible, with the understanding that some distortion is inevitable. Then the gain may be decreased to give a tolerable noise level. Now the slit is widened to restore correct servo energy, and finally, the maximum speed suppression invoked, consistent with the noise level chosen. A test spectrum is run and compared with the standard.

In order to obtain an ir spectrum in times of the order of 1 min or less, extensive modification of the spectrometer is required, and spectrometers usually have to be custom engineered for this purpose. For such applications, spectrometer manufacturers should be consulted directly. It should be recognized that any ir spectrum obtained in less than a minute will be significantly inferior if thermal detectors, presently employed in most spectrometers, are being utilized.

In any application requiring rather extreme alteration of spectrometer conditions (especially high resolution and limited optical energy), it is mandatory to set carefully the servo energy level to ensure that the spectrum will be recorded with reasonable fidelity. The simple overshoot and dead spot check discussed above under general purpose setup is a quick method which will suffice for many purposes. But the best check is to scan through a few bands at the selected scan speed and at a speed about one-fourth as fast, and to compare the band intensities in the two scans; if the band intensities differ materially, no really good results can be obtained under the conditions chosen.

## Summary

We have suggested a method for establishing optimum conditions of resolution, scan time, and noise level in spectrometer operation, and shown how one may quantitatively trade a position in one parameter to gain advantage in another. These procedures are summarized in Tables I and II. It will be found that use of a carefully established standard program will not only result in more accurate and reproducible spectra but increase the productivity of the spectrometer as well.

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Stewart of Beckman Instruments, Inc., H. B. Kessler of Perkin-Elmer Corporation, and by L. W. Herscher, H. D. Ruhl, and A. Bartz of The Dow Chemical Company.

## References


1. K. Frei and H. H. Gunthard, J. Opt. Soc. Am. **51**, 83 (1961).
2. G. Petrash, Opt. Spectry. **6**, 516 (1959).
3. W. J. Potts, Jr., *Chemical Infrared Spectroscopy*, (John Wiley & Sons, Inc., New York, 1963), Vol. I.
4. J. U. White and J. N. Howard, Opt. Soc. Am. **46**, 662 (1956).
5. H. Sakai and G. A. Vanasse, J. Opt. Soc. Am. **56**, 357 (1966).
6. D. A. Ramsay, J. Am. Chem. Soc. **74**, 72 (1952).
7. S. Broderson, J. Opt. Soc. Am. **44**, 22 (1954).
8. H. J. Kostkowski and A. M. Bass, J. Opt. Soc. Am. **46**, 1060 (1956).
9. R. A. Russell and H. W. Thompson, Spectrochim. Acta **19**, 1013 (1963).
10. K. S. Seshadri and R. N. Jones, Spectrochim. Acta **19**, 1013 (1963).
11. D. Montegny, Spectrochim. Acta **20**, 1373 (1964).
12. Commission on Molecular Structure and Spectroscopy, International Union of Pure and Applied Chemistry, *Tables of Wavenumbers for Calibration of Infrared Spectrometers* (Butterworths, Washington D.C., 1961).
13. H. M. Mould, W. E. Price, and G. R. Wilkinson, Spectrochim. Acta **15**, 313 (1959).
14. J. E. Stewart, Appl. Opt. **4**, 609 (1965).
15. H. J. Sloane, Appl. Spectry. **16**, 5 (1962).
16. J. U. White and M. D. Liston, J. Opt. Soc. Am. **40**, 29, 36, 93 (1950).
17. N. Wright and L. W. Herscher, J. Opt. Soc. Am. **37**, 211 (1947).
18. M. J. E. Golay, J. Opt. Soc. Am. **46**, 422 (1956).
19. A. E. Martin, Trans. Faraday Soc. **47**, 1182 (1951).
20. H. K. Hughes, Appl. Opt. **2**, 937 (1963).
21. D. Z. Robinson, Anal. Chem. **23**, 273 (1951).
22. S. Broderson, J. Opt. Soc. Am. **43**, 1216 (1953).
23. J. E. Stewart, Paper No. 205 at the 12th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, 1961.
24. A. L. Smith, in *Treatise on Analytical Chemistry*, I. M. Kolthoff and P. J. Elving, Eds. (Interscience, New York, 1965), Part 1, Vol. 6.
25. K. F. Luft, Angew. Chem. **B19**, 2 (1947).
26. R. N. Jones and A. Nadeau, Spectrochim. Acta **20**, 1175 (1964).


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ORDINATE EXPANSION - This is for illustration only. This is for the Berkin-Elmer 467. For other instruments, check the instrument manual.

ORDINATE EXPANSION
Pushbutton switch, when depressed, (switch glowing) expands any 20% portion of the transmittance range to cover the entire chart ordinate.

Depress the ORDINATE EXPANSION push button to expand the ordinate sca 5 times the original scale.

If a specific transmittance range is to be expanded to fit full scale, determin from a spectrum run without scale expansion, this transmittance range ar locate the closest equivalent range in column B of Table 2. Scan to any wav number position at which the transmittance corresponds to that given column A for the desired expansion range and depress the ORDINATE E PANSION push-button switch (indicator lit). Determine the noise level by o serving the pen fluctuation about its mean value. If the fluctuation is considere excessive it can be decreased by increasing the TIME CONSTANT contr setting and/or by increasing the setting on the SLIT PROGRAM selector. the time constant is increased, the scan speed should be decreased to allo for slower pen response; if a wider slit program is used, the GAIN settir should be decreased. (The ORDINATE EXPANSION switch should be off whe changing the setting of the GAIN control.)

**Table 2 – Transmittance Values and Ranges**

| Column A Transmittance value at which to engage scale expansion | Column B Approximate Transmittance range covered by scale expansion | Column A Transmittance value at which to engage scale expansion | Column B Approximate Transmittance range covered by scale expansion |
|---|---|---|---|
| 0 | 0-20 | 52 | 42-62 |
| 4 | 3-23 | 56 | 45-65 |
| 8 | 6-26 | 60 | 48-68 |
| 12 | 10-30 | 64 | 51-71 |
| 16 | 13-33 | 68 | 54-74 |
| 20 | 16-36 | 72 | 58-78 |
| 24 | 19-39 | 76 | 61-81 |
| 28 | 22-42 | 80 | 64-84 |
| 32 | 26-46 | 84 | 67-87 |
| 36 | 29-49 | 88 | 70-90 |
| 40 | 32-52 | 92 | 74-94 |
| 44 | 35-55 | 96 | 77-97 |
| 48 | 38-58 | 100 | 80-100 |

METHOD OF ANALYSIS

Spectra that are developed for casework must be compared to standard scans run on a particular instrument or handled by the instrument's library. Standard spectra must be kept on file for the instrument if no library is available. Any spectra used to develop conclusions for casework must be kept with case files.

Periodically a polystyrene film should be run on the IR. These too, should be kept on file.

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# (2) Laboratory Techniques and Preparation of Samples

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In order to obtain a spectrum with well-defined peaks, one must have a thorough knowledge of the laboratory techniques used in infrared spectroscopy. Proper sample preparation may be considered the most important step toward obtaining a good spectrum. The preparation of naphthalene for analysis furnishes a good example of how sample preparation can change the spectrum. Dispersion in potassium bromide or a nujol mull, or deposition of a film by evaporation of a suitable solvent, often results in a spectrum which one can hardly associate with the familiar one for this compound; however, when naphthalene is carefully melted on a salt plate, the true spectrum—a readily identifiable curve with very sharp maxima—results.

The complexity of the problem of sample preparation is indicated by the variety of techniques that may be used, and by the multiplicity of conditions under which a sample may be run. Thus, samples may be investigated:

(1) in a transparent solvent;
(2) as a pure material, if the sample is a liquid or gas;
(3) as a melt;
(4) as a powder;
(5) as a mull;
(6) as a cast or pressed film;
(7) as a suspension in a liquid;
(8) as a film which has been microtomed from a thick sample;
(9) by reflectance (specular or ATR);
(10) as a single crystal;
(11) by pyrolyzing the sample and determining the spectra of the pyrolysis products;
(12) as a film which has been lifted from a surface;
(13) by diluting with an inert gas and condensing on cold salt plates (matrix isolation); and
(14) dispersed in a halide disk.

In addition, many modifications of the above methods are possible.

This chapter will attempt to present the various techniques, the special precautions which they require, and their limitations.

## SAMPLING TECHNIQUES

It is possible to determine the infrared spectra of materials in the solid, liquid, or gaseous state. There is a limit to the thickness of sample which the infrared beam can traverse, and therefore it is necessary that the sample be prepared in such a manner that the path length is not so large that all the energy of the beam is absorbed. For liquids which are strong absorbers in the infrared, this path length may have to be as small as 0.01 mm, while for gaseous materials it is not unusual to use path lengths of 10 cm or even several meters. Obviously, a strong liquid absorber can be dissolved in a suitable solvent and its spectrum determined in solution. Generally, if a material is a liquid or is soluble in a suitable solvent, the liquid state is the most suitable one for determination of the spectrum.

Determining the spectra of solid materials involves sampling problems quite different from those associated with liquids and gases. A solid may be crystalline or amorphous, or it may be present as a fine powder or as a film. Each of these conditions represents a different sampling problem, and techniques have been devised to handle all of them.

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### Liquid Cells

Liquid cells may be obtained in matched pairs from 0.01 to 4 mm in path length. Variable liquid cells that may be adjusted to any number of path lengths are also available. Microcells may be obtained with a variety of path lengths and volumes. For very small cells a beam condenser may be required to reduce the normal beam of the spectrophotometer to the size of the microcell window. Generally, beam condensers are not required for sample volumes greater than 0.01 ml, but use of such systems makes possible sample volumes as small as 0.002 ml.

Liquids with strong infrared absorbance generally require cells of 0.025-mm path length. However, when such a material is dissolved in a transparent solvent, the permissible path length may be increased to 0.1 mm.

A 1-mm cell is useful in trace impurity analysis, since the longer path length in such a cell allows one to detect bands due to materials present in very low concentrations.

A demountable cell is used for mull materials or liquids of high viscosity. Such cells may or may not use spacers. When spacers are used, when a thicker sample is to be run, the demountable cells differ from the sealed cells in that the spacer is not sealed to the windows of the cell. In addition, entrance ports are not necessary in the demountable cell since the cell can be taken apart and the liquid placed between the plates. Spacers of Teflon or any other plastic material can be used with this type of cell to control the thickness of the liquid.

(5)

## Liquid cell types

Three types of Beckman-RIIC general-purpose liquid cells are available; sealed, semi-permanent and demountable.

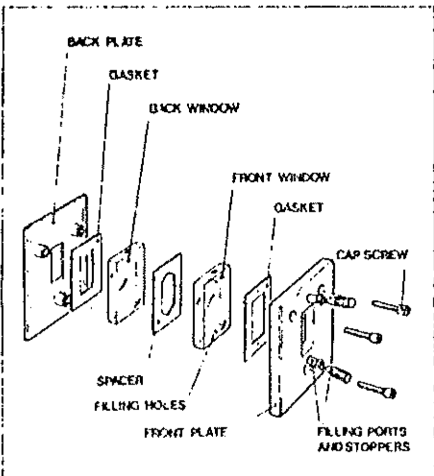
**Sealed.**—In this type of cell, windows and spacer are sealed together in a more or less permanent sandwich. The sealed cell has an amalgamated metal spacer between the windows, and an amalgamated metal gasket between the front of the cell and the front window. The amalgamation forms a permanent leakproof seal, although if necessary, it is possible to separate the windows by mechanical cleavage. The sealed cell is particularly suitable for use with volatile samples.

**Semi-permanent.**—In the semi-permanent cell, the spacer is not cemented to the window and the sandwich is clamped firmly together in the cell body. A PTFE spacer is used instead of the amalgamated metal spacer and gaskets. By changing the spacer, the path-length can be readily altered. A semi-permanent cell can be used as an alternative to a sealed cell, except with very volatile samples.

**Demountable.**—A demountable cell is one in which the sample is introduced when the cell is demounted. The sample is examined when the cell has been reassembled. Spacers can be either metal or PTFE, although lead is supplied as standard. This type of cell is also used for the "mull" technique.

## Matched cells

Sealed liquid sampling cells can be supplied in matched pairs and a pathlength match of better than 3 microns or 3%, whichever is the greater, can be guaranteed.




Typical Beckman-RIIC liquid cell

## How to select a liquid sampling cell

The selection of an infra-red (IR) liquid sampling cell from the wide range of types, window material, and pathlengths available, can be simplified by using the following step-by-step procedure:

**1. Determine the most appropriate window material** best suited to the sample by referring to the Table on page nineteen. This choice will depend upon the compatibility of the sample with salt windows and the wavelength range of the instrument.

**2. Select the optimum path-length.**—The choice is dependent on the spectral properties of the sample. For an unknown sample, where a variable pathlength cell cannot be considered, the following rule-of-thumb method may be used:

- Pure liquids (100%)—use 0.025 mm. pathlength.
- 10% solution in a convenient solvent—use 0.1 mm. pathlength.
- Trace amounts of sample (less than 1% solution)—use 1.0 mm. pathlength.

In practice, the 0.1 mm. pathlength is most generally used.

**3. Select a cell size** suitable for the available volume of the sample. If the volume is greater than 500 $\mu$l., use a standard cell. The fill volume for microcells is given in the appropriate text.

**4. Select a cell type** according to the physical properties of the sample. For low viscosity, low boiling point solutions, a sealed cell must be used. For high viscosity, high boiling point solutions, a demountable cell may be used.

**5. Select a special-purpose cell** for difficult-to-handle samples. For example, if spectral data is requ[illegible] at temperatures other than amb[illegible] a Variable Temperature Cell [illegible] should be used in conjunction w[illegible] a Variable Temperature Unit [illegible]. The use of Throwaway Cell TAC-[illegible] is advised if samples are of a radio-active, highly toxic, or hard-to-remove nature.

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### Throwaway Cells

The Beckman-RIIC range of throwaway, or disposable, liquid cells, is particularly useful when handling radioactive or highly toxic samples, especially those which are in aqueous solution or very viscous. Throwaway cells are also useful in routine or teaching situations where normal hygroscopic precision cells may be damaged.

The throwaway cell, including the two ports, is made from a single piece of silver chloride film, fused and protected by thermosetting plastic covers. In this way, the sample comes in contact only with AgCl. Two ports ensure rapid and convenient filling with sample; the sampling aperture is 2 × 14 mm. The cell has a volume of 0.005 ml. at 0.1 mm. pathlength, the volumes for other pathlengths being proportionate. Although identical in construction, each cell is colour-coded for immediate recognition of pathlength.

Throwaway cells are transparent up to the transmission limit of AgCl (approx. 22 microns) and can be used with practically all aqueous and non-aqueous samples. The low cost and low volume sample requirement makes their use an economical alternative to the standard sealed cell.

**THROWAWAY CELLS, TAC-1**

| Part No. | Type Designation | Pathlength (mm.) | Colour Code |
|---|---|---|---|
| 195470 | TAC-1 (In packs of ten) | 0.025 | Ivory |
| 195471 | | 0.05 | Red |
| 195472 | | 0.1 | Orange |
| 195473 | | 0.2 | Yellow |
| 195474 | | 0.5 | Green |
| 195475 | | 1.0 | Blue |
| 195476 | TAC-1A (Assorted sizes) | 0.025, 0.05, 0.1, 0.2 (two of each) 0.5 and 1.0 (one of each) | |

| | |
|---|---|
| Throwaway Cells, Ivory TAC-1 | 30.50 |
| Throwaway Cells, Red | 38.00 |
| Throwaway Cells, Orange | 30.50 |
| Throwaway Cells, Yellow | 38.00 |
| Throwaway Cells, Green | 30.50 |
| Throwaway Cells, Blue | 30.50 |
| Throwaway Cells, Assorted Sizes, | 38.00 |

(E) Silver Chloride cells such as the one shown made by Wilks is extremely useful for small samples which may contain water such as arson distillates and engine oils.

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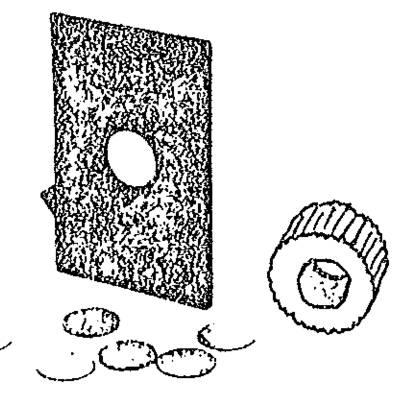
## (6) MINI-CELLS

One of the most commonly-practiced techniques for obtaining qualitative infrared spectra of liquids is to place a few drops of the sample between salt windows and press the windows together using an appropriate holder. The Mini-Cell represents the most economical approach to this method of sampling.

The Mini-Cell has a threaded, two-piece Delrin body, and two silver-chloride cell windows, discs of special design. The windows fit into one portion of the cell. The second portion of the cell is then screwed in to form the seal.

The AgCl cell windows each contain a 0.025mm circular depression. The rim of the window is flat, and the circumference is beveled to insure proper sealing. Because AgCl flows slightly under pressure, a tight seal is formed. (The Mini-Cell holds acetone for over an hour without measurable loss, and $CCl_4$ overnight.)

The 0.025mm circular depression in each window enables the cell pathlength to be varied, as shown in the adjoining diagram. The windows can be placed back to back for a conventional smear, or arranged as illustrated for a 0.025mm or a 0.05mm pathlength. (The 0.1mm depression windows are used in the same manner.)



Mini-cell kit

The cell windows are reusable and stand up well under heavy treatment such as might be encountered in the student or criminalistics lab·atory

Windows positioned for smears

| | | |
|---|---|---|
| 4041 | Mini-Cell* Kit complete with sample holder, cell slide and six AgCl mini-cell windows with 0.025mm depression ...... *Patented | $27.00 |
| 4045 | Mini-Cell AgCl Windows, pkg. of 6 with 0.025mm depression | $20.00 |
| 4073 | Mini-Cell AgCl Windows, pkg. of 6 with 0.1mm depression.. | $20.00 |
| 4074 | Mini-Cell AgBr Windows, pkg. of 6 with 0.025mm depression | $40.00 |
| 4075 | Mini-Cell AgBr Windows, pkg. of 6 with 0.1mm depression.. | $40.00 |
| 4046 | Mini-Cell Student Pak Deluxe includes two windows, sample holder cell slide and instructions ................ | $15.00 |
| 4047 | Mini-Cell Student Pak Standard includes two windows, sample holder and instructions ........................ | $10.00 |
| 4039 | Mini-Slide Holder (additional holder for either the Mini-Press or Mini-Cell) .................................... | $ 3.00 |
| 4044 | Mini-Cell Sample Holder (windows not included) ......... | $ 7.00 |
| 999-3119 | Mini-Cell "O" Ring — lot of six ......................... | $ 2.50 |

Windows positioned for 0.025mm and 0.1 mm pathlength

Windows positioned for 0.05mm and 0.2 mm pathlength

Pathlength variations of Mini-Cell Windows

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(3) Demountable Sealed Cell

The Perkin-Elmer Demountable Sealed Cell (Fig. 8) fills many of the needs normally met by sealed cells, yet retains advantages of demountable cells.

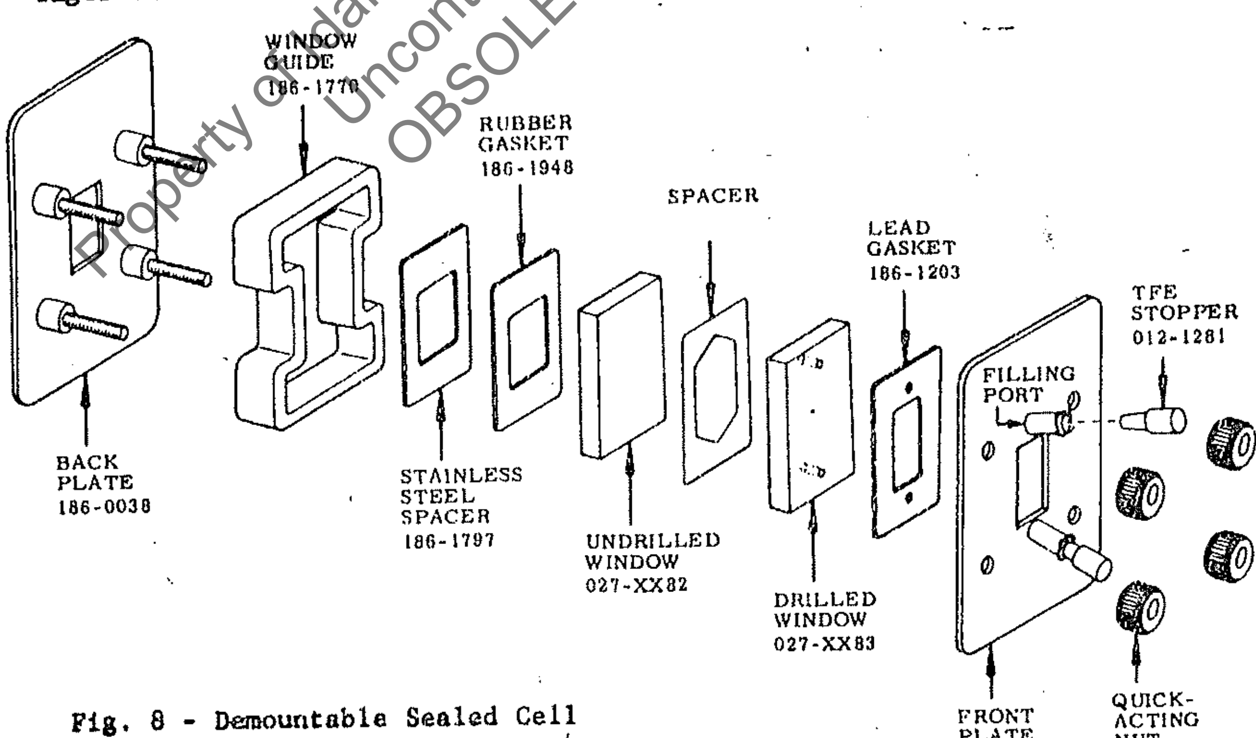


Fig. 8 - Demountable Sealed Cell

Interchangeable spacers yield a variety of cell thicknesses nominally from 0.015 mm to 0.2 mm. These thicknesses are reasonably reproducible after cell disassembly.

(5)

## IR TRANSMITTING MATERIALS

| Materials | Useful Range μ | Reflection Loss at 10 μ | Refractive Index at 5 μ | Solubility | General Properties |
|---|---|---|---|---|---|
| NaCl Sodium Chloride | 0.25 to 16 | 7.5% | 1.519 | Soluble in water and glycerine. Slightly soluble in alcohol. Insoluble in HCl. | Widest used window material for transmission and dispersion studies. Low cost. Easily polished to optical flatness. |
| KBr Potassium Bromide | 0.25 to 30 | 8.4% | 1.53 | Soluble in water, alcohol glycerine. Slightly soluble in ethers. | Chemical properties similar to NaCl, but not so hard. Slightly hygroscopic. Used for longer wavelength transmission work. |
| CsBr Cesium Bromide | 1 to 35 | 11.6% | 1.667 | Soluble in water and acids. | Soft and hygroscopic. |
| CsI Cesium Iodide | 0.24 to 55 | 13.6% | 1.74 | Soluble in water and alcohol. | Soft and extremely hygroscopic, but has largely replaced CsBr for long wavelength transmission work. |
| $CaF_2$ Calcium Fluoride | 0.13 to 10 | 5.6% | 1.399 | Insoluble in water. Soluble in ammonia and salt solutions. | Hard. Used for high-pressure cells. Difficult to grind and polish. Excellent transmission over short range. |
| $BaF_2$ Barium Fluoride | 0.13 to 12 | 7.7% | 1.45 | Insoluble in water. Soluble in acids and $NH_4Cl$. | Chemical characteristics similar to $CaF_2$. Very sensitive to mechanical and thermal shocks. |
| AgCl Silver Chloride | 1 to 22 | 19.5% | 1.997 | Insoluble in water and alcohol. Soluble in $NH_4OH$ and strong bases. | Mechanical properties resembl[illegible] lead. Colourless, ductile, and corrosive to metals and alloys. Corrosion retarded by coating with silver sulphide, or silver plated metal in contact. Cleaned and polished using a 0.2 strength acid fixer. Good resistance to thermal shock. |
| KRS-5 Thallium Bromide Iodide | 1 to 38 | 28.4% | 2.380 | Sparing soluble in water. Soluble in bases. Insoluble in acids. | Double salt containing TlBr (48%) and TlI (52%). Excellent transmission. Of wide use in the infrared laboratory, particularly in ATR work. |
| Irtran-2 | 2 to 13 | 12% | 2.245 | Insoluble in water, decinormal acids, and bases. Virtually insoluble in all organic solvents. Reacts with strong oxidising agents. | Zinc Sulphide. Extremely hard and durable. Withstands thermal shocks. Useful for temperature range – 200°C to +800°C. |
| Polyethylene | 16 to 1,000 | — | — | Insoluble in water. Tends to swell and become contaminated with some organic solvents. | High density type most suitable for far-infrared windows. Cannot be used for high temperature work (m.p. 110°C). |
| AgBr Silver Bromide | 0.45-35 | 25% | 2.2 | Insoluble in: water, Acetone, Nitrobenzene, Methanol, Saturated alcohols. | Mechanically similar to AgCl. More expensive than AgCl—Darkens in time with UV irradiation. |

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(7)

# ARSENIC SELENIDE

## INFRARED CELL WINDOWS

Arsenic selenide ($As_2Se_3$) is an optical material particularly suited for cell windows and ATR in infra red spectrophotometry with excellent transmission from 0.8 to 16.7 microns (12,500 - 600 $cm^{-1}$). It is insoluble in water and acids, thus making it excellent for use where water is present in organic samples for determining IR spectra for aqueous solutions. It is technically a glass, having no defined crystalline structure. Hardness is three times that of NACl, twice that of KRS-5.

### PHYSICAL CHARACTERISTICS OF ARSENIC SELENIDE

| | | |
|---|---|---|
| Optical Properties: | Useful range | 0.8 - 16.7 microns<br>12,500 - 600 $cm^{-1}$ |
| | Refractive Index | 2.8 |
| Physical Properties: | Knoop Hardness | 80 - 100 |
| | Density | 4.75 |
| | Thermal Conductivity | 0.0016 (cal/Sec. cm. °C) |
| | Coefficient of Expansion | $19 \times 10^{-6}$/°C |
| | Strength | Unpliable, Brittle |
| | Young's Modulus | 20.49 $kg/mm^2$ (7psi) |
| Chemical Properties: | Water Resistivity | Insoluble |
| | Acid Resistivity | Insoluble* |
| | Base Resistivity | $<$ pH 11 - Excellent<br>$>$ pH 11 - Poor |
| | Organic Solvent Resistivity | Insoluble |

*Soluble only in concentrated nitric acid and aqua regia.

At room temperature, arsenic selenide may be used in 35% HCl, 95% $H_2SO_4$ (up to two hours), 10% $HNO_3$ and all organic solvents.

### PRICE LIST

| DISCS (mm) | PRICE | RECTANGLES (mm) | PRICE |
|---|---|---|---|
| 13 x 2 | $26.00 | 15 x 10 x 3 | $32.00 |
| 25 x 2 | 26.00 | 22 x 8 x 9.1 | 41.00 |
| 25 x 3 | 26.00 | 41 x 23 x 6 | 43.00 |
| 25 x 4 | 26.00 | 45 x 20 x 6 | 43.00 |
| 25.2 x 5 | 27.00 | 38.5 x 19.5 x 4 | 53.00 |
| 25 x 6 | 27.00 | 50 x 25 x 6 | 55.00 |
| 32 x 3 | 36.00 | 39.5 x 39.5 x 6.5 | 68.00 |
| 38 x 6 | 45.00 | 65 x 32 x 5 | 94.00 |
| 41 x 3 | 50.00 | 66 x 30 x 4 | 95.00 |
| 49 x 6 | 57.00 | ATR 45 Degree Ends | |
| 48.5 x 6.5 | 64.00 | | |
| 56.7 x 6 | 79.00 | 52 x 20 x 2 | $114.00 |

Cost for drilled windows: $3.50 per hole.
Specify hole diameter and location.
Maximum hole diameter 1.6 mm.

(2)

***Polishing Salt (NaCl, KCl) Plates.*** While fully assembled cells may be purchased, the ability to polish and reassemble cells is extremely useful. Repolishing of old cell plates will result in better transmission, so that even when a sealed cell is not leaking it is sometimes of value to disassemble it and repolish the plates. Several methods of polishing have been published. One suggested manual method will be detailed here.

If the salt plate is very rough, it is first polished with 2/0 emery paper, using a light hand stroke, until all polish lines are straight and in the same direction. The operation is then repeated in the crosswise direction with 320 carborundum paper. Next, the plate is polished, with a light motion so that little heat is generated, on a flat piece of ground plate glass, using a little water as the polishing agent. Merely breathing on the glass is usually sufficient to deposit the necessary thin layer of water. This step requires a longer time than the previous operations and should be continued until interference fringes can be seen between the salt plate and glass. The salt plate will appear fairly clear at this point, with only slight haziness. The interference fringes indicate that the salt plate is now sufficiently flat.

The final polishing is done with a soft polishing cloth. A polishing compound such as Linde Fine Abrasive, Type A-5157, can be used. The cloth is moistened with absolute alcohol and the abrasive is added to its surface. Too hard or too rapid polishing may cause fine fracture lines to form in the salt plate. When the polishing is

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done properly, the plate will be crystal clear when finished. Powder may be removed from it by using a second polishing cloth free of abrasive. A new polishing cloth may wear down the edges of the salt plate more than the middle section, so that care must be taken in using new cloths until their nap is worn.

Another technique used to polish sodium chloride very quickly is to use a ground-glass plate and large amounts of water as a lubricant. This technique, while very fast, results in etch lines in the salt plate and is not recommended when the plates are to be used in sealed cells.

(3) CALCULATION OF CELL PATH LENGTH

The thickness of sealed cells is one of the basic quantities required for quantitative analysis. The value should be verified from time to time since the thickness may change due to a gradual erosion of the internal surfaces of the crystal windows in the cell. In addition to the thickness of cells, the thickness of polymer films is also important, both for quantitative analysis of the film and for a knowledge of the thickness itself.

The basis for the measurement is the interference fringe pattern produced when the transmission of the film or empty cell is recorded over a range of frequencies. This pattern, an effect of the wave nature of light, results from the interaction between radiation which is reflected by the inner surfaces and then transmitted. Unless absorption occurs, most of the radiation at any given wavelength is transmitted. A small amount, however, is reflected and then transmitted. The amount reflected depends on the difference in the refractive indexes of the two materials at the reflecting interface. The reflected radiation, as finally transmitted, may be exactly in phase with the radiation not reflected; it may be exactly out of phase; or it may be somewhere in between. If in phase, reinforcement occurs and the cell (or film) transmission is maximum; if out of phase, destructive interference occurs and the transmission is

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minimum. Between the maxima and minima, the transmission changes gradually with frequency as radiation, which is neither entirely in phase nor entirely out of phase, interacts. As the spectrophotometer scans the cell or film transmission at one wavelength after another, a wavy interference fringe pattern emerges, such as that shown in Figure 10 or Figure 11.

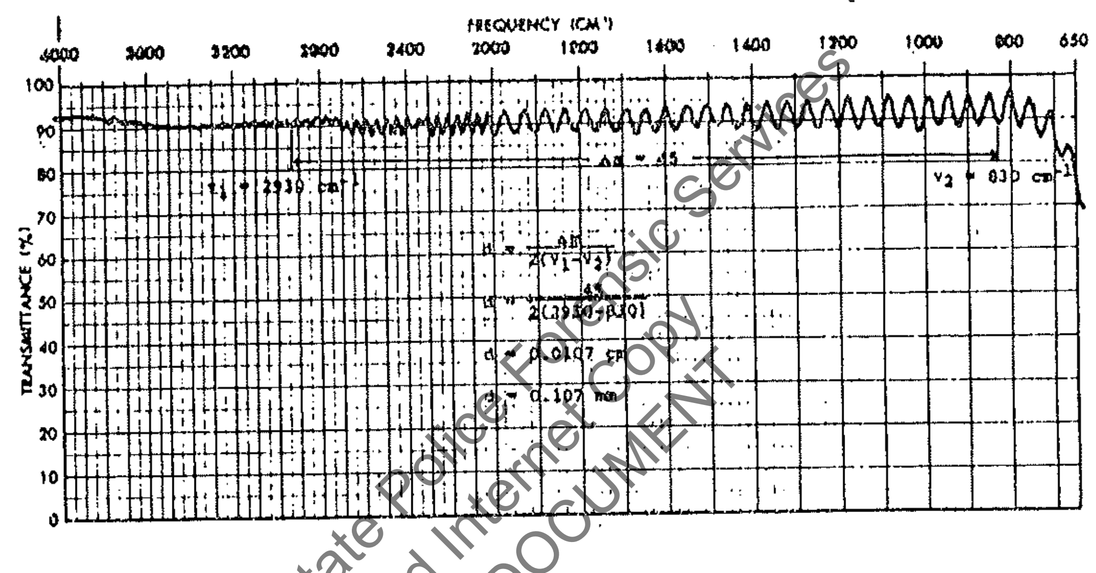


Fig. 10 - Fringe pattern obtained for an empty 0.1 mm thick sealed cell

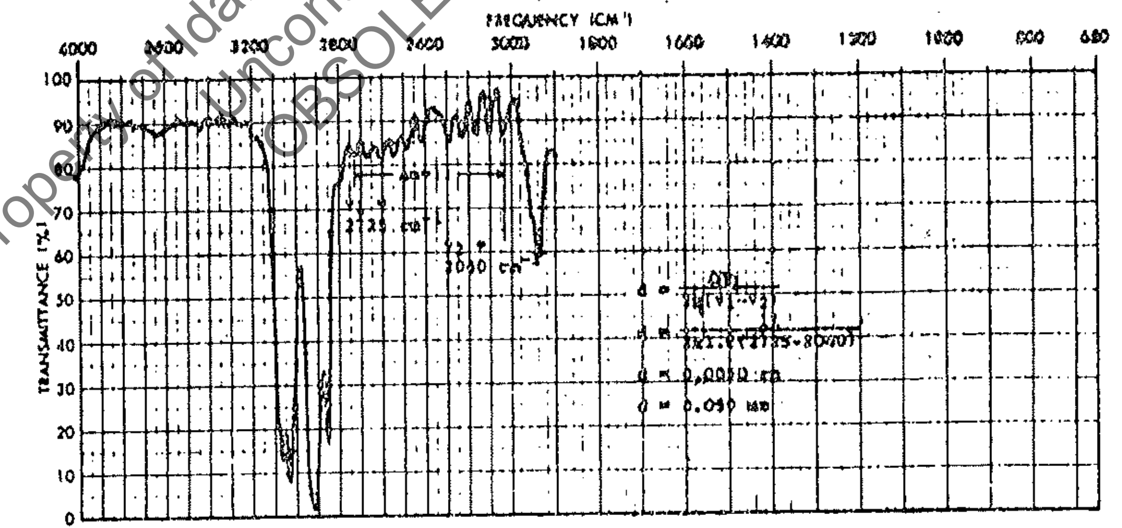


Fig. 11 - Fringe pattern obtained with the 0.05 mm thick polystyrene calibration sample

The thickness, t, of a cell or film can be calculated from data obtained from the fringe pattern by using Equations 1 and 2, below.



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For cells: $t = \frac{n \cdot 10^4}{2(\nu_1 - \nu_2)}$ = cell thickness in microns — Equation 1

For films: $t = \frac{n \cdot 10^4}{2\eta(\nu_1 - \nu_2)}$ = film thickness in microns — Equation 2

where: $t$ = thickness, in microns (u).

$\nu_1$ = frequency at which first maximum (or minimum) occurs, in wavenumber units.

$\nu_2$ = frequency at which last maximum (or minimum) occurs, in wavenumber units.

$n$ = number of maxima (or minima) in the interval from $\nu_1$ to $\nu_2$, a whole number.

$\eta$ = index of refraction for the film.

(2)

**Optimum Regions for Observing Interference Fringes**

| Cell Thickness, (mm) | Wavelength, (μ) |
|---|---|
| 0.0125 | 2 to 6 |
| 0.025 | 2 to 6 |
| 0.050 | 2 to 8 |
| 0.075 | 3 to 10 |
| 0.10 | 4 to 12 |
| 0.30 | 6 to 12 |
| 0.50 | 7 to 14 |

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*Cleaning and Drying of Liquid Cells and Salt Plates.* Because many materials are strong infrared absorbers, it is necessary to clean liquid cells quite thoroughly between spectral runs. As might be expected, sealed liquid cells are more difficult to clean than demountable cells, since the latter can be disassembled and the salt plates treated by some thorough and convenient method. One method of insuring that the plates of a demountable cell are clean is to polish them lightly with a polishing cloth containing a fine abrasive and a lubricant, taking care that the abrasive is one which will not become imbedded in the plate.

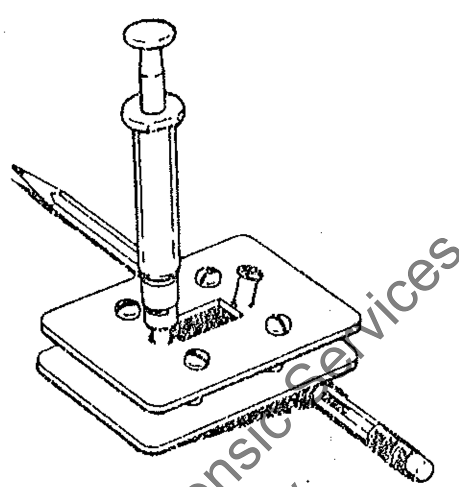
The recommended procedure for cleaning a sealed cell is to flush it thoroughly with a solvent that will dissolve all the materials that have been present in the cell since its last cleaning, and then to follow this flushing by passing dry air or nitrogen through the cell. Vacuum can also be used to remove solvent from the cell. The likelihood of spurious bands in the spectrum of the next sample run in the cell will be decreased if the solvent used in the flushing is one that is fairly transparent in the infrared.

To test if a solvent will attack the salt plate, a roughened salt plate is used. If a drop of the solvent appears to smooth the rough surface, the plate is soluble in the solvent.

*Filling of Cells.* A syringe of a few milliliters capacity fitted with a suitable adapter is extremely useful in filling liquid cells. The syringe is inserted with a gentle twist into the bottom well of the cell and the liquid is forced upward at a slow rate in order to avoid the accumulation of air bubbles. The rise of the liquid level can be observed visually and the action should be continued until the cell is filled. The plugs are then replaced and the cell is carefully wiped

before the spectrum is determined. A clouding of the salt plates indicates that the solution contains water or some other solvent capable of attacking the plates, in which case it should immediately be removed from the cell.

(3)



- Correct way to fill a sealed cell

The cell can be cleaned by removing the Teflon plugs and flushing the cell thoroughly several times with an appropriate solvent.

(E) The solvent can be removed by drawing dry air through the cell. A simple air dryer is easily made from a clean 50 ml syringe (disposable). The plunger is removed and a small piece of cotton pushed into the tube. After the cotton add Anhydrous $CaCl_2$ to fill approximately 2/3 of the remaining volume. The plunger can be used as a cap when the dryer is not in use. The dryer should be mounted directly on the cell's leur lock port. A vacuum pump attached to the second port will quickly dry the cell.

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(2)

**Solid or Semisolid Samples**

For some analyses it is necessary to obtain the infrared spectrum of a solid or a semisolid. Spectra of solid materials are affected by phenomena that are not encountered in the spectroscopy of liquids. One of these is *scattering*, which occurs whenever the particles of the solid are not small enough compared to the wavelength of the incident infrared radiation. Generally, if the particle size is below 3 $\mu$, very little scattering will occur since the wavelengths scanned will be longer than 3 $\mu$. Scattered radiation is a problem whenever the sample consists of a dispersion of a material of various particle sizes. No large amounts of scattered radiation occur in single crystals or amorphous films, although whenever a change in medium occurs, some radiation will be scattered. Whenever the particles are of such a size that they partially scatter the infrared beam, the spectrum will appear to have a rising baseline in the region from 3 to 6 $\mu$. Thus, when a spectrum of a solid shows a very sharply rising baseline, the particle size may not be small enough and the sample should be rerun after the particle size has been reduced. One technique used to reduce radiation scattering is to surround the particles with a medium such as a hydrocarbon oil (termed a *mull oil*). When this is done a particle size slightly larger than 3 $\mu$ can be used without encountering excessive scattering.

The effectiveness of the mull oil in reducing the amount of scattered radiation rests on the fact that scattering occurs when the refractive index of the solid differs from that of the surrounding medium. By replacing air by a medium which has a refractive index somewhere between that of air and that of the solid, the amount of scattering is reduced.

A second phenomenon that is observed when crystalline solids are used for infrared spectral determinations is the so-called *Christiansen effect* [27], which involves an anomalous dispersion of the infrared beam. Spectra showing this effect have regions, in the vicinity of absorption bands, which seem to be more transparent than the over-all 100% transmission if the instrument has been set so that the normal baseline is at 100% transmission. The Christiansen effect can occur even if the solid is surrounded by a mull oil used to reduce scattering. The phenomenon appears to involve a selective behavior for vibrations and appears to be different for ionic and covalent crystals. Since it is related to the rapid change in refractive index that occurs near an absorption band, the highest sample transmission is found on the high-frequency side of absorption bands.

A third phenomenon observed with crystalline solids is that of false or disappearing absorption bands. These effects occur if the polarization of the infrared beam and the orientation of the crystalline material are such that parallel and perpendicular radiation give different absorption bands.

The following sections will concern themselves with the practical steps necessary for an adequate preparation of samples to be analyzed in the solid state.

***Powders and Films.*** If the sample has a particle size near 3 $\mu$ its spectrum may be determined by depositing a fine powder of the material on a salt plate. Grinding mills are available which can be used to reduce sample materials to this fine particle size. The powder can be deposited on the plate by dispersing it in some volatile liquid, wetting the plate with the dispersion, and allowing the liquid to evaporate.

One variation of this technique employs water containing a dispersing agent such as sodium metasilicate to prepare fine dispersions of some samples. The dispersing agent is then removed by centrifugation and the fine particles of the sample can be separated into layers of uniformly sized particles by sedimentation.

Any technique which will produce a fine powder or a film on a salt window can be used. For example, one can prepare a slurry of the solid and a low-boiling liquid. When this slurry is spread on a

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hot salt plate, the rapid vaporization of the solvent leaves a fluffy coating of fine particles [20].

When the solid is soluble in a solvent which is not satisfactory for infrared analysis, it may be possible to evaporate the solvent, leaving a fine film of the solid. This technique can also be used to build up a film layer by layer when the solid is not sufficiently soluble to obtain a single film of the required thickness. Heat may be applied as the film is being formed. A number of problems are encountered when this technique is used, including the following:

1. Impurity bands due to trapped solvent or impurities from the solvent may appear in the spectrum of the sample.
2. If the film thickness is a multiple of the wavelength of the infrared beam, interference fringes may appear in the spectrum. Usually these fringes appear only for free films, and not for films deposited on salt plates.
3. Anomalous bands may appear in the spectrum due to crystallization or other polymorphic changes in the solute.
4. Decomposition of the sample may take place in the solvent, especially if heating is required to remove the last traces of the solvent.

***Mulls (Dispersions in Liquids).*** The technique of dispersing a solid in a liquid can be used to reduce the amount of light scattered from the solid, thus giving a spectrum with sharper absorption bands as well as preventing band shifting due to scattering. The name commonly given this technique is *mulling* the sample.

Hydrocarbon oils as well as halogenated hydrocarbons have been used as the liquid in mulls. Some of the more common oils used are paraffin oils such as nujol, fluorocarbons, and hexachlorobutadiene. More than one oil is frequently used to cover the entire spectral range.

Generally the technique of preparing a sample in mull oil consists in grinding the dry solid and then adding the oil and regrinding for several minutes. The sample so prepared is then gently squeezed between two salt plates until the thickness is suitable for a spectral determination.

In any technique where the particle size must be reduced by grinding it is possible that the compound may be changed by subjecting it to the forces of grinding. When the sample is not decomposed by grinding the absorption bands of the spectrum should be sharpened considerably by long grindings. If the spectrum of the mull does not show sharp bands even after repeated grindings, it is a strong indication that the mull technique is not suitable for that particular sample.

Usually grinding the sample before adding the mulling agent prevents orientation effects. For ideal mulling, to eliminate scattering, the dispersing agent should have a refractive index close to that of the sample.

Quantitative work using the mull technique is quite difficult since the amount of scattered light may differ even in samples of the same thickness, because of uneven grinding and other effects.

One of the disadvantages of mulling is that it cannot be used very conveniently on rubber, plastics, or resinous materials, although it is possible to freeze these materials, shatter them with a hammer, and grind them while cold to obtain good mull dispersions.

The fact that a change of state or a chemical decomposition of the compound can occur is another disadvantage that must be considered. Orientation and polymorphic effects have been noted in mulls [2]. Differences have been observed between the spectra of hand-ground and machine-ground samples [3]. Some workers suggest that vigorous grinding will reduce most samples to the amorphous state, which may be the most convenient physical state to use in

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making spectral determinations [4]. The proper mull-oil-to-sample ratio must be obtained. By examining the spectrum it is possible to ascertain whether the sample or mull oil peaks are too strong or too weak with respect to the other.

A variation of the mulling technique consists in dispersing solid samples in liquids by means of ultrasonic radiation or by adding dispersing agents. Any technique which keeps the solid in colloidal suspension is satisfactory provided the particle size is not so large that light scattering occurs. Dispersing agents that have been successfully used include aluminum stearate [5] and Alkaterge C [6]. With the aid of these dispersing agents, materials have been dispersed in carbon disulfide and carbon tetrachloride. Fibrous or high-molecular-weight materials may absorb the dispersing agents and are therefore not suited for this technique.

(3)

ADVANTAGES:

- Non-reactive medium
- Ratio or thickness easily changed
- No expensive equipment required

DISADVANTAGES:

- Preparation difficult -- grinding
- Mull bands interfere unless split mull used
- Sample not recoverable
- "Large" sample required, 25-50 mg
- Split mull - twice the work
- Impossible to do quantitative work without internal standard

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(2)

*Pellets.* Samples may be mixed with an alkali halide such as KBr and pellets pressed from the powder mixture. When the powdered mixture is dried and then placed in a die to which a vacuum can be applied, a transparent pellet can be obtained which shows very little light scattering. The sample appears to form a solid solution in the halide.

Several variations of the basic method of preparing pellets are in use. In one, the sample and halide are ground under a solvent such as $CHCl_3$ or $CCl_4$. In another, the halide and sample are melted and then allowed to cool into a clear pellet.

If some other technique can be used to obtain a fine powder of the sample and halide, it may not be necessary to grind them at all. For example, if both are soluble in water, then by evaporating a water solution of them a fine powder of the mixture can be obtained. A technique of removing the water very quickly, called *freeze-drying*, has been suggested [7, 8]. In this procedure the water solution of the sample and halide is frozen, and a strong vacuum is applied to the frozen solid. The water sublimes very quickly, leaving a fine powder.

Solvents other than water also may be used in the freeze-drying technique.

Obviously, any water remaining in the pellets will give unwanted absorption bands. Hence, since in the procedures usually used to grind a sample in the halide moisture pickup is very difficult to avoid, it is important that water be removed by drying the mixture after grinding but before the pellet is made.

In addition to KBr, a number of other materials, including NaCl, KCl, KI, and ammonium halides, have been used as the halide matrix for various samples. The pellet material must meet the following requirements:

1. It should have a high transmittance throughout the spectral range.
2. It should have a low sintering pressure.
3. It should be available in a pure state and be fairly nonhygroscopic.
4. It should have high chemical stability.
5. Its refractive index should be near that of the sample.

The advantages of using the pellet technique include:

1. Spectra are obtained which are free from interfering bands.
2. Less light is scattered.
3. For some materials superior resolution can be obtained, compared to spectra determined in other media.
4. Pellets can be stored conveniently for long periods of time.

Difficulties encountered with the pellet technique include the following:

1. For ionic samples, exchanges of the halide with the sample ions can occur, giving spectra representative of the various species present.
2. For some samples the observed spectra do not agree with those measured in other media. This is apparently due to physical isomerization or other changes in the structure of the sample. It appears that in some instances addition compounds are formed between the halide and the sample [9].
3. Physical or chemical changes can occur in the sample during the grinding and/or pressing process.
4. Because of the difficulty of completely removing water from the halide and the sensitivity of pellets to moisture, some water always remains as an impurity.

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In many cases the spectrum obtained by the use of a pellet does not agree with the spectrum of the compound measured in some other medium. This anomalous behavior has been investigated extensively [10-13]. One group of workers [12] suggested that in the case of some compounds the anomalous behavior is due to surface adsorption phenomena, notably the adsorption of a monomeric species of the solute on the halide molecules. For another series of compounds, Baker [3] suggested that the anomalous spectra were due either to polymorphic or amorphous forms of the sample in the halide disk. Tolk [9] has shown that the aging of pellets in a moist atmosphere removes some of the observed anomalies. In some instances heating of pellets achieves the same result.

Baker's studies of this anomalous behavior were quite extensive, and he reported the following factors that should be considered in preparing samples in pellet form.

*Crystal Energy of the Sample Phase.* Compounds with high lattice energy have the same mull and pellet spectra unless polymorphic transitions occur. Normally, compounds which melt above

200°C are stable if the grinding is not too vigorous. Low-melting-point (89–90°C) compounds normally show broadening and shifting of peaks.

*Energy of Grinding (Sample and Matrix).* If a compound shows broadening and shifting of peaks, vigorous grinding will give a spectrum nearly identical to the liquid spectrum, thus showing that the grinding is merely randomizing the molecular orientation. Hand grinding, because its intensity and uniformity are much harder to control, is inferior to mechanical grinding.

*Lattice Energy of the Matrix.* The lower the matrix lattice energy, the greater the proportion of the grinding done on the sample. To prevent excess grinding and working of the sample, it may be desirable to have high matrix lattice energy. The higher the lattice energy, the greater the sintering pressure required (KCl > KBr > KI).

*Particle Size of the Matrix.* As the initial size of the matrix particles is decreased, spectral distortion increases. Baker prefers to start with 20-mesh material rather than 250-mesh on the basis that the halide absorbs some of the grinding energy and does not permit too much to go into the sample. Others [14] have noted that the use of too large a grain size, even though uniform, gives rise to pellets scattering an appreciable fraction of the incident light.

*Ability of the Sample to Recrystallize in the Pellet* (related to crystal energy). It is frequently stated that one advantage of KBr is that the pellets can be stored, repressed, and used as an unchanging standard reference [10]. This is not necessarily correct, because recrystallization in the pellet can occur within a matter of minutes for high-crystal-energy compounds or over a period of months if the compound has low crystal energy. A possible means of reducing this effect is to heat the pellet below the melting point of the solid, thus allowing stress relaxation and recrystallization.

*Relative Stability of Polymorphic Forms.* For many compounds, under the conditions existing during grinding and fusing, there exist states that are more stable than the original form. This means that these compounds can be physically isomerized by vigorous pelleting. In many organic preparations crystalline structures result that are unstable at room temperature and will probably revert to the more stable forms, thus giving changing spectra.

Hydrates and samples that contain such ions as $NO_3^-$ which can undergo low-temperature transformations cannot be studied by the pellet method [10]. Some oxidation of the KBr can occur when it is mixed with a strong oxidizing agent.

Baker mentions two other effects which are less important but may play a role, namely, the surface adsorption of the sample on the matrix powder and the role of dielectric forces. We shall discuss only the latter effect here.

*Dielectric Forces of the Alkali Halide.* Several investigators [10,14–17] feel that the most serious difficulty with the pellet technique lies in anion exchange between the compound (especially inorganic salts) and the alkali halide. This causes the appearance of new bands and the shifting of others, the magnitude of the effect depending on the amount of exchange. The exact position of the new bands will depend on which alkali halide is used [10]. The exchange is promoted and accelerated by water adsorbed on the surface of the sample and the halide. All the halide pellet materials give rise to this phenomenon [10] to a greater or lesser extent, depending on water pickup and the pressure required for sintering. Because the effect is greatly dependent on the adsorbed water, the degree of exchange is quite variable. There is a possibility that the effect can be eliminated [15] in some cases by evacuating the die for a long period and by maintaining low water content and humidity. However, as yet, no definite solution to the problem has been found.

Generally speaking, the pellet technique appears to be unsatisfactory to a greater or lesser degree for inorganic compounds.

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many solid organic acids and imides, ~~some~~ carbohydrates, some amides, solid phenols, some amines, ~~and~~ ~~solid~~ amine hydrochlorides.

(3)

## THE POTASSIUM BROMIDE PELLET TECHNIQUE

### a. Introduction

Potassium bromide powder can be pressed at about 12 tons of force into clear discs having high transmission throughout the 4000 to 650 $cm^{-1}$ range of the infrared instrument. Before pressing, samples may be mixed with the KBr powder at a sample concentration level of 0.1% to 2%, and their spectra obtained in the KBr matrix. As in the mulling technique, the sample must be very finely ground in order to reduce scattering losses and absorption band distortions. This experiment demonstrates a wet-grinding method which has been found particularly effective for reducing the particle size of inorganic materials as well as some organic compounds. As with the mulling technique, the choice of wet or dry grinding depends on the sample and the quality of the spectrum desired by the operator.

The spectrum of the blank disc should exhibit no absorption bands from 4000 $cm^{-1}$ to 400 $cm^{-1}$. Should broad bands appear at 3350 $cm^{-1}$ and at 1640 $cm^{-1}$, the KBr contains water and should be dried overnight at 105° C in a vacuum oven. A sharp band at about 1400 $cm^{-1}$ indicates a chemical impurity, probably potassium nitrate. Bands between 3000 $cm^{-1}$ and 2900 $cm^{-1}$ usually indicate the presence of organic impurities. This contamination could also come from the previous sample. Always use maximum care to keep the KBr dry and free from contamination.

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Handling Procedures for KBr

Once the KBr has been found to be acceptable, divide the KBr into 10 smaller lots and place in clean, dry, screw-cap vials and store in a desiccator. Use only one vial at a time to prevent contamination of the entire lot.

Handling Procedures for Die

Never allow KBr and/or sample to remain on die. Moisture will be absorbed resulting in rusting of the die parts. Always remove remaining KBr and sample by washing die parts in warm water, followed by an acetone or methanol rinse to remove residual water. A final rinse with a volatile solvent like chloroform removes residual acetone or methanol, and then the die may be allowed to air dry.

Condition/Appearance of Pellet

(1) Fogged - moist KBr or sample; chemical impurities in KBr.

(2) Spotty - improper grinding or mixing.

(3) Crumbling - powder not leveled in die.

(4) Too thick - scattering, fogged; too intense bands--loss of apparent resolution, readability; recrush, regrind, repress. Thinning pellet by abrasion--watch for scattering (Christiansen effect).

(5) Too thin - fringe pattern at longer wavelengths.

(6) Improper ratio of sample to KBr

Organics: 1 mg sample to 300 mg KBr

Inorganics: 0.1-0.5 mg sample to 300 mg KBr

These will vary with sample.

ADVANTAGES:

- Inorganics as well as organics can be analyzed
- Easily storable for long periods when dry
- No solvent band interferences to 400 $cm^{-1}$ (25 um)

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- Sample recoverable
- Very little sample required

DISADVANTAGES:

- Requires dry KBr - vacuum drying with heat best
- Possible chemical reaction with moist KBr, or moist sample
- Preparation fairly difficult
- Expensive equipment required - die, press, oven, vacuum oven
- Limited to 25μ (400 $cm^{-1}$) scan
- Difficult to do quantitative analysis - usually requires internal standard, or band ratio technique

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(2)

***Cells for Gas Analysis.*** Cells for gaseous samples are commercially available. They differ from sealed liquid cells in that the path lengths are longer and the inlet system for introduction of the sample is designed to handle gases. Gas cells may be designed to operate at atmospheric or higher pressures. The high-pressure cells must be capable of withstanding the forces of the gas against the seals, cell windows, and walls.

Gas cells generally have path lengths of 2 cm, 5 cm, 10 cm, 1 m, or longer. A 10-cm cell is standard for many gas analyses. Long-path-length high-pressure cells are used for analysis of trace components (down to the parts-per-billion range) and for weak infrared absorbers, while short-path-length cells of course are used for strong absorbers.

A simple cell can be constructed from a Pyrex tube by using sealing wax to attach two NaCl end plates. This cell will not withstand high pressures but is adequate for low-pressure gas work.

Simple heated cells can be made by using a heating tape on conventional cells.

Metals such as brass can be used for cells, provided the brass is chemically blackened to decrease gas adsorption on the walls.

The long path length of cells designed for trace analysis of gases and vapor is achieved optically by reflecting the beam back and forth in the cell, using mirrors. This type of cell has been used to obtain an equivalent path of 1600 m. The optical diagram for a long-path cell is shown in Figure 3-3. Some cells of this type can be evacuated or pressurized. The number of passes through a cell of this type that can be made without decreasing the efficiency of the system can be calculated from the formula

$$R^n = \frac{1}{e}$$

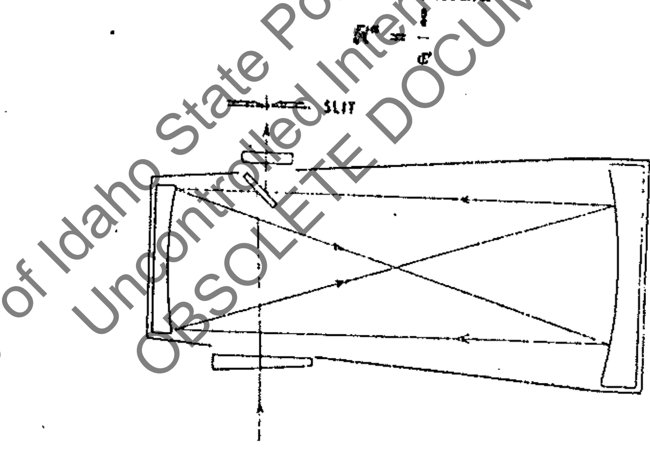


Figure 3-3. Optical path of a long-path-length gas cell.

where $R$ is the reflectivity of the mirror, $n$ is the number of passes, and $e$ is the base of natural logarithms.

(5)

**Selecting a Gas Cell**

**10 cm. pathlength universal cell.**—Gives a reasonable absorbance level for the majority of gases and is suitable for most sampling situations. Body of uniform cross-section.

**10 cm. pathlength low volume cell.**—Requires less sample for a given pathlength, and therefore, suitable for use when sample volume is restricted. Body has tapered cross-section conforming closely to radiation beam.

**9 cm. pathlength heated cell.**—Most suitable for vapour phase studies of relatively volatile samples. Temperature range from ambient to +250°C. Conical body cross-section.

**5 cm. pathlength high-pressure cell.**—For all high-pressure sampling with gases or liquids up to a pressure of 150 atmospheres, but limited by window material. Body of square cross-section.

**Variable pathlength cell.**—For studying strong and weak bands in the same sample, or for low concentrations. Internal mirror arrangement gives long pathlength in relatively compact cell. Pathlength is adjustable from 0.75 to 21.75 m.



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(3) MICROSAMPLING TECHNIQUES - GENERAL

a. What is Micro?

1 ul is a cube 0.001 in. on a side. Much easier to manipulate 1 ug of solid than 1 ug of a liquid.

For solids, mix with 1-2 mg KBr so that the sample can be scavenged together and kept in a visible condition until micro pellet is prepared.

For liquids, manipulate in 5-10 ul of volatile solvent or directly in a microsyringe.

(E) Infrared is excellent technique for microsampling but not good for trace analysis. (This is chemical trace as opposed to forensic trace) If the trace component can be isolated from its matrix, i.e., gas chromatography, thin layer chromatography, liquid chromatography, etc., then a spectrum of the unknown trace component or contaminant can be obtained by the proper choice of microsampling technique and proper manipulation of the sample once the technique has been chosen.

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(8) One of the advantages of infrared spectroscopy is the ability to work with minute amounts of sample. Routine analyses, without specialized equipment, can often be made on a few milligrams of most solids or liquids. But as the sample size shrinks to the microgram or submicrogram level, beam condensers will usually be required. One microgram of a sample with unit density, for example, would be a cube only 0.1 mm on each side. In contrast, the beam size in most infrared spectrophotometers is of the order of 12 to 15 mm high and as much as 1 to 4 mm wide. To obtain useful spectra from such small samples, it is necessary to use an accessory which will reduce the beam size at the sample point, and then re-expand it to properly fill the spectrophotometer aperture. Ideally one would like to reduce beam size to match the actual area of the sample, but this is not always possible, and in such instances, it is necessary to work with less than full beam energy. When this is the

case, a reference beam attenuator can be used to expand the spectrum back to full-chart size.

The 4X All Reflecting Beam Condenser (186-0220) can be used in all Perkin-Elmer infrared spectrophotometers except the Model 160. It can be used as a single unit in the sample beam only, or a pair of accessories can be mounted simultaneously in sample and reference beams except in the 7XX series of spectrophotometers where mechanical interference restricts use of the accessory to the sample beam only.

(3) AVAILABLE ACCESSORIES

a. Ultramicro KBr Die

1.5 mm pellet for samples down to 10 ug.

0.5 mm pellet for samples below 10 ug.

b. Beam Condensers

1 x 4 Refracting Beam Condenser: KBr focusing lenses require care in handling to prevent fogging. Does not lengthen sample beam to cause uncompensation in sample and reference beams. KBr lenses cut off scan at 25 u (400 $cm^{-1}$).

(8)

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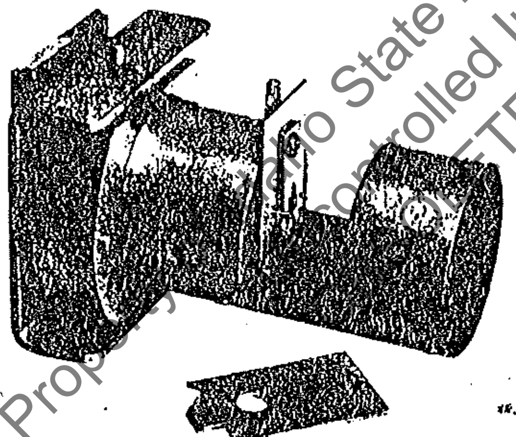
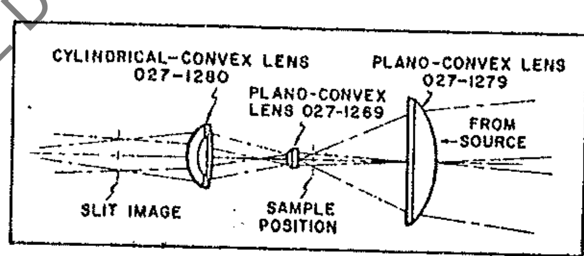


Beam Condenser, showing clip mounting in place, and alternate mounting plate below.




Optics, Beam Condenser Accessory.

(3) 4X Reflecting Beam Condenser: All front surface reflecting mirrors (no lenses). Permit scan out to range of any Perkin-Elmer instrument, including Model 180 (32 $cm^{-1}$). Lengthens sample beam by folding path in sample compartment, thereby causing uncompensation of sample beam to reference beam.

(9)

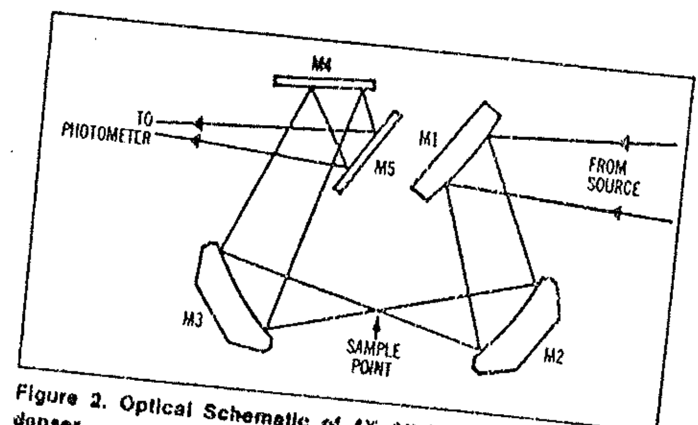



Figure 2. Optical Schematic of 4X All Reflecting Beam Condenser.

(3) 3X Beam Condenser/Specular Reflectance (Low Cost Combination Accessory): Provides 3X demagnification of source image at focus using front surface reflecting mirrors, with flat mirror in place on horizontal sample stage. With sample on horizontal stage, unit becomes a micro specular reflectance accessory.

c. 1.5 mm micropellet discs and 0.5 mm micropellet discs. See 2.a. above.

d. Microdemountable cells for analyzing micro volumes of liquids (non-volatile).

e. Micro sealed cells for analyzing micro volumes of volatile liquids.

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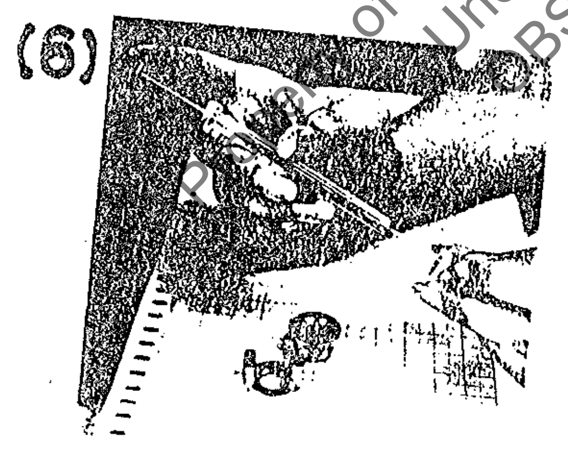
(6)



Filling An Ultra-Micro Sealed Cell

## SEALED ULTRA-MICRO LIQUID CELLS

This cell is constructed from two 13mm polished discs with a stainless steel precision etched spacer cemented between them. The top window is drilled to accept the sample. The clear aperture of the cell is 1.3 x 5.3mm.

The volumes of the cell with different spacers are as follows:

| Spacer Thickness | Volume Required to Fill Aperture |
|---|---|
| .015mm | 0.17 µl |
| .025mm | 0.28 µl |
| .050mm | 0.57 µl |
| .100mm | 1.14 µl |
| .200mm | 2.28 µl |

NOTE: The two filling holes have a total volume of 1.72 µl which need not be filled since capillarity will hold the sample in the cell aperture. Since pitch polished optically flat windows and precision etched, burr-free stainless steel spacers are used in the Wilks Ultra-Micro Cells, interference fringes are readily produced for precise cell calibration and the cells are highly reproducible in thickness.

(3)

## EFFECTS OF IMPROPER SAMPLE PREPARATION

1. LIQUIDS AND SOLUTIONS

Using demountable cell for volatile liquids--evaporation of sample, weak spectrum, or none at all.

Using wrong thickness sealed cell for pure liquids or solutions. Pure liquids with polar properties (alcohols, ketones) usually require 15 to 25u cells, rarely thicker. Non-polar liquids may require up to 50u sealed cell. Solution (~ 10%) usually require 100u sealed cell with second sealed cell containing only solvent in reference beam.

Leaking sealed or demountable sealed cells will show weakening of spectrum toward longer wavelength with possible superimposed fringe pattern indicating voids in cell.

2. SOLIDS: KBr PELLET

Faults Caused by Powder

| FAULT | CAUSE | REMEDY |
|---|---|---|
| 1. Marked scattering of light throughout disc. Poor visibility of distant objects. | 1. Impurities. Even mixtures containing as little as 5% of a second alkali halide show this effect. | 1. Use pure halides, "spectrum scopic grade." |
| 2. Irregular 'blotchy' appearance. | 2. Usually associated with dampness or coagulation of powder. | 2. Dry and break up powder. |
| 3. Appearance of fringe pattern. | 3. Pellet too thin. | 3. Use 300 mg KBr. |

Faults Caused by Sample

| FAULT | CAUSE | REMEDY |
|---|---|---|
| 4. Disc shows number of white spots, otherwise clear. | 4. Few coarse grains amongst otherwise fine mixture. Poor mixing. | 4. More even grinding of sample. Better distribution of sample in KBr. |

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| | | |
|---|---|---|
| 5. Irregular 'blotchy' or totally cloudy appearance. | 5. Damp sample | 5. Dry sample or pump under vacuum longer. |
| 6. Translucent or cloudy disc. | 6. Nature of sample. Forms poor solid solution with KBr. | 6. Sample with some other technique. |
| 7. Christiansen scattering, poor baseline, poor band shapes. | 7. Sample particles too large. | 7. Regrind sample to about 1u particle size. |
| | Pressing Faults | |
| 8. Disc opaque over part of area. | 8. Insufficient pressure possibly coupled with bad distribution. | 8. Grind and re-press. Take more care in distributing powder. Do not exceed 25,000 pounds total load. |
| 9. Disc perfectly clear when removed from press, developing irregular internal cloudiness after an interval of from one minute to several hours. | 9. Lack of vacuum. | 9. Check vacuum. More prolonged pumping. |
| 10. Cloudy area in center | 10. Anvil or plunger faces not flat. | 10. Replace or repolish anvil or plunger, or both. |

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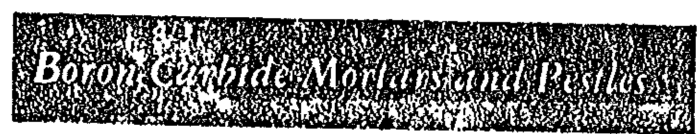
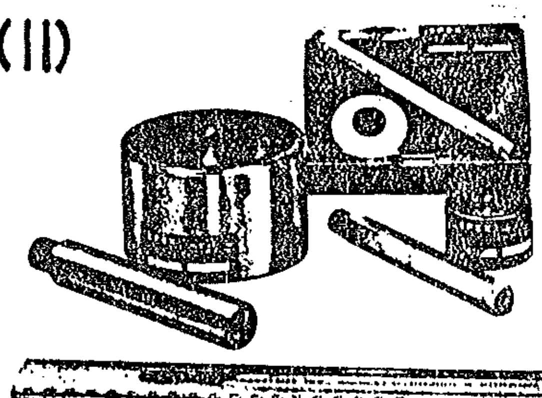
3. NUJOL MULLS

Nujol mulls are subject to the same problems as for samples prepared as KBr pellets, since the requirements for sample preparation are the same. This relates to the grinding of the sample to a small (~ 1u) uniform particle size. In addition, the ground sample must be uniformly suspended in the correct ratio of mulling agent in order to get a suitable spectrum.




(II)



Boron carbide is one of the best materials for hand grinding. Possessing a hardness close to diamond, it is also extremely inert, resisting attack by most acids and alkalies. In addition, boron carbide is unbonded so that the only possible metallic contaminating element is boron itself. Here it differs from, say, tungsten carbide which is usually bonded with cobalt. Following is a comparison with other materials as to Knoop hardness number:

| MATERIAL | KNOOP HARDNESS |
|---|---|
| Tungsten Carbide | 1050–1500 |
| Aluminum Oxide | 1265–1630 |
| Silicon Carbide | 2130–2140 |
| Sapphire | 1600–2200 |
| Boron Carbide | 2250–2260 |
| Diamond | 6000–6500 |

The material from which the mortars are produced is made by the Norton Company. Compressed under great pressure, its density approaches the theoretical value.

The 1/2" and 1" mortars are mounted in removable plastic bases for ease in handling; 2" d. and larger are encased in stainless steel. Pestles are attached to an aluminum handle.

**3201** Mortar and pestle, boron carbide. Mortar cavity 1/2" d. by 5/32" deep, highly polished. Pestle 1/4" d. ........ Set $ 55.00

**3202** Mortar and pestle, boron carbide. Mortar cavity 1" d. by 1/4" deep, highly polished. Pestle 1/2" d. ........ Set $110.00

**3205** Mortar and pestle, boron carbide. Mortar cavity 1-1/2" d. by 3/4" deep, highly polished. Pestle 9/16" d. ........ Set $293.00

**3203** Mortar and pestle, boron carbide. Mortar cavity 2" d. by 1" deep, highly polished. Pestle 9/16" d. ........ Set $470.00

**3204** Mortar and pestle, boron carbide. Mortar cavity 3" d. by 1-1/2" deep, highly polished. Pestle 3/4" d ........ Set $550.00

(E) The mortar and pestle 3201 is excellent for grinding small IR samples. The hardness of the boron carbide allows one to grind inorganics, paints and organics easily. Its small size is helpful when grinding small samples, ie. 50 $\mu$g or less.

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(3) COLLECTION AND MANIPULATION OF GC FRACTIONS

a. Best and least expensive collection device is open end 100 mm melting point capillary tube. May be used with or without coolant depending upon volatility of fraction to be collected.

b.

| B.P. of Fraction | Coolant |
|---|---|
| Above 150° C | Air (no coolant) |
| 75° to 150° C | Water or wet ice |
| 0° to 75° C | Dry ice |
| Below 0° C | Dry ice or liquid $N_2$ |

c. Coordinating fraction collection with appearance of chromatogram.

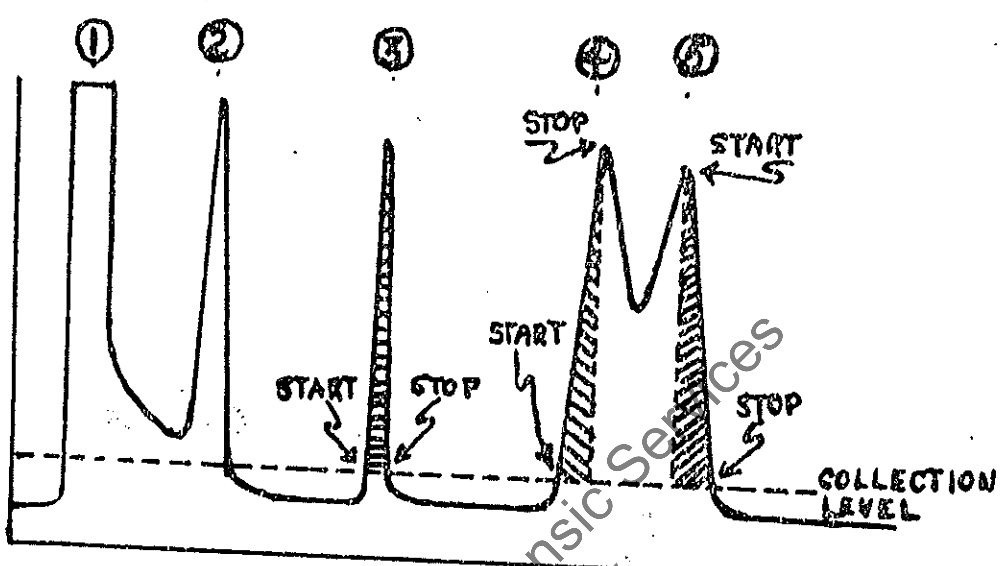


Fig. 17 -
Typical Chromatogram

Peak 1 - Solvent

Peak 2 - Unwanted peak

Peak 3 - Collection starts when pen rises to approximately 20% above baseline on chart.

Peak 4 and 5 - Unresolved doublet. Collection of peak 4 started as usual, stopped at peak maximum to prevent cross contamination with peak 5. Collection of peak 5 starts at peak maximum for same reason.

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d. Use of coolants

May be used in straight flow-through configuration for collection of solids. Solids may be collected directly on few mg KBr placed in capillary. Remove by tapping end of capillary on hard surface, or by inserting fine wire in capillary.

Where coolants are necessary, capillary may be shaped into "u" configuration and placed in small paper or plastic drinking cup or disposable plastic beaker as shown:

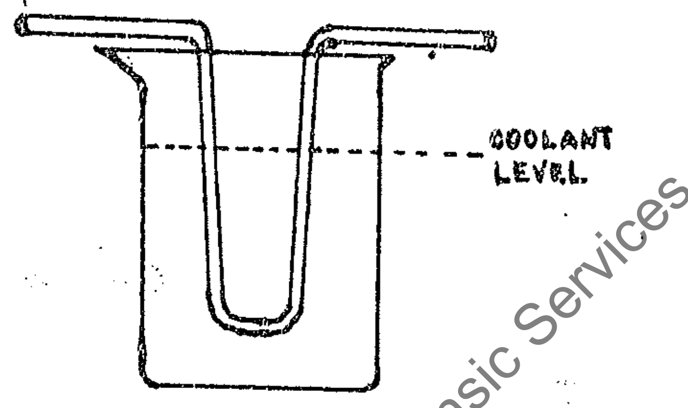


Fig. 18 - Collection Capillary in beaker with coolant

(3) If more than one pass is required for sufficient collection for IR spectrum, and fraction is liquid, use second capillary for second run, since carrier gas may evaporate fraction collected from first chromatogram.

(4) Don't use excessive cooling; i.e., don't use liquid nitrogen when dry ice or wet ice will do. Reason is that fraction may form aerosol and pass through capillary without being collected.

(5) When using KBr in capillary, use care not to block capillary.

(6) Keep equipment clean.

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(E) A new instrument, the CIRA 101, is available to use for running a GC type effluent directly on existing IR instrumentation. It is limited in the sense that it has a proprietary GC. Duane Mauzey (Riverside DOJ Lab) looked at the instrument but at the time none of the columns that were available were compatible with the types of separations needed by a criminalistics lab. I believe columns are now available.

PRICE: ABOUT $67.0

(21) The CIRA 101 chromatographic INFRARED analyzer is an infrared accessory designed specifically for separating multi-component mixtures into their pure constituents for infrared spectral analysis. The unit contains a proprietary gas chromatograph and an integrated infrared cell which together fit into the sample compartment of any standard infrared spectrometer.

Features of the CIRA's proprietary chromatograph include: high sample injection capacity, high resolution of eluting constituents, high concentration of sample to carrier gas, no consequential loss of resolution when flow of chromatograph is interrupted. The CIRA's chromatograph utilizes nitrogen as a carrier gas and incorporates a strip chart recorder which specifies the location and relative concentration of eluting constituents.

All operating procedures have been simplified and standardized so that the complexities and inconveniences of gas chromatography have been eliminated.

The CIRA contains a gold coated infrared cell with NaCl windows. The cell is maintained at a temperature of 250°C for compatibility with the chromatograph. It has an average transmission of 50%.

The CIRA contains a valving system which permits flow through the chromatograph to be stopped and a specific effluent to be locked in the IR cell for standard spectral analysis by the spectrometer. The unique features of the CIRA is that this "stop flow" operation is achieved with no consequential diffusion or loss of resolution of subsequent eluting peaks. Thus, the infrared spectroscopist is free to stop the flow of the CIRA on each of a series of peaks and run a full, normal infrared scan on each constituent. The CIRA will routinely provide standard identifying IR spectra of 1% or smaller components in a 50 μl sample injection.

## Operation

The CIRA 101 chromatographic INFRARED analyzer has been specifically designed for ease of operation for the infrared user. Absolutely no chromatograph experience or knowledge is required. Absolutely no modifications or special connections for your infrared spectrometer are required. The CIRA 101 chromatographic INFRARED analyzer operates as follows:

1. The chromatograph/IR cell section of the CIRA 101 is placed into the sample chamber of your infrared spectrometer.

2. The carrier gas flow is set to a standard flow rate.

3. The mixture to be analyzed is injected into the inlet of the CIRA 101. Concurrently, the CIRA 101 is switched on, starting the chromatograph. The chromatograph recorder begins and the columns start programming through a standardized temperature ramp.

4. The CIRA 101 then operates unattended until the first component of the injected mixture starts eluting. As the chromatograph recorder starts responding to the eluting peak and the pen passes through a pre-set amplitude, it sets off an alarm, thus notifying the operator that a component is starting to elute.

5. The flow of the chromatograph is manually stopped by closing two valves. (The IR cell can be purged if previously eluting constituents are present.)

6. Your infrared spectrometer is turned on and takes its normal infrared scan. While the IR spectra of the component is being scanned, the CIRA 101 is in a "stop flow" condition and the chromatograph's recorder is automatically stopped.

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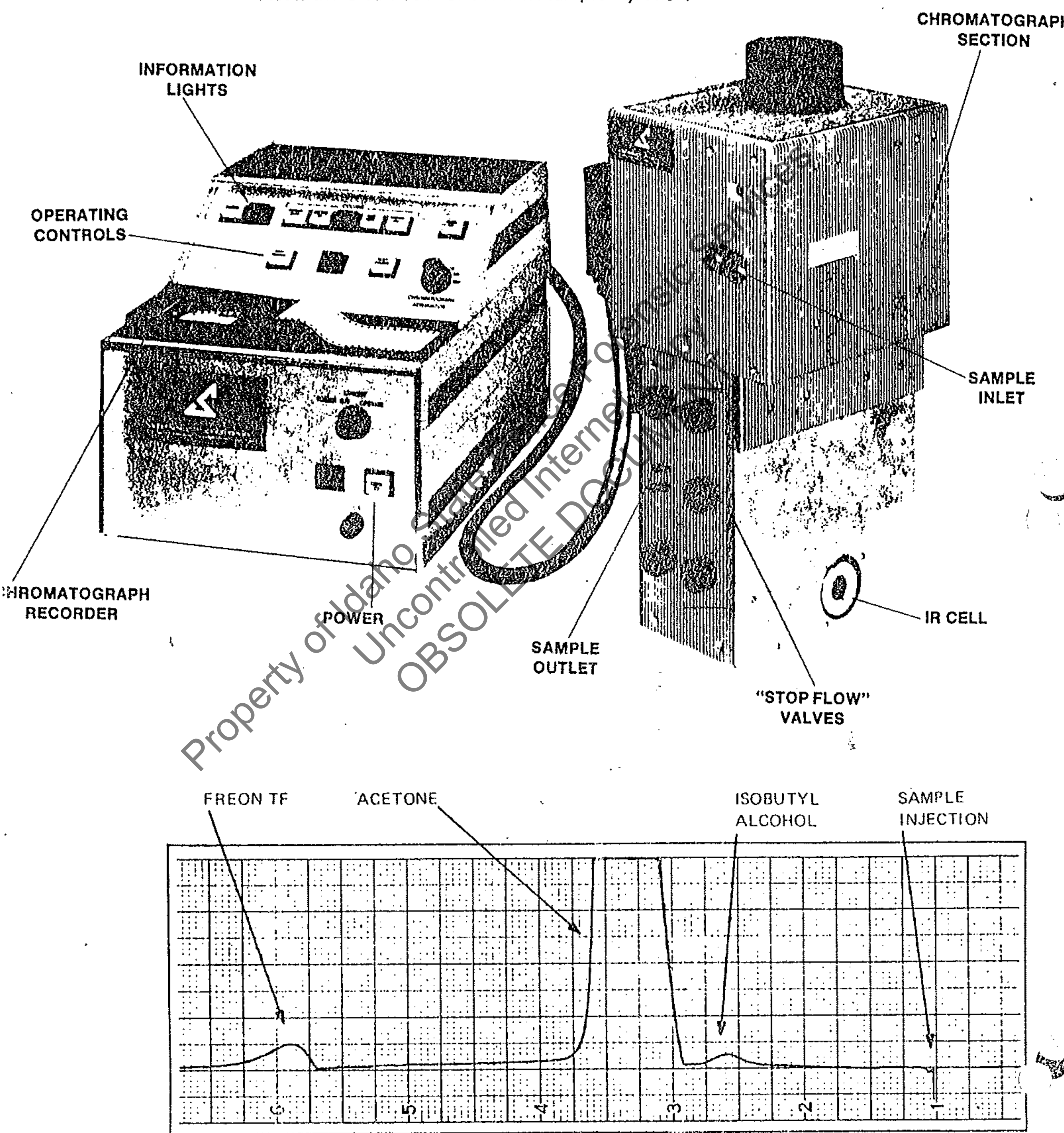
7. When your infrared scan has been completed, the stop flow valves are reopened, the chromatograph recorder automatically starts, the flow continues until the next constituent starts eluting.

8. The stop flow process is repeated until all peaks have been analyzed. Activating a switch resets the CIRA 101 for the next sample injection.

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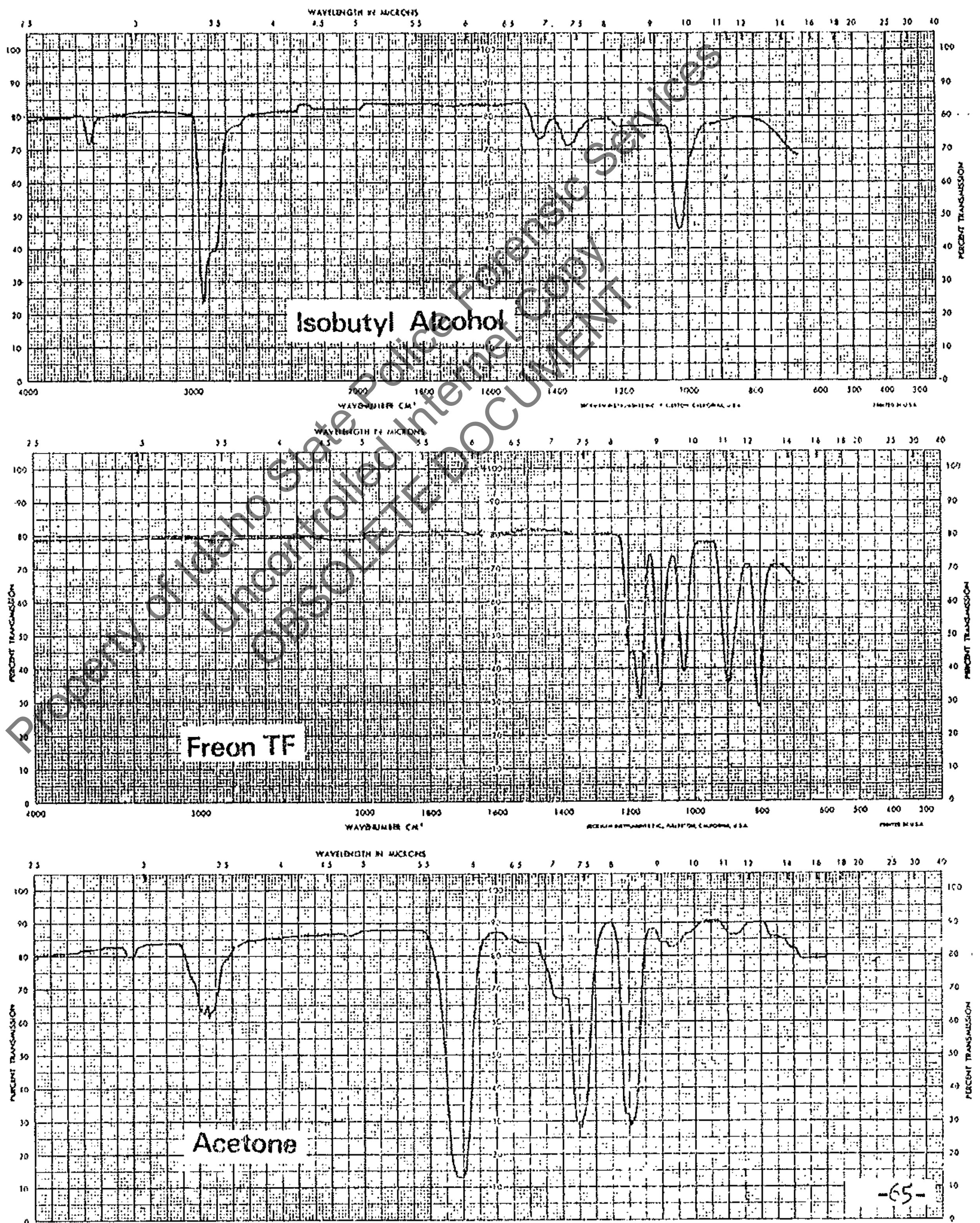
As an example, the CIRA 101 is here operated together with the Beckman AccuLab 6 spectrometer.

Fifty microliters of a mixture containing 98% Acetone, 1% Freon TF and 1% Isobutyl Alcohol was injected into the CIRA 101. The chromatogram produced for this mixture by the CIRA 101 is shown above. The chromatogram was produced by the CIRA's 2" strip chart recorder which operates at the rate of ¼" per minute.

The CIRA 101 was sequentially placed in its "stop flow" mode for each of the three constituents. When each constituent was locked in the CIRA's infrared cell, a standard 8 minute scan was made by the AccuLab 6 spectrometer;

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# (12) WICK-STICK® ELEMENTS

## New Technique for the Preparation of KBr Pellets from Microsamples

**Harry R. Garner and Herbert Packer**
*The Harshaw Chemical Company, Division of Kewanee Oil Co.*

The most popular techniques for collection of microsamples on KBr powder prior to infrared spectroscopic examination are evaporation from a solution of the sample in the presence of KBr powder and lyophilization.[1]

Many microsamples originate from thin-layer chromatographic (TLC) separations. The sample is separated by TLC, the adsorbent removed from the area of the chromatogram containing the separated material, the sample eluted with a suitable solvent, filtered to remove the suspended adsorbent and finally mixed with KBr by one of the above techniques.

By using a porous triangle of pressed KBr, a Wick-Stick®, in a small glass vial capped so that evaporation is restricted to the center of the vial, the filtration of adsorbent and deposition of the sample on KBr can be accomplished in a single step. The adsorbent containing the sample is scraped from the thin-layer chromatographic plate and transferred to a glass vial containing a Wick-Stick using a thin-stemmed funnel to prevent the adsorbent from dusting on the top half of the Wick-Stick. A suitable eluting solvent is added and a vented cap is placed on the vial (Figure 1).

Figure 2 shows the Wick-Stick appartus with a sample of dye partially collected from silica gel G using ethyl ether as the eluting solvent. The pressed KBr triangle is 2.5 cm high, 0.8 cm wide at the base, and 0.2 cm thick. The glass vial is 3.5 cm high with a 1.0-cm inside diameter. The vent hole in the stainless steel cap is 0.3 cm in diameter. A stainless steel spring clip holds the Wick-Stick upright and centered in the vial.

The solvent climbs the Wick-Stick by capillary action and evaporation takes place preferentially at the apex of the KBr triangle depositing the sample. The adsorbent is effectively filtered by the porous KBr. Emission spectrographic analysis has shown that less than 10-ppm adsorbent is detected in the tip of the Wick-Stick after elution from either alumina gel G or silica gel G.

One to two mm of the tip is cut off with a sharp scalpel, mashed on a clean metal surface, and pressed into a transparent disk using a microdie. Most of the sample is concentrated on a quantity of KBr which is slightly in excess of the amount required to press 1.5-mm microdisks. For ultimate sensitivity, the KBr can be carefully scraped from the edges and surface of the Wick-Stick tip and incorporated into a 0.5-mm microdisk.

FIGURE 1

"BEFORE". WICK-STICK ELEMENT IS SHOWN HERE READY FOR USE. The triangular-shaped KBr WICK-STICK element is placed in a holder clip and set inside the glass vial. Solvent and sample can now be carefully put into the vial and solvent allowed to evaporate from the tip of the WICK-STICK element.

FIGURE 2

"AFTER". SAMPLE HAS NOW MIGRATED TO THE TIP OF THE WICK-STICK element. (Note darkened tip area.) When the solvent has evaporated, the tip of the WICK-STICK element containing the sample is now sliced off, placed in a KBr die and pressed into a micro pellet.

Evaporation of the solvent can be accelerated by maintaining the temperature 10°–20°C below the boiling point of the solvent and directing an air jet across the cap of the vial. The vial holds 1–1.5 ml of solvent which will evaporate under these conditions in less than 1 h. Two or three solvent passes effectively concentrate the sample at the tip. While concentration may require a half-day, the operator does not have to attend the samples during this time and is free to perform other duties.

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Satisfactory spectra for qualitative analysis are obtained from 10–50 μg of sample when a 1.5-mm-diam micro-KBr-die and a beam-condensing unit are used.

Figure 3 shows the spectrum obtained from 20 μg of pyrogallol collected from an ethyl ether solution and Figure 4 presents the spectrum of 50 μg of cholesterol which was separated from a steroid mixture by TLC and collected by the Wick-Stick method.

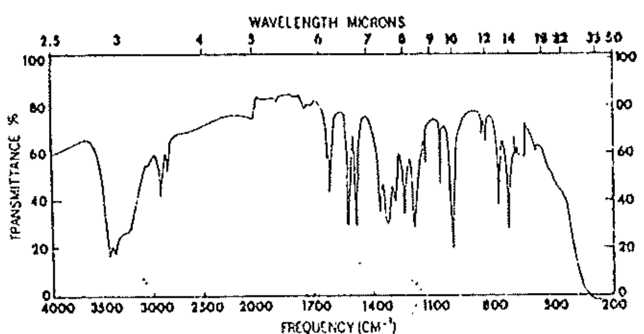


Fig. 3. 20 μg of pyrogallol collected on a Wick-Stick from ether solution.

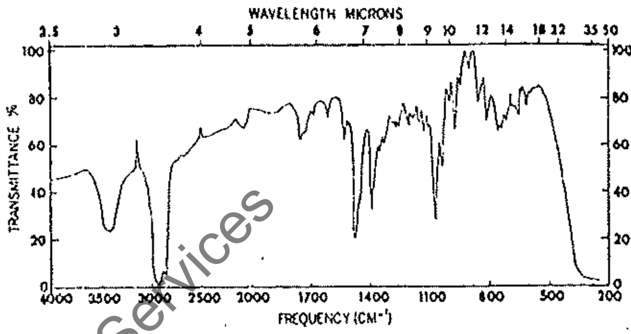


Fig. 4. 50 μg of cholesterol separated from steroid mixture by TLC and collected on a Wick-Stick.

1. W. B. Mason, Pitts. Conf. Anal. Chem. and Appl. Spectry., March 1958.

# ATR

(10)

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When a radiation beam in a high index medium suffers total reflection at its interface with a lower index one, an evanescent wave is set up in the latter which propagates parallel to the interface and is exponentially attenuated in the direction normal to it. The energy of this wave eventually returns to the first medium, which explains the totality of the reflection (however, the propagation of the evanescent wave produces a lateral shift in the reflected beam).

If the second medium is absorbing, the evanescent wave is weakened before its return, and the reflection will no longer be total. This phenomenon is called attenuated total reflection (ATR). If the evanescent wave is weakened by means other than absorption, the phenomenon is called frustrated total internal reflection (FTIR).

The first observation of the evanescent wave was made by Newton [1], who intercepted it with a second high index medium very close to the first. A transmitted wave then appeared in this medium (a case of FTIR) whose intensity depended on the distance between the first and third media.

Nearly half a century later, Maxwell [2], in his electromagnetic theory of light, gave a theoretical explanation of the phenomenon, which strongly spurred research in the field. After many investigators repeated Newton's experiments with more and more perfect setups, Hall [3] succeeded in photographing the evanescent wave. Soon afterward fluorescence [4] and scattering [5] excited by this wave were observed, and the first really quantitative study was made in the microwave region by Schaeffer and Gross [6]. The evanescent wave was also studied by diffracting it from curved or knicked interfaces [7], or from gratings superimposed on them (however, many of these experiments are not understood quantitatively even today). The first reports describing ATR were published in 1933 by Taylor [8], who applied it to the obtention of spectra. The lateral shift of the totally reflected beam was first observed by Goos and Hanchen in 1947 [9].

The first practical application of the phenomenon was that of Greski [10], who used it for light-beam modulation in optical communication applications. The device essentially consisted of a FTIR setup in which the distance between the first and the third media constituted the variable air gap of a microphone. The device was used by the

German Army in World War II [11]. After Taylor's the next reported application was by Leurgans and Turner with an FTIR interference filter [12], essentially a Fabry-Perot filter in which FTIR replaced the semitransparent mirrors. These were not very successful, owing to difficulties in the theory not resolved until very recently [13].

However, the present boom in internal reflection spectroscopy started in 1960 with Fahrenfort's [14, 15] and Harrick's [16-18] fundamental papers, which by their thorough, detailed description of the basic theory and the wide range of suggested and demonstrated applications opened the gates to this technique for practicing spectroscopists. The result was a flood of papers, totaling some 600 in about 6 years.

## I. VALUE OF THE TECHNIQUE

The rapid growth of the technique is due to several features that make it fairly unique, such as:

1. It gives transmission-like spectra for a very wide variety of samples, including those whose absorption is too intense for practical transmission work. This includes pure liquids, most solid samples, aqueous solutions, the peaks of strong absorption bands, etc., in the infrared, and concentrated solutions and fundamental absorption bands of semiconductors in the ultraviolet.

2. For samples thicker than about a wavelength, the spectra obtained are independent of sample thickness, which enormously simplifies sample preparation. Indeed, many of the samples studied by ATR would have been nearly impossible to study by other techniques [19-22]. An outstanding example is the ability of ATR methods to sample small gas chromatographic fractions [23] without requiring difficult sample transfer steps or very costly special-purpose spectrophotometers.

3. Like all reflection methods, but with more sensitivity, ATR reflectivities are sensitive to both sample index and absorptivity. The former sensitivity can be minimized by working far from the critical angle of total internal reflection [24, 25], in order to obtain transmission-like spectra, or one can work near it to have high sensitivity to both optical constants, which can then be determined by a pair of measurements [15, 26-29].

4. Normally the depth to which the evanescent wave penetrates is only a small fraction of a wavelength, allowing one to study selectively the absorption due to surface states [17, 18] and thin coatings [30].

5. The light loss per reflection being very low in the absence of sample absorption, it is possible to use multiple reflection to study thin samples and even monolayers [31].

6. The effective penetration being inversely proportional to the difference between the incidence angle used and the critical angle, the strength of the absorption can be regulated and the dynamic range of the method increased [32].

7. In Raman and fluorescence spectroscopy, evanescent wave excitation offers the advantage of inherent exciting radiation filtering (not dependent on wavelength differences), low sensitivity to scattering and reabsorption, and a very convenient geometry [33, 34].

8. ATR's potential for microanalysis is rivaled only by the more complicated techniques of microscopic spectrometry. Unlike this, it can work also with weakly absorbing samples [35-39].

9. ATR has the further advantage over transmission of being able to isolate absorption by dipoles lying in any of the three spatial directions [40-42].

These advantages are so great that in many cases the applications of ATR have outrun the theory, and many interesting aspects of the phenomenon are only now being cleared up. In this field we have Harrick's treatment of the equivalent penetration [43], Hansen's work on the general theory of thin-layer systems [44, 45], several authors' work on the accuracy of the optical constant determinations [27, 42,



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(3) INTERNAL REFLECTANCE SPECTROSCOPY

When light is reflected from a crystal-sample interface at an angle greater than the critical angle, total internal reflection occurs at the interface. If, however, the frequency of the reflected beam matches a vibrational frequency in the sample, there will be interaction and the totally reflected beam will be attenuated, or as some prefer to say, frustrated. If an internal reflectance accessory is placed in an infrared spectrophotometer, the resulting scan will be an internal reflectance spectrum and, if properly run, will closely resemble the transmission spectrum of the same sample.

An important difference, however, is that the internal reflection spectrum is characteristic of only a surface layer of the sample, usually no more than a fraction of a micron to a few microns thick. This can be both an advantage and a limitation--it makes internal reflectance an ideal technique for studying surface films or samples too opaque for ordinary transmission measurements, but information about the bulk of the sample can be obtained only when the surface is representative of the interior. Internal reflectance spectroscopy may be employed to study powders, adhesives, greases and smears, and liquid samples.

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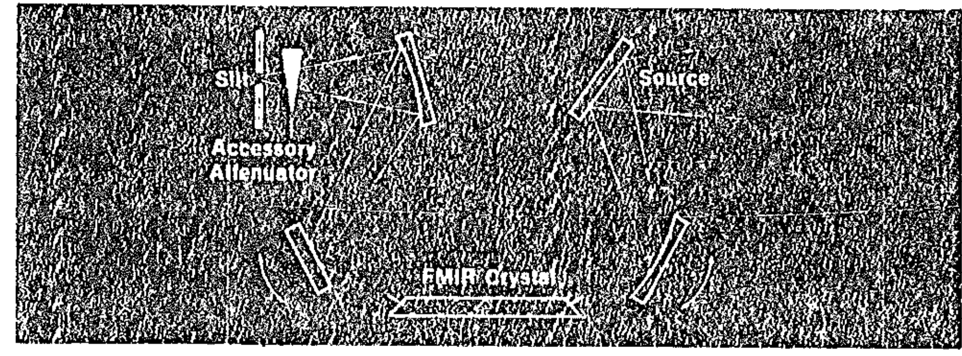
Sample preparation is extremely simple--it is only necessary that the sample be pressed into intimate contact with the internal reflectance crystal.




FMIR Accessory and Schematic Diagram

ADVANTAGES:

- Little or no sample preparation
- Micro reflectance possible
- Surface only - no substrate (could be a disadvantage)
- Thickness of sample doesn't matter
- Films-Powders-Liquids-Solutions

DISADVANTAGES:

- Expensive accessory
- Expensive crystals, scratch easily
- Surface contact sometimes difficult
- Optical alignment
- Hard, rigid samples difficult
- Dependence of spectrum appearance on:
  - angle of incidence
  - refractive index of XTAL
  - refractive index of sample
  - wavelength and penetration

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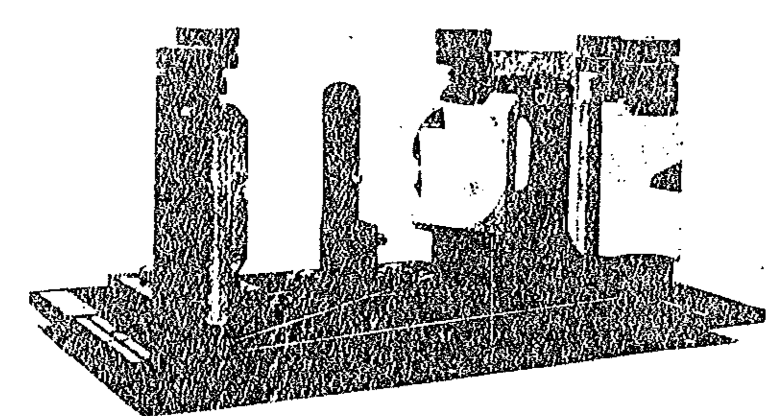
(13)

## Diamond ATR Crystal

In the continuing search by Wilks for ATR crystal materials able to withstand chemical and physical attack, the diamond represents the ultimate breakthrough. The chemical inertness of the diamond crystal assures that no reagent will mar the surface, and the physical hardness of the diamond assures that it will remain scratch-free even after contact with the most abrasive samples. In cleaning the diamond crystal after use, any desired solvent can be used. This combination of properties will enable the spectroscopist to rapidly and easily examine polymers, TLC fractions, powders, minerals, etc. Please contact Wilks Scientific for further details.



Diamond ATR Crystal Mounted In Wilks Universal Micro Sampling System — Model 45A

(3)

2. SPECULAR REFLECTANCE

Requires coating or film on reflective substrate. This includes coated metal surfaces or epitaxial layers on transistor wafers. This technique appears to have relatively little forensic use and will not be discussed.

(E)

(20)

# THE FORENSIC MICROANALYSIS OF PAINTS, PLASTICS AND OTHER MATERIALS BY AN INFRARED DIAMOND CELL TECHNIQUE

Although the analysis of paints, plastics, fibres and other polymeric substances by infrared spectroscopy is an established technique, the forensic chemist has special requirements. His analyses must be fast, reliable and applicable to very small amounts of material. The physical evidence that he encounters is, by its nature, very limited in quantity, so that a microscope may be needed to see the evidence let alone characterise it chemically. For example, paint transferred between vehicles in a hit and run collision can consist of submicrogram smears, generally contaminated with paint from other layers or from other sources. Readily visible materials from the scene of a crime such as paint, fibres, *etc.*, that have rubbed off on to a suspect's clothing will usually be detected and removed by the suspect himself. Particles that remain will tend to be small and their characterisation will require microchemistry.

Forensic methods for the infrared analysis of solid materials such as polymers have been reported by several authors in the past dozen years[1-4]. Sample preparation has been by cast film, KBr pellet or by supporting the sample in the beam and running "as is". Cleverley[2] used KBr discs with 10 to 50 $\mu g$ of sample. Fox *et al.*[3] obtained KBr spectra with as little as 3 $\mu g$ of sample. Smalldon[4] repeated the work of Cleverley and Fox and reports spectra obtained from 1 to 20 $\mu g$ of single acrylic fibres.

Our experience with KBr pellets has been that it is difficult to obtain consistently good spectra with small samples. We have found that the 50 $\mu g$ used routinely by Cleverley[2] is a realistic estimate of the required sample weight. Smaller samples produce poor spectra often with considerable loss of material in the process.

## METHOD

We report an infrared diamond cell method presently in use in R.C.M. Police Laboratories on paints, plastics, fibres, *etc.*, as an alternative to the micro KBr technique. The technique was reported by Eves at the First Interpol Seminar on the Scientific Aspects of Police Work (Paris, 1963) and at the Fifth International Meeting of Forensic Sciences (Toronto, 1968) by Tweed *et al.*[6] Corrigan[14,15] was the first to apply the diamond cell in forensic work.

The diamond cell is a high pressure device obtained commercially*[7-9]. It consists of two diamonds set in a holder by means of which even pressure of up to 50 kilobars can be applied. The diamond windows are two type II diamonds having adjacent flat surfaces, the smaller of the two surfaces having an area that can range from 0.25 to 1.5 $mm^2$. The sample is placed directly on to one of the two diamond faces, generally the smaller face. The diamonds are then pressed together lightly in the holder sandwiching the sample in between. The cell (holder plus diamonds) is mounted in the infrared sample beam using a 4X or 6X reflecting beam condenser.

Since the diamonds themselves have a wide absorption band between 2700 and 1800 $cm^{-1}$ (Fig. 1) the usable spectrum is from 1800 to 200 $cm^{-1}$ and thus includes the entire fingerprint region. The 4000 to 2700 $cm^{-1}$ region may also be used (Fig. 2).

The chief advantages of the diamond cell over conventional infrared techniques are as follows:

(1) It is a micromethod (1 to 5 $\mu g$);

(2) No sample preparation is required and the entire sample is preserved;

(3) The method is quick and reliable.

The procedure for recording a spectrum is straightforward. One to 5 $\mu g$ of sample are loaded, with the aid of a microscope, on to the smaller of the two diamond faces. The diamonds are then pressed together in the holder using very light pressure and the holder

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is mounted on the beam condenser.

The diamonds themselves transmit only 5–6% of the total beam energy. It is therefore necessary to produce as much energy as possible in the sample beam. This is achieved by (a) operating at maximum slit-width setting; (b) removing the 100% comb entirely from the sample side; (c) aligning the beam condenser mirrors to give maximum transmittance with the empty diamonds in the diamond cell holder. While step (c) is critical, re-alignment will not be necessary so long as the beam condenser mirrors are not disturbed. High gain is required and the scan speed should be sufficiently slow for good resolution. (With the Perkin-Elmer Model 567 a 9-minute scan between 2000 and 200 $cm^{-1}$ is adequate.) Finally an adjustable attenuator comb is hung in the reference beam to compensate for the low energy transmission of the diamonds.

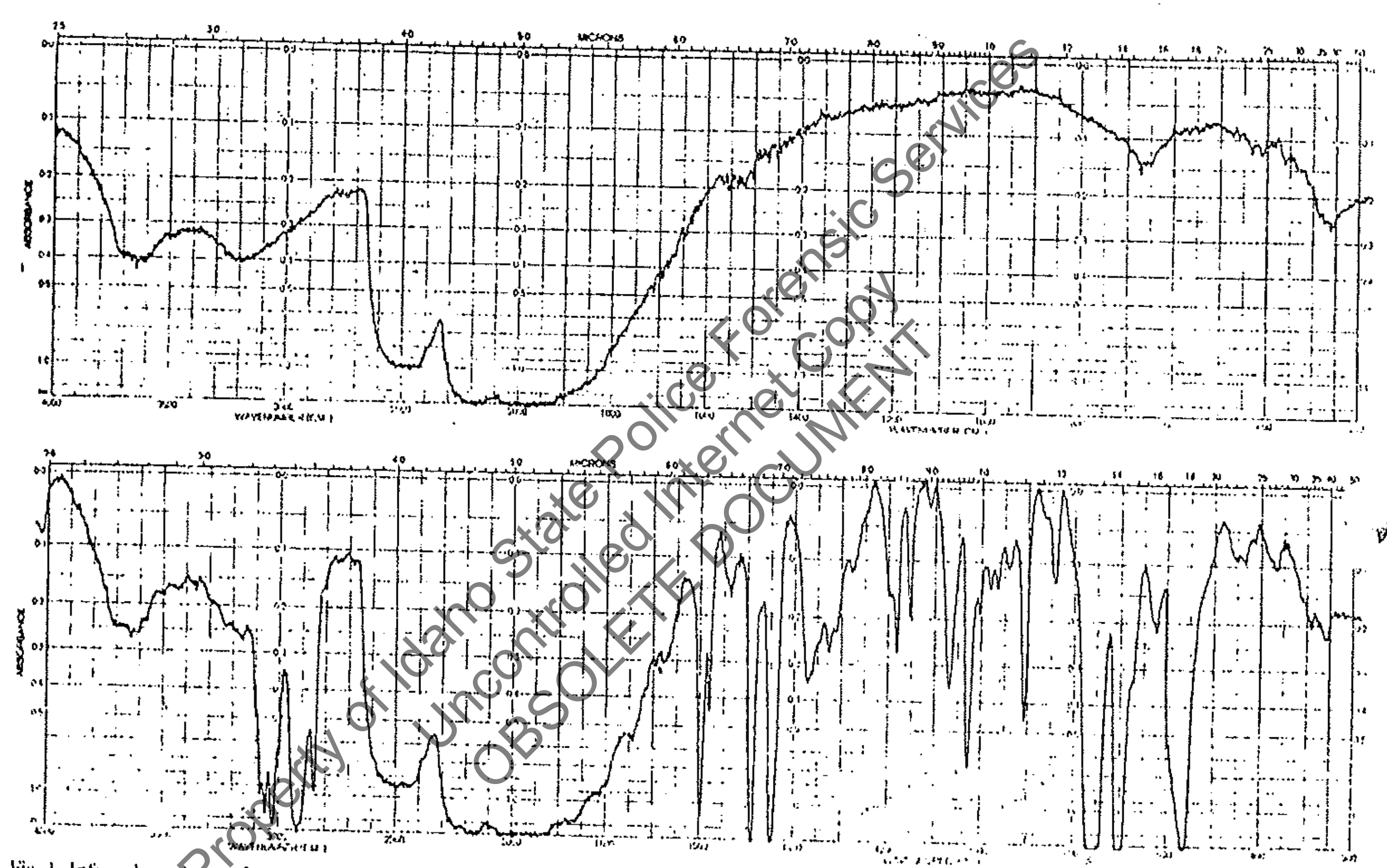


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Fig. 1. Infrared spectrum of type II diamonds with no sample present. P-E 567.

Fig. 2. Diamond cell spectrum of 0.05 mm polystyrene film (approximately 15 μg). P-E 567.

## RESULTS AND DISCUSSION

The types of sample taken by the diamond cell may be defined broadly as any material that can be flattened to a film by pressure. Spectra illustrating various sample types are shown in Figs 2–8.

The spectra were obtained with a Perkin-Elmer Model 567 (scan mode medium; slit 7; time constant 1), or with a Perkin-Elmer Model 621 (slit program 2 X 10; gain 6, attenuation speed 1100; scan time 16; suppression 0; scale 1X; source current 0.8 amp). Sample weights were determined on a Mettler ME22 electrobalance with a standard deviation of 0.3 μg (18 determinations).

The top curve of Fig. 3 is a diamond cell spectrum of 2.2 μg of a white acrylic melamine topcoat taken from a 1974 Ford automobile. The lower curve of Fig. 3 is a

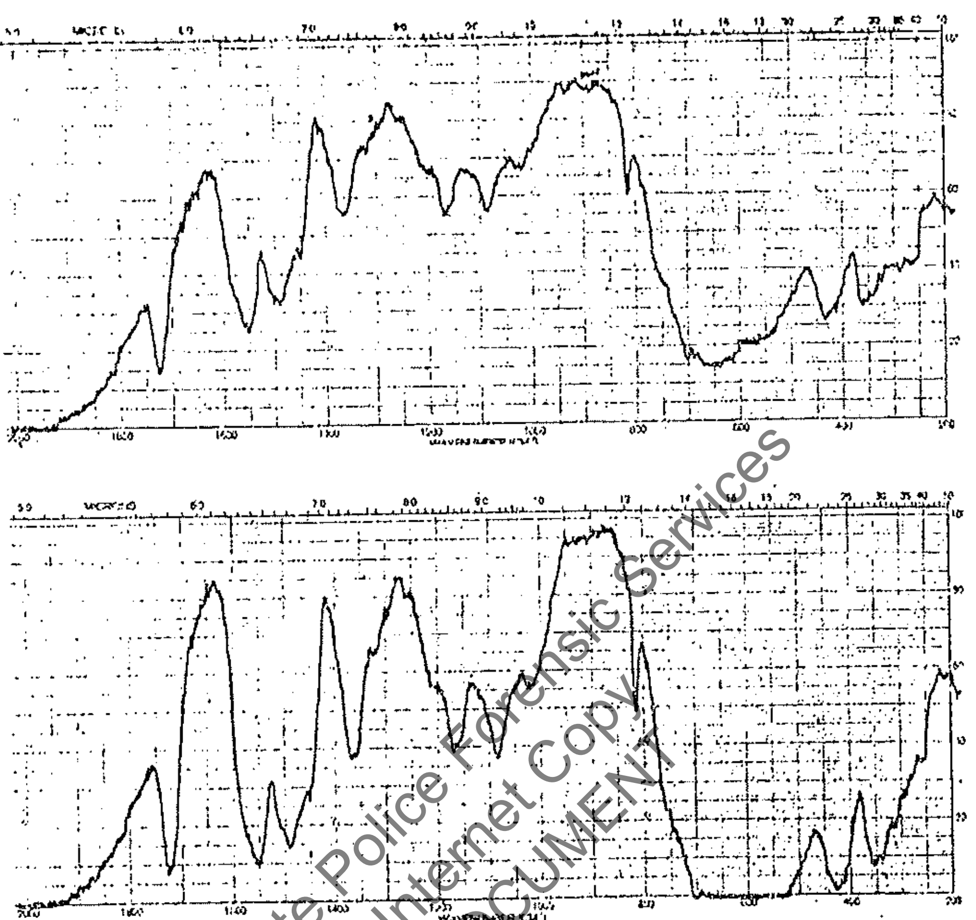

Fig. 3. Above: Diamond cell spectrum of 2.2 µg of a white acrylic melamine topcoat (1974 Ford). Below: Spectrum of 4.0 µg of the same material. P-E 567.

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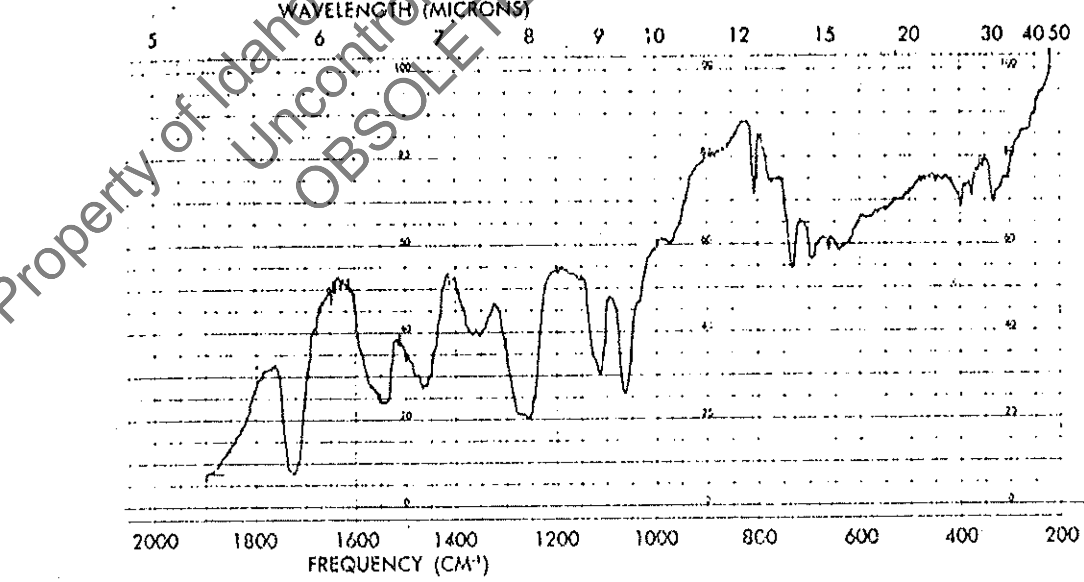



Fig. 4. Diamond cell spectrum of a blue alkyd melamine topcoat (1972 Toyota Celica). P-E 621.

spectrum of 4.0 µg of the same material. The C=O absorption at 1725 $cm^{-1}$, C—O ester around 1100 $cm^{-1}$ and triazine ring at 1550 and 815 $cm^{-1}$ are characteristic of this type of cured acrylic paint[10-12]. Titanium dioxide absorption is apparent below 700 $cm^{-1}$ (ref. 13).

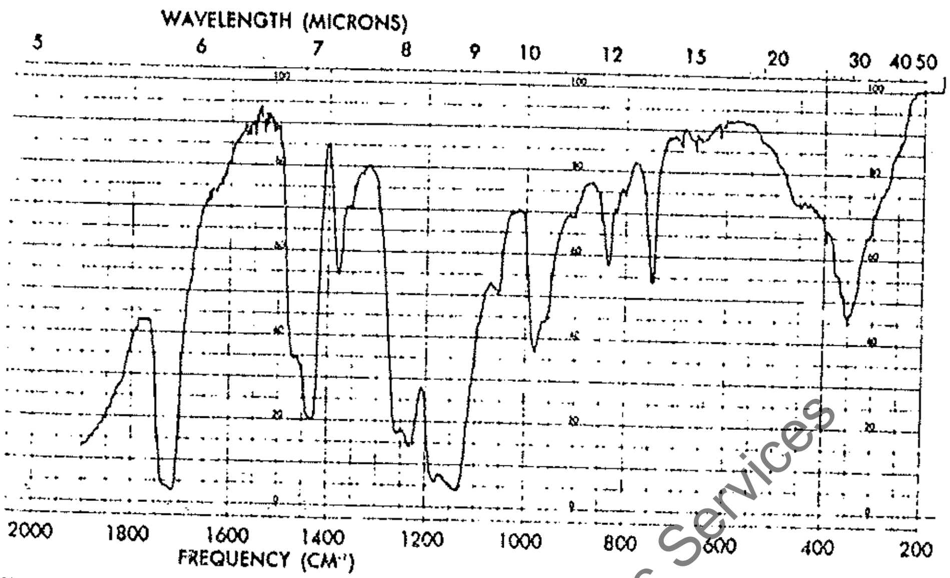
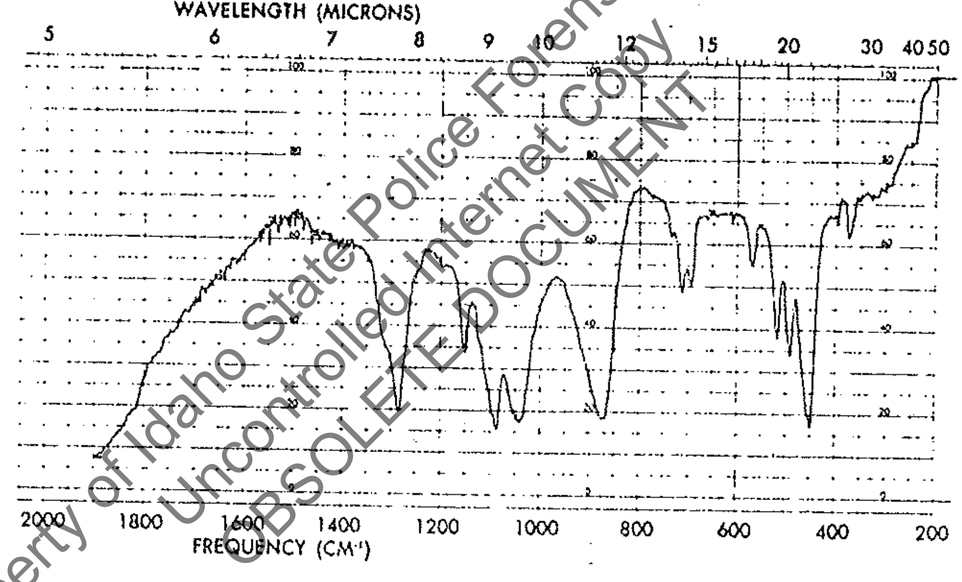



Fig. 5. Diamond cell spectrum of methylmethacrylate (from a tail-light lens). P-E 621.

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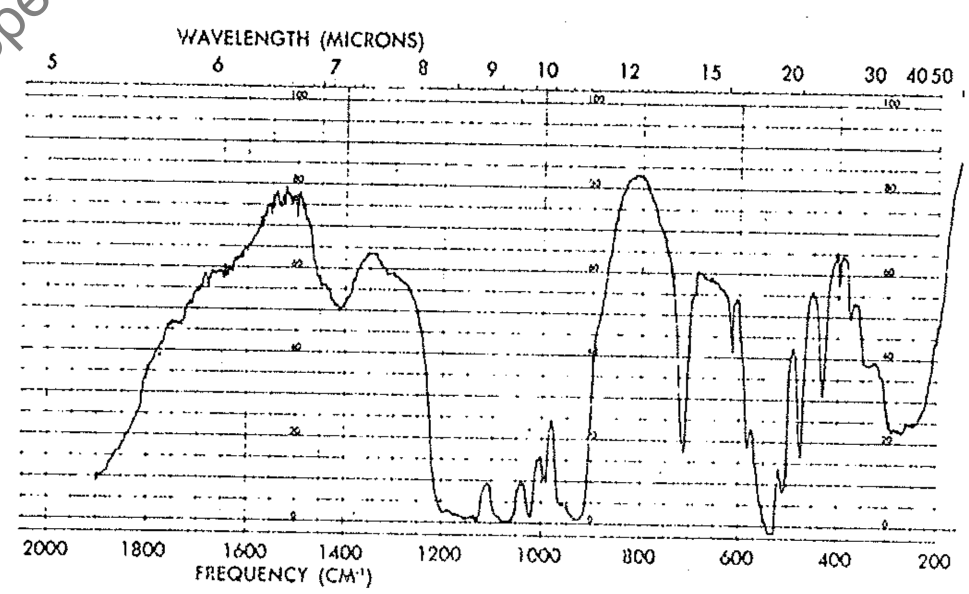


Fig. 6. Above: Diamond cell spectrum of "Fac[illegible] [illegible]oothpaste. P-E 621. Below: Spectrum of "Crest" toothpaste. P-E 621.

Fig. 4 is a spectrum of the blue alkyd melamine topcoat from a 1972 Toyota Celica. The C–O ester peaks near 1260 and 1120 $cm^{-1}$ together with a third unassigned peak near 1070 $cm^{-1}$, constitute the highly characteristic trio found in many alkyd spectra[10-12].

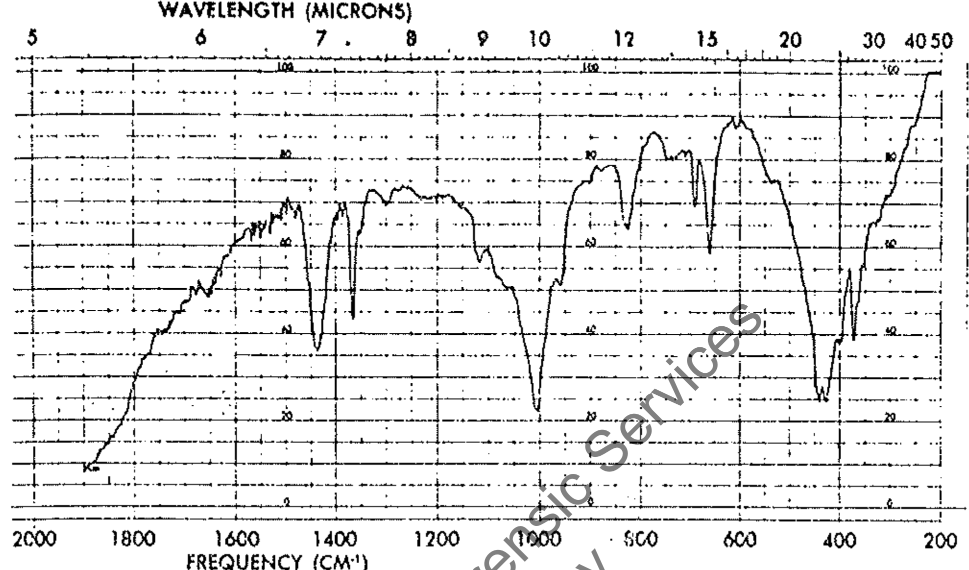


Fig. 7. Diamond cell spectrum of polyisoprene containing a large amount of talc (from a foam rubber sample). P-E 621.

Fig. 5 illustrates the spectrum obtained from 4 μg of a methylmethacrylate tail-light lens.

The top curve of Fig. 6 is a spectrum of "Fact" toothpaste (dried on a slide) the main contributor being sodium metaphosphate[13]. The lower curve of Fig. 6 is a spectrum of

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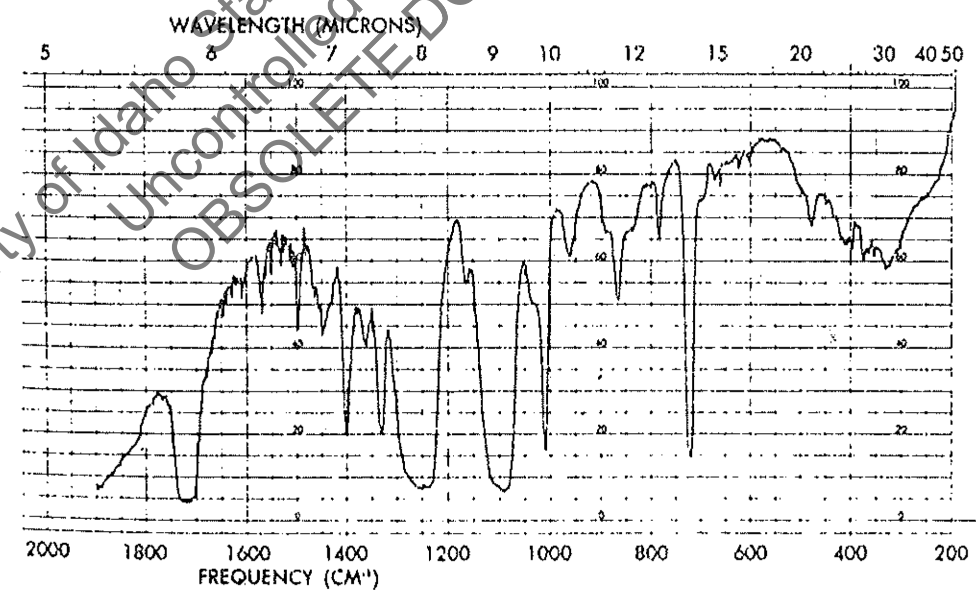


Fig. 8. Diamond cell spectrum of a polyester fibre (Millhaven Fortrel). P-E 621.

"Crest" toothpaste much of whose absorption is due to calcium pyrophosphate[13].

A polyisoprene/talc mixture from a sample of foam rubber is shown in Fig. 7 and, finally, the diamond cell spectrum obtained from a sample of a polyester fibre (Millhaven Fortrel) is presented in Fig. 8. A 1 μg weight of a 20 μm diameter polyester filament is approximately 3 mm long. The sample for Fig. 8 was therefore obtained by cutting up a 5–10 mm length of the single fibre into 0.5–1.0 mm lengths and stretching them out side by side on the diamond face.

(14)

Type II diamond cells for use in infrared crime detection work........$2,485

(E) The diamond cell appears to be very useful in the forensic lab however it should be noted that beam condenser and diamond cell alignment is very critical and can be very difficult. It should also be realized that the diamond can be cracked and chipped (replacement is about $2000 or more). When figuring the cost, one must also consider a beam condenser. The only beam condenser that is compatable with the diamond cell (that I know of) is the Perkin-Elmer 4X All Reflecting Beam Condenser (#186-0220) which has as an accessary a Diamond Cell holder (#186-0312). Because of the large energy loss (only 6 - 8% get through diamonds), the diamond cell may not work satisfactorily with certain instruments with low energy sources.

# PYROLYSIS I.R.

(15) There is, [illegible], a serious limitation in the characterization of polymers by their infrared spectra. Because of the intractable physical state of many polymeric materials, the procedures customarily used for the preparation of samples, such as casting thin films by evaporation of a solvent, by melting, or by mulling with a dispersing agent, are impossible. Such materials are the cured, infusible, insoluble, and largely nonextractable synthetic resins and elastomers. These materials generally are too resilient to be dispersed by mulling in Nujol or perfluorokerosene, or contain fillers, particularly carbon black, which are opaque to infrared radiation. It has been found impractical in most cases to attempt the preparation of thin sections with a microtome. Hence, an alternative method to permit infrared identification of such complex substances is required.

The author found that such intractable polymers may be readily characterized by pyrolysis, followed by examination of the infrared spectrum of the pyrolyzate. By this digression an otherwise difficult situation is circumvented entirely, but the desired result, identifying the material, is nevertheless achieved.

The behavior of plastics on dry distillation and the chemical reactions of gases from the pyrolysis of plastics have been described as an aid to rapid identification (*17*). An excellent review article by Penn (*19*) cites some 25 references pertaining to the study of polymer pyrolysis. Infrared absorption has been employed as a tool in the study of thermal and oxidative degradation of several polymers, notably polystyrene, by Achhammer (*1*), polyvinyl formal by Beachell (*5*), cellophane by Kmetko (*16*), and silicone resins by O'Neill (*18*). These papers aid in the interpretation of spectra prepared from products of more severe conditions of thermal degradation—pyrolysis. Literature relative specifically to the comparison of infrared absorption spectra of pyrolyzates with classified reference spectra as a means of identifying polymers is conspicuous by its almost complete absence. A method dealing with the identification of a parent material by an infrared study of its pyrolysis products has, however, been referred to in at least one case—namely, examples IX and XI in a paper by Barnes *et al.* (*3*).

In principle, when heated a given substance will decompose to a specific number of products in a definite ratio, provided that all operating conditions are controlled exactly. If all the pyrolysis products from a given substance are utilized, the infrared spectrum should always be the same from a standpoint of both band position and intensity ratios.

One might expect to obtain a poorly defined and featureless spectrum, since the yield of pyrolysis products from a given polymer is undoubtedly very complex in many cases, yet remarkably discrete spectra are the rule rather than the exception. In a few instances the spectrum closely resembles the unformulated and uncured or low molecular weight counterpart of the high polymer. This similarity arises from the fact that a number of linear homopolymers crack or pyrolyze into simple monomeric or low molecular weight components (*19*). Changes in molecular weight of a given linear polymeric type ordinarily do not alter the infrared spectrum materially. Notable for this behavior are polyethylene and polystyrene (omitted from the present discussion because of solubility considerations), polytrifluorochloroethylene (Kel-F, Figures 5 and 6), and isoprene-isobutylene copolymers (Butyl rubber, Figure 18).

Rather low pyrolysis temperatures (Table I) were experienced in some instances. It is evident that at these temperatures some of the pyrolysis products are formed by simple distillation of low molecular weight fractions. Boonstra and coworkers (*6*) have reported higher cracking temperatures for rubber. The optimum cracking temperature is stated to be 725° C. at 8 mm. of mercury. At these conditions up to 59% of isoprene can be obtained from natural rubber. The experimenters state, however, that at 300° to 400° C. the solid rubber "evaporates" and is cracked into lower polymers. At still higher temperatures, these again split up into lower fractions. With the exceptions of the pyrolysis temperatures reported in Table I for Kel-F, Butyl rubber, and polythene, which are in line with previous findings (*19*), little or no information is available as to optimum pyrolysis conditions for the other materials listed.

Slight variations in the composition of the pyrolysis products of a series of identical samples caused by failure to reproduce exactly the conditions of pyrolysis do not change the infrared absorption pattern materially. This method may then have some limitations not experienced when pyrolysis products are examined by some more sensitive means, such as mass spectrometry (*33*). For this reason no attempt has been made to distinguish between members of closely related groups such as the phthalate-type alkyd resins or to put this method on a quantitative basis, but there is reason to believe that with carefully controlled pyrolysis

**Table I. Temperatures Employed to Pyrolyze Representative Polymeric Materials**

| Polymer | Temperature, ° C.[a] | Residue |
|---|---|---|
| Saran tubing | 375-400 | Little |
| Filled rubber except silicone | 425-450 | Tarry mass |
| Polythene[b] | 440-450 | None |
| Nylon 66 (molded) | 460-490 | Little |
| Kel-F | 450-500 | Little |
| Silicone rubber | 475-500 | Fillers, little tar |
| Formex enamel on copper wire | 490-500 | Tarry mass |
| Wood flour phenolic | 500-550 | Charred mass |
| Teflon | 550-585 | Some charring |
| Asbestos phenolic[c] | 600-650 | Charred mass |
| Silicone resin (high methyl)[c] | 725-750 | Silica, very little charring |

[a] Temperatures measured in pyrolyzing mass with Chromel-Alumel thermocouple and indicate range of a number of readings for each material represented.
[b] Not included in this discussion.
[c] Tube walls at sample location were 850-900° C.

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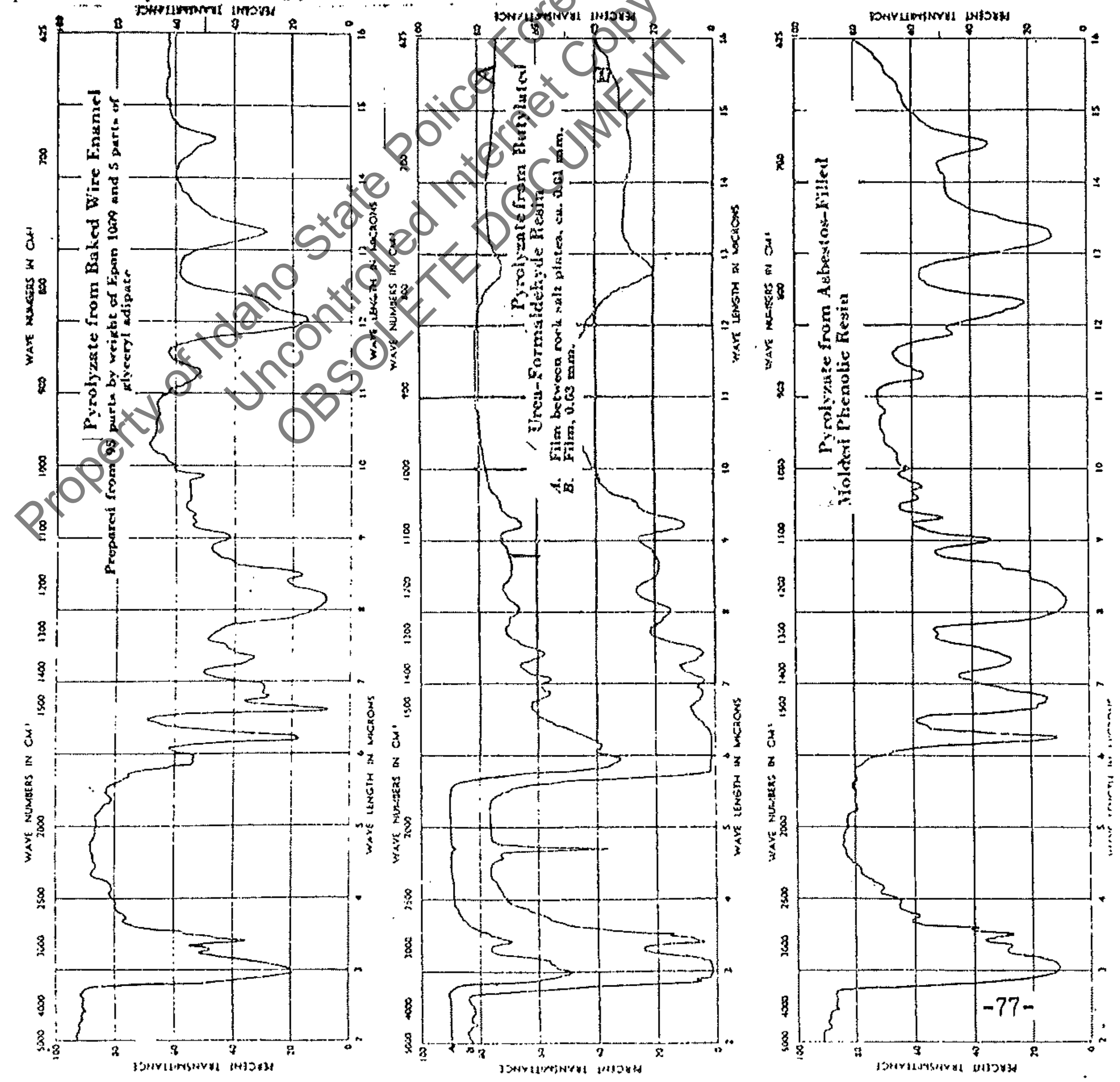
conditions, a semiquantitative estimation could be made in some cases. The condensates of volatile products are used for preparing the infrared spectrum. Utilization of gaseous products resulting from the pyrolysis would ordinarily require a relatively larger sample, and the difficulties of filling the gas cell would detract from the speed of testing, which is one of the especially attractive features of the present method. The pyrolyzate could be passed into some infrared transparent solvent such as carbon tetrachloride, but this alternative was avoided because of preferential solubility of some products and complete insolubility of others.

In the present paper the spectra have been prepared with the intent of utilizing the characteristic general pattern of the spectrum obtained in each case as a means of identifying a number of complex organic materials with little regard to the actual constitution of the pyrolysis products. In some cases the analyst may wish to ascertain some of the products of pyrolysis of a polymer, as this information in itself may be a definite clue to the identity of the original material. However, ordinarily there is no need to interpret spectra thoroughly with respect to the exact composition of the pyrolysis products. Moreover, as has been aptly pointed out by Shreve (*26*), infrared spectra are very valuable in the classification of many industrially important materials which in terms of definitive chemical make-up are poorly characterized as individuals. For many practical purposes, the absorption spectrum can be used with the aid of a file of classified reference spectra as a unique characterizing pattern with little regard for the compositional background which it reflects. Following thi approach, the infrared spectrum of the pyrolyzate is used simpl as a unique characterizing pattern, without special regard for th actual chemical compositon which it reflects.

Using the pyrolysis technique, 20 minutes is usually sufficien time for an experienced operator to make an identification Frequently, rapid answers at relatively low cost are a definite requirement in industrial laboratories, and it is felt that this is an asset to be exploited and is an advantage not enjoyed by other methods, such as the somewhat more laborious and detailed methods of mass spectrometry (*33*).

## METHOD

The sample may vary from 2 grams to a few hundred milligrams, depending upon whether the sample material is estimated to contain a large amount of inert filler or is 100% polymer. Small fragments of the material to be tested are placed in a 15- by 120-mm. borosilicate glass test tube. The tube is held nearly horizontal and heated at its closed end over the inner blue cone of the Bunsen burner flame to 375° to 750° C. The temperature of pyrolysis depends upon the type of sample (Table I). Heat must be applied as rapidly as possible to minimize charring. The vaporous pyrolyzate condenses as a liquid on the cooler portion of the tube and is transferred directly to a sodium chloride window. A "sandwich" is then made by pressing a second sodium chloride window over a few drops of the pyrolyzate on the first window.

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(19) A modification of this procedure allows smaller (1 - 5 mg.) samples to be seen. A pyrex tube with a 1 mm I.D. is closed by melting and acts as the pyrolysis tube. A sample is introduced and pyrolyzed. After the tube cools the chared end is removed by cutting the glass tube. A small amount of KBr is introduced into the end of the open tube containing the pyrolysis products (visible as a yellowish oil). The KBr is pushed through the tube with the sealed end of a 1 mm cap tube (which should slide through like a syringe plunger. It might be well to check its fit before pyrolysis). The KBr plus the oil can be pushed out into a small mortar, ground and a pellet pressed as usual.

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## QUANTITATIVE INFRARED SPECTROSCOPY

### 1. INTRODUCTION

The amount of sample present in the sample beam of an infrared spectrophotometer is directly related to the strength of an absorption band (or the absorbance at any given frequency) in the infrared spectrum of the sample. The relation is expressed mathematically, in terms of sample concentration and radiation path length through the sample, by the Beer-Lambert Law. Depending on the units of measurement, this law may be expressed in a variety of ways:

$$\text{Absorbance} = A = \frac{ecl}{\text{mol. wt.}} = \underline{a}bc = \log \frac{I_o}{I} = \log \frac{1}{T} = \log \frac{100}{\%T}$$

Where A = absorbance, observed from the spectrum.

e = molar absorptivity or molar extinction coefficient. It is a characteristic of the sample at a particular frequency. It is often nearly the same for a group of compounds if the absorption band is characteristic of that group.

c = concentration in grams per liter.

l=b = sample path length in cm (inside cell thickness).

$\underline{a}$ = absorptivity, characteristic of the compound for that particular absorption. $\underline{a}$ = e/mol. wt.

$I_o$ = intensity of incident radiation.

I = intensity of transmitted radiation.

T = transmittance = $I/I_o$ or the ratio of the radiant energy transmitted [illegible] the radiant energy incident on the sample.

Most infrared spectrophotometers record the spectrum linearly in transmittance units and nonlinearly in absorbance units. In order to relate such spectra directly to concentration, the transmittance measurements must be converted to absorbance units. This can be done either by (1) mathematical conversion; (2) using chart paper having a nonlinear absorbance scale, or (3) using a special ruler calibrated in absorbance units, which has the same length as the transmittance scale on the chart paper.

2. ESTABLISHING A QUANTITATIVE METHOD OF ANALYSIS

a. Composition of sample must be known qualitatively, i.e., what components are present.

b. Reference spectra must be available or be recorded for each component in the sample.

c. An absorption band for the component of interest must be selected for measurement.

d. This absorption band must not be subject to interference by nearby bands from any other component in the mixture. Consult the reference spectrum of each component in order to select the correct band.

e. Prepare at least three or four standard solutions which bracket the expected concentrations of the unknowns.

f. Record the spectra of the standard solutions over the region of interest (the whole spectrum need not be recorded) in duplicate.

g. Measure and plot the absorbance values of the analytical band on the ordinate (vertical) scale of linear coordinate graph paper. Plot the corresponding concentration values of the standard solutions on the abscissa scale of the graph paper.

h. Repeat the measurements for the unknown solutions under



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exactly the same conditions.

j. Determine from the calibration curve the concentrations of the unknown samples.

3. PRECAUTIONS

a. Use non-linear absorbance paper for quantitative analysis.

b. Adjust instrumental conditions to give high signal-to-noise recordings. This means wider than normal slits, lower than normal amplifier gain setting, and a medium to slow scan speed. These conditions will insure highest photometric accuracy resulting in accurate and precise analytical results.

c. Absorbance values should be kept between 0.2 and 0.8. Cell thicknesses or sample concentrations can be adjusted to maintain absorbance values within this range.

d. Establish precision and accuracy for the method of analysis by multiple repeat analyses.

e. Determine experimentally at what concentration level the plot of Absorbance vs. Concentration no longer adheres to Beer-Lambert Law, i.e., at what concentration level calibration curve is no longer linear.

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(16)

# Application of Fourier transform infrared spectroscopy

INFRARED SPECTROSCOPY is an essential analytical tool in the research and development laboratory. It finds use in the identification of compounds, the analysis of functional groups, the determination of structures and detection of intra- and intermolecular interactions. Infrared spectroscopy is particularly convenient since it is a nondestructive analysis; it is rapid and simple, and gases, liquids or solids can be examined. Most chemists are introduced to infrared spectroscopy at the sophomore level in organic chemistry and have continued to utilize the technique more or less effectively in their research.

Most researchers are not familiar with the application of Fourier transform techniques to infrared spectroscopy and are therefore not familiar with the new potential of infrared for analysis. The purpose of this article is to outline the new areas and cite some examples of current application of Fourier transform infrared spectroscopy.

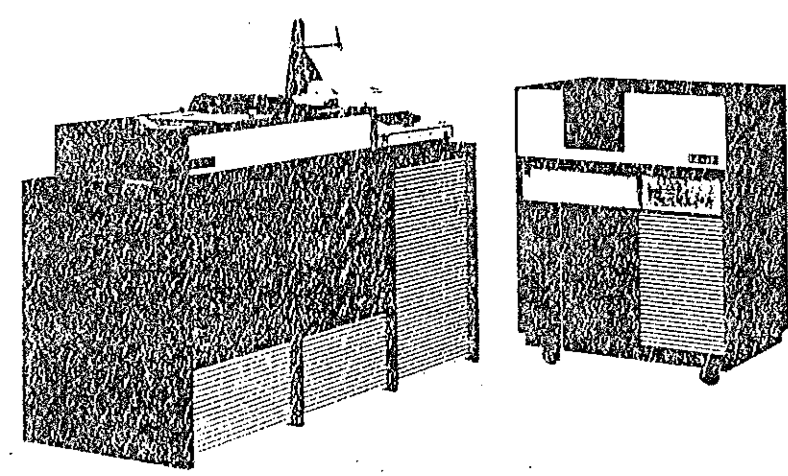
## Comparison of Fourier transform and conventional dispersive infrared spectroscopy

The spectral information is obtained quite differently for dispersive and Fourier transform techniques. Conventional dispersive spectroscopy employs a prism or grating, which disperses the polychromative infrared radiation spatially into a spectrum of frequencies. The energy in each individual frequency interval is then scanned sequentially. To obtain quality spectra with a dispersive instrument, narrow slits are required. These slits ensure that the frequency intervals, detected at any one time, are sufficiently narrow so that the desired resolution is obtained.

More recently, interferometric spectroscopy, commonly called Fourier transform spectroscopy, has been developed. Here, an interferometer is used instead of a grating or prism monochromator. The spectral information for all the frequencies is obtained at the same time — during one scan of the interferometer whose duration is on the order of 1 sec. The actual spectrum is obtained by taking the inverse Fourier transform of the interferogram, which is the output of the interferometer.

The most common interferometer used in Fourier transform systems is the Michelson interferometer[1]. A simplified diagram of such an interferometer is shown in Figure 1. The interferometer consists of two mirrors at right angles to each other. One mirror is stationary while the other moves in a direction perpendicular to its front surface. A beamsplitter is positioned at an angle of 45 deg to the two mirrors. The incident beam is amplitude-divided at the beamsplitter. Ideally, the beamsplitter should transmit 50% and reflect 50% of the light. The two beams pass to the stationary and moving mirrors and reflect to the beamsplitter. The beams are then recombined at the beamsplitter and exit the interferometer to the detector.

Various degrees of interference (from totally constructive to totally destructive interference) are produced for each frequency by the optical path difference in the two arms. Consider a monochromatic source. When the optical path lengths of each arm of the interferometer are identical, there will be constructive interference of the two light beams when they recombine at the beam splitter. If the movable mirror is moved ¼ of a wavelength, the two beams will be 180 deg out of phase when they recombine at the beam splitter; they will destructively interfere. Each individual incident frequency will produce an output flux with a cosine variation whose frequency is dependent on the incident frequency. Since each frequency can only interfere with itself, the output of the interferometer, the interferogram, for a polychromatic source is the sum of the fluxes at each individual frequency. Such an interferogram is shown in Figure 2. The point of maximum intensity in the interferogram occurs at the position where the optical path lengths for the two arms of the interferometer are identical. It is only at this mirror position, that every frequency constructively interferes. Thus, it can be seen that the interferometer establishes specific phase relationships for each frequency as a function of mirror displacement. The resultant interferogram is related to the intensity as a function of frequency spectrum by a Fourier transformation. Fourier analysis of the interferogram picks out the pattern for each frequency and determines the magnitude of the flux at that frequency, the Fourier coefficient. The Fourier analysis is a tedious process and must be handled by high speed computers.



*Digilab FTS® -14 Fourier Infrared Spectrophotometer*

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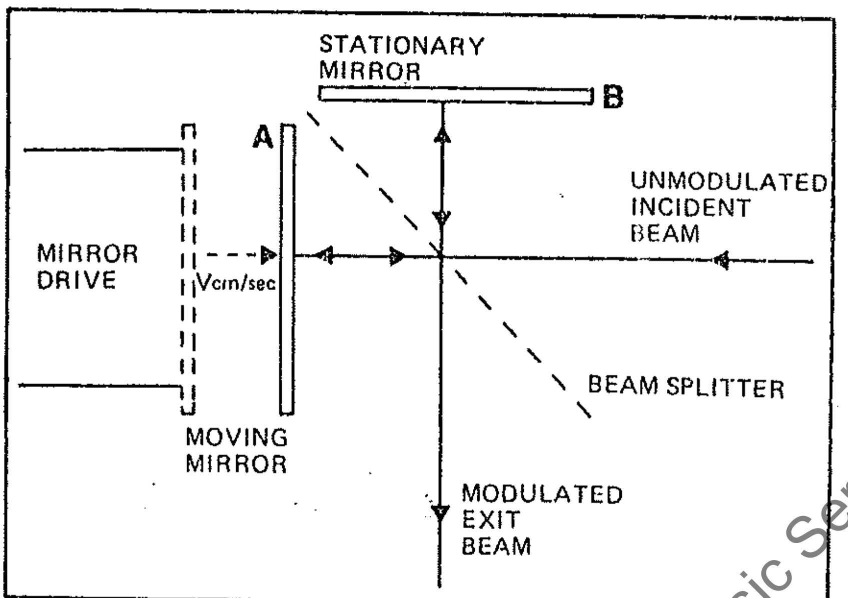


*Figure 1 — Diagram of Michelson interferometer.*

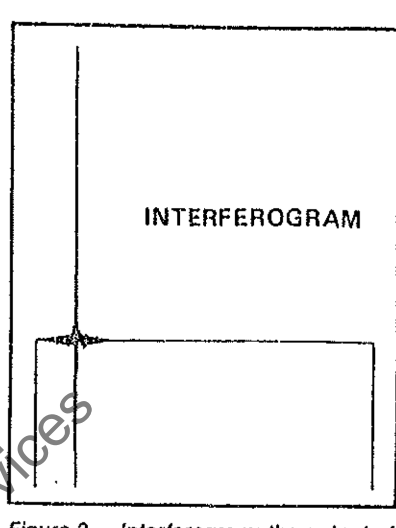


*Figure 2 — Interferogram; the output of a Michelson interferometer.*

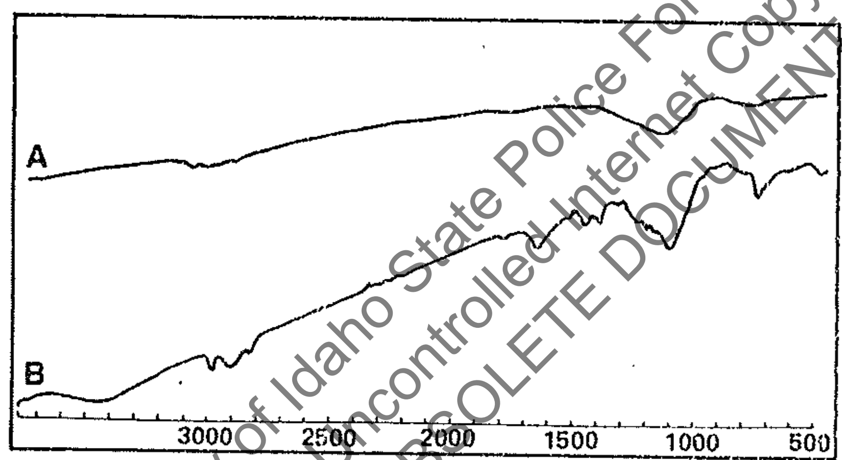


*Figure 3 — Comparison of dispersion and FTS spectra of carbon filled rubber. Spectrum A; dispersion. Spectrum B: from a Fourier transform instrument.*

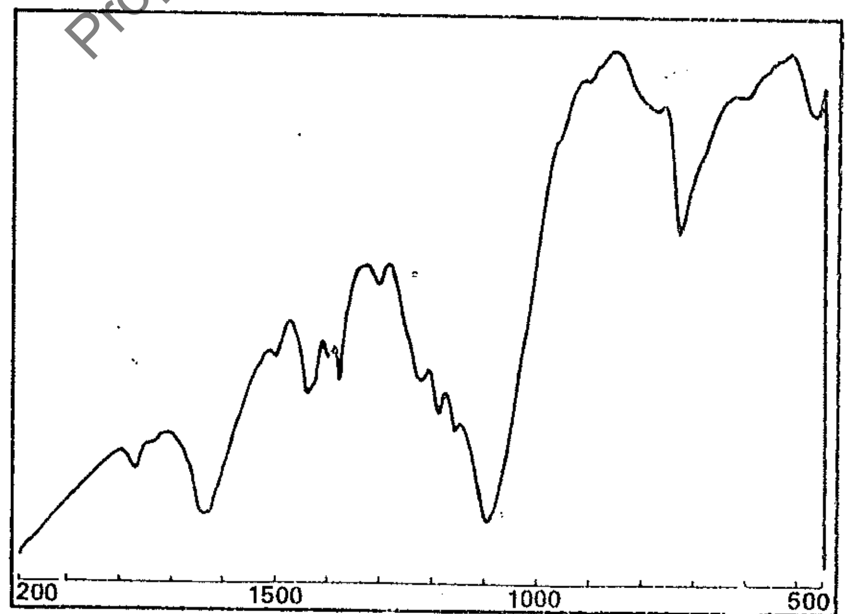


*Figure 4 — Computer expanded F᠆ ᠆tra with carbon filled rubber.*

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Fourier transform spectroscopy is a less direct way of obtaining a spectrum than conventional dispersive spectroscopy. However, there are certain advantages that make the interferometric method favored over the dispersive method. The most important points are (1) the increased energy throughput, (2) the multiplex advantage, (3) the improved accuracy of the frequency scale, and (4) the computer capabilities for processing the digitized spectral data.

The throughput or Jacquinot's advantage[3] refers to the improvement in the light throughput that is possible with an interferometer. The throughput in a dispersive instrument is limited because of the entrance and exit slits of the monochromator. With an interferometer there are no slits, and the throughput is limited by the size of the mirrors. The increase in the energy throughput for interferometric systems over dispersive systems is of the order of 100 to 200 times[1]. This advantage becomes greater at higher resolution; the throughput for a dispersive system is decreased (slits must be narrower to improve resolution) while it stays constant for an interferometer, except for the very highest resolutions.

The multiplex or Fellgett's advantage[4] reflects the fact that an interferometer receives information from all frequencies in the spectrum simultaneously during each scan whereas a conventional dispersive instrument only views information from the narrow frequency interval that is within the exit slit of the instrument at any one time. For a dispersive instrument each resolution element can be observed for a time T/N, where T is the total scan time and N is the total number of resolu-

tion elements scanned. The signal for each resolution element is then proportional to T/N. If the noise is random and independent of signal level[1], the noise is proportional to $(T/N)^{1/2}$. Thus, for a dispersive instrument, the signal-to-noise ratio, $(S/N)_D$, can be expressed as:

$$(S/N)_D \propto (T/N)^{1/2} \qquad (1)$$

For an interferometer, the signal for each resolution element is proportional to T since each resolution element is detected during the entire scan time, T. Likewise, the noise is proportional to $T^{1/2}$. The signal-to-noise ratio for an interferometric system, $(S/N)_I$, can be expressed as:

$$(S/N)_I \propto T^{1/2} \qquad (2)$$

If the interferometric and dispersive instruments have the same optical throughput and the same detection system, the proportionality constants in equations (1) and (2) would be the same[1]. Comparison of the signal-to-noise ratios for interferometric and dispersive instruments can be made thusly

$$\frac{(S/N)_I}{(S/N)_D} = N^{1/2} \qquad (3)$$

Hence, for equivalent scanning times and optical throughput, the interferometer has a higher signal-to-noise ratio. For a typical infrared spectrum with a resolution of 2 $cm^{-1}$ and a frequency range of 4,000 $cm^{-1}$, the improvement in signal-to-noise would be $\sqrt{4{,}000/2} \sim 145$. The interferometer employed in this work is of the fast scanning type — individual scan times on the order of 1 sec. To obtain longer scan times with this system, individual scans are co-added. This multi-scan feature enables the signal-to-noise to be improved proportionately as the square root of the number of scans.

The position of the movable mirror in the interferometer is controlled by a reference interferometer that has a monochromatic laser as a light source. This invariant interference pattern (cosine wave of known frequency) is used as an internal calibration for the spectral frequency scale. The frequency scale in an interferometric system is thus internally calibrated and is not subject to changes in the system's temperature or humidity as is a dispersive system[1].

The fourth major advantage of Fourier transform systems is the capability of processing the spectral data. The inverse Fourier transform of the interferogram must be computed to obtain the intensity as a function of frequency spectrum. Until the past few years, only conventional techniques were available for this computation. The computation problem limited Fourier transform spectroscopy to the far infrared region because of the enormous computation time required for the other regions. This problem has been eliminated by utilization of the fast Fourier transform algorithm of Cooley and Tukey[6]. Forman[7] applied this method to Fourier transform spectroscopy and found a savings in computation time of two or three orders of magnitude over the conventional methods. Use of the Cooley-Tukey algorithm with the dedicated minicomputer makes on-line Fourier analysis a reality.

The digitized spectral data can also be processed with the computer. Once a spectrum is recorded, it is stored. A spectrum can be plotted in transmission, absorbance, or log absorbance modes as an aid in interpretation. The intensity scale can be expanded, and the spectrum then replotted. Two spectra can be divided. For example, an emission spectrum of a coating has contributions from the coating plus the substrate; the contribution from the sub-

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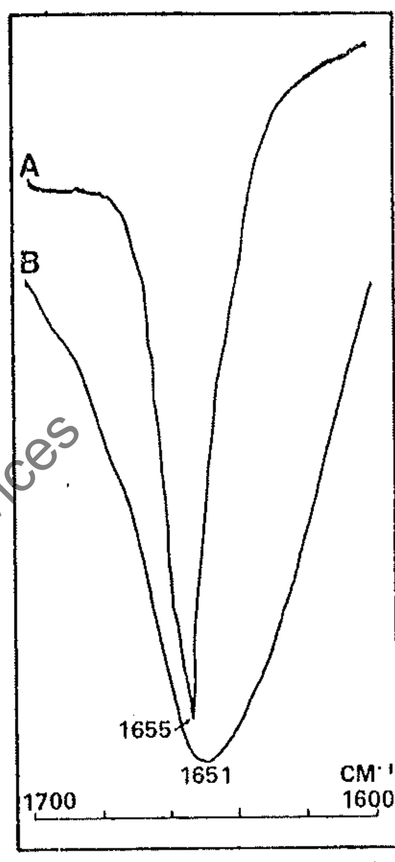


*Figure 5 — Infrared spectrum in the carbon-carbon double bond stretching region for carbon filled and unfilled polybutadiene B — carbon filled. A — Unfilled.*

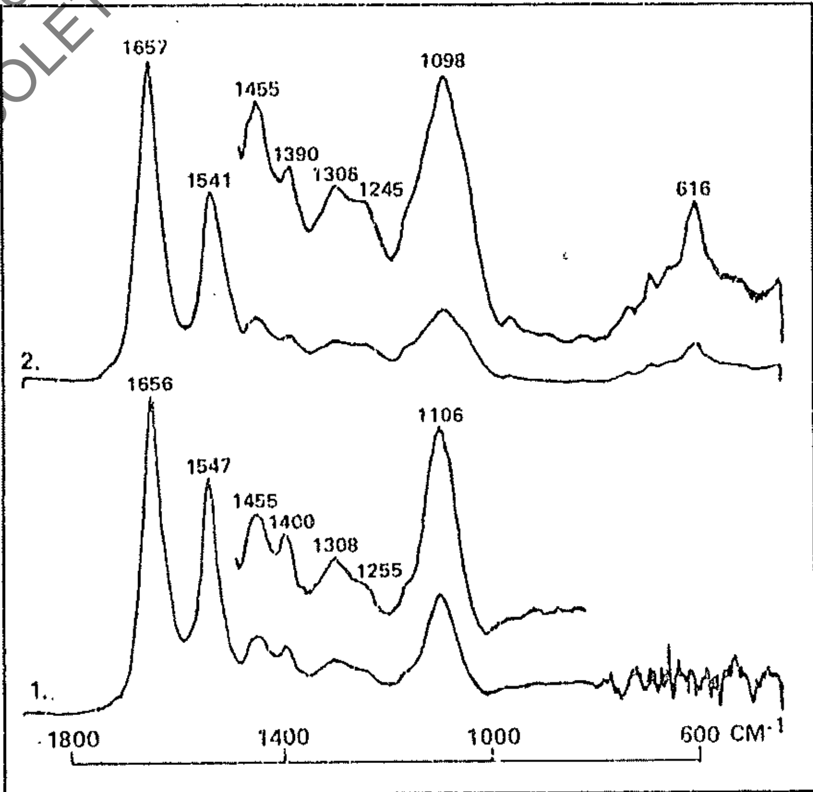


*Figure 6 — Infrared absorbance spectra of hemoglobin. 1 — aqueous solution (pH 4.8). 2 — cast films.*

strate can be eliminated by division of the coating's emission spectrum by the substrate's spectrum. Absorbance spectra can be subtracted to remove interferring absorbance bands of one of the components. A multicomponent spectrum could likewise be generated by addition of the individual components' spectra; this summed spectrum can then be compared with an experimentally obtained spectrum of the composite system as an aid in elucidating the molecular interactions that occur in the system. The potential also exists for numerical integration of band areas and automatic location of band peaks.

In summary, the Fourier transform infrared system is capable of performance unmatched by dispersive instrumentation. However, as might be expected, the cost of Fourier transform equipment is several times that of dispersive equipment and thus will not be available to all laboratories. The following applications were done on a Digilab Inc. FTS-14 Fourier transform spectrophotometer.

## Applications

The advantages of Fourier transform spectroscopy are most easily demonstrated for highly energy limited samples. For example, samples which transmit very little of the infrared radiation to the detector. Carbon-filled rubber samples have long presented difficulties for the infrared spectroscopist due to the strong absorbance of carbon black. In Figure 3, we show a comparison of the spectra obtained on the same KBr pellet of a carbon-filled rubber for a dispersion in the FTS-14 system. The differences in signal-to-noise ratio as reflected in the spectra are obvious in the quality of the spectra. The additional advantage is the capability of computer-expansion of the scale to give the spectrum shown in Figure 4. The FTS-14 spectrum can easily be used to identify the elastomeric component. We have observed frequency shifts of the carbon-carbon double bond stretching mode resulting from the complexing of the carbon black with the rubber as shown in Figure 5. This complexing apparently gives rise to the well-known stiffening effects of carbon black. Studies of filled samples can lead to better utilization of the carbon black and to improved elastomers.

Another area of utilization of the arithmetic package of such systems as the FTS-14 is the removal of interfering absorbance from substances such as solvents. A classical limitation of infrared analysis has been in the study of water solutions due to strong, broad infrared absorbance of water. With the FTS-14, if the aqueous solutions are examined such that total absorbance does not occur, the water spectrum can be subtracted. For biological systems where water is the only interesting solvent, this feature can be utilized and demonstrated for protein solutions. The aqueous solution infrared spectrum of hemoglobin obtained with a Fourier transform spectrometer is shown in Figure 7 (spectrum 2). No useful detail can be observed until the water spectrum (spectrum 1) is subtracted to yield spectrum 3 where the classical amide I and II modes at 1,657 and 1,547 $cm^{-1}$ are observed. A comparison of the infrared absorbance spectrum of hemoglobin as a cast film (less water) is shown in Figure 6. The secondary structure of protein can be determined using conformationally sensitive frequencies[6].

Finally, infrared spectroscopy has previously been limited when mixtures are being studied. In the past it has been necessary to separate by physical or chemical means, the components and examine each of them separately. However, with the FTS-14 the spectrum of a mixture can be synthesized digitally by coadding the spectrum of the pure components. This effect is illustrated in Figure 8 where the ortho-, para-, and meta-xylenes are shown in the 3,200-3,700 $cm^{-1}$ region in the bottom of the figure. The top spectrum is the spectrum of a mixture of these three components with the solid line being the measured spectrum and the dashed line the calculated spectrum based on a least squares fit of the spectrum. The top spectrum is

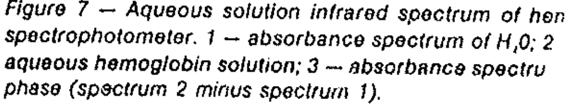

*Figure 7 — Aqueous solution infrared spectrum of hem[o]globin obtained with FT spectrophotometer. 1 — absorbance spectrum of $H_2O$; 2 [—] absorbance spectrum of aqueous hemoglobin solution; 3 — absorbance spectru[m] [o]f hemoglobin in aqueou[s] phase (spectrum 2 minus spectrum 1).*

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for an equal amount of each component, and the middle spectrum is for a 1% solution of meta-xylene in para-xylene with no ortho-xylene. The dashed line is again the calculated spectrum and the agreement is excellent. In the lower wave number region between 850 and 605 $cm^{-1}$ the spectrum of the xylenes is influenced by the presence of the other components and the pure spectra are perturbed and the agreement between the calculated and observed is therefore not as good. This latter result, although disconcerting initially, may well serve ultimately to allow the measurement of the amount of interaction between components of a mixture. Fourier transform infrared spectroscopy will find many additional uses in the research and development laboratory and our old friend, infrared spectroscopy, should experience a rebirth of interest as a result.

## References


1. S. Tolansky, "An Introduction to Interferometers", Longmans, Green and Co., London, 1966.
2. P. Jacquinot, Rep. Prog. Phys., 13, 267 (1960).
3. R. J. Bell, "Introductory Fourier Transform Spectroscopy", Academic Press, New York, 1972.
4. P. B. Fellgett, J. Phys. Radium, 19, 187 (1958).
5. J. Connes and P. Connes, J. Opt. Soc. Am., 56, 896 (1966).
6. J. W. Cooley and J. W. Tukey, Math Comput., 19, 297 (1965).
7. M. L. Forman, J. Opt. Soc. Amer., 56, 978 (1966).
8. T. Miyazawa, K. Fukushima, S. Sugano, and Y. Masuda in Conformations of Biopolymers, Ed. G. N. Ramachandran, Academic Press, London, 1967 Vol 2 p.557.


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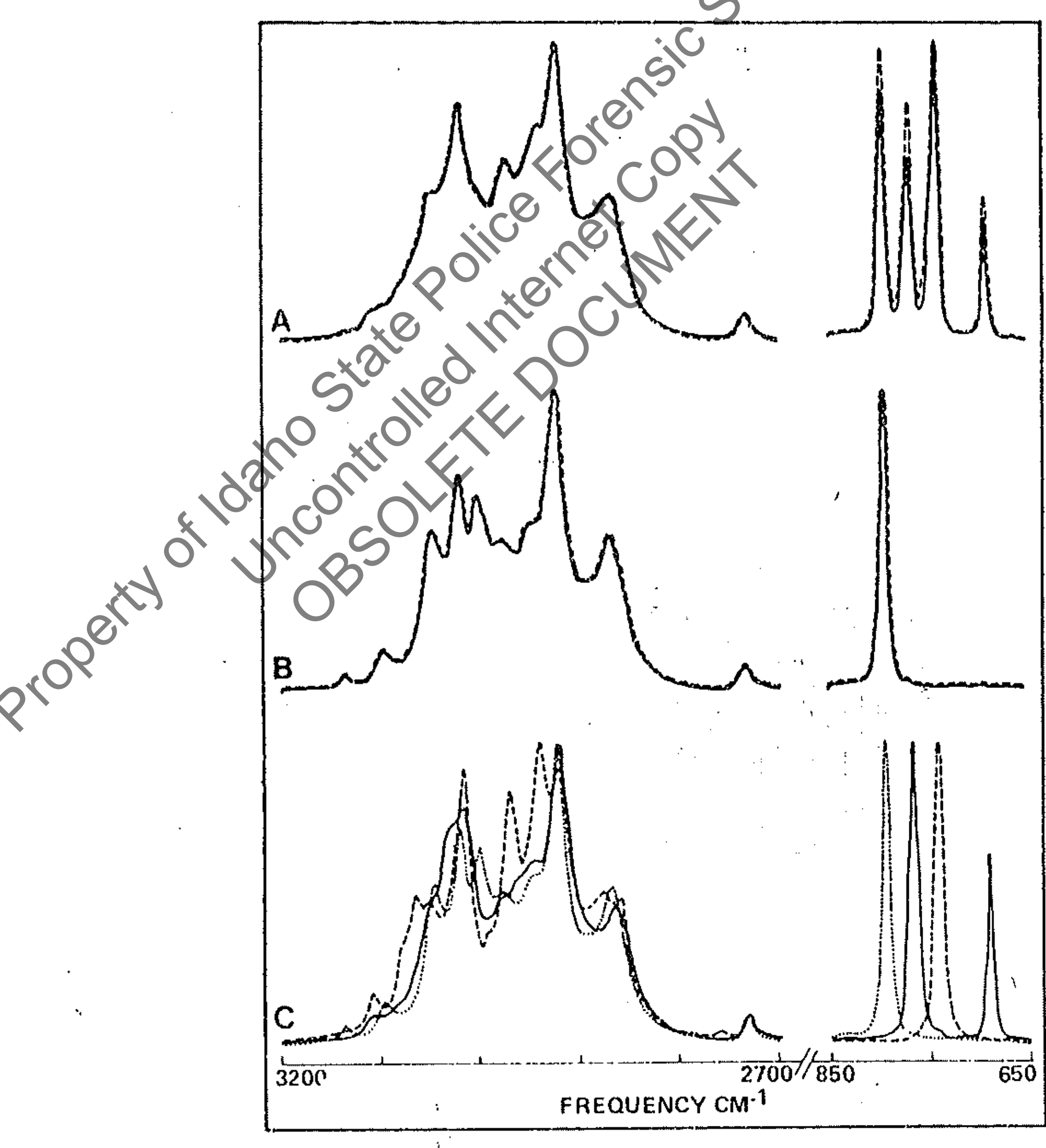


*Figure 8 — Infrared spectra of ortho-, meta-, and para-xylenes. A — solid line is experimental spectrum of mixture; dashed line is computed spectrum of mixture. B — 1% concentration of meta-xylene in para-xylene. Dashed line is calculated spectrum; solid line is computed. C — solid line is meta-xylene; dashed line is ortho-xylene; dotted line is para-xylene.*

This section describes a strictly non-theoritical approach to the identification of unknown spectra. The Sadtler Spec-Finder can be found at most universities and a partial index (of drugs) can be found in The Handbook of Analytical Toxicology (by Sunshine). The Spec-Finder is available on tape for computer searching. It will be apparent that spectra quality will be important - especially the absorbance, since it is necessary to know for sure which is the strongest band.

# DIRECTIONS FOR USE OF THE SADTLER SPEC-FINDER®

Consider this curve of eucalyptol the spectrum of an unknown compound. With the aid of the Sadtler Spec-Finder the identity would be determined using the following procedure.

STEP 1: On the coding slip shown below the strongest absorption band for each one micron region is coded to the nearest 0.1 micron.

STEP 2: The strongest band in the entire curve is selected and entered on coding slip.

STEP 3: The Spec-Finder page listing all reference spectra having a corresponding strongest band is located.

STEP 4: The entries in this group are searched in an effort to match the number in each micron region of the coding slip with the number in each micron region listed on the page. In searching one must many times allow ± 0.1 or 0.2 in the various regions. The closest match(s) reveals a spectrum (or several spectra) serial number(s).

STEP 5: The unknown spectrogram is compared on a point-to-point basis with the Sadtler Reference spectrum to establish identity. If positive identification is not made the comparison will give the user valuable information about the functional groups present and absent, enabling him to establish the materials general structure.

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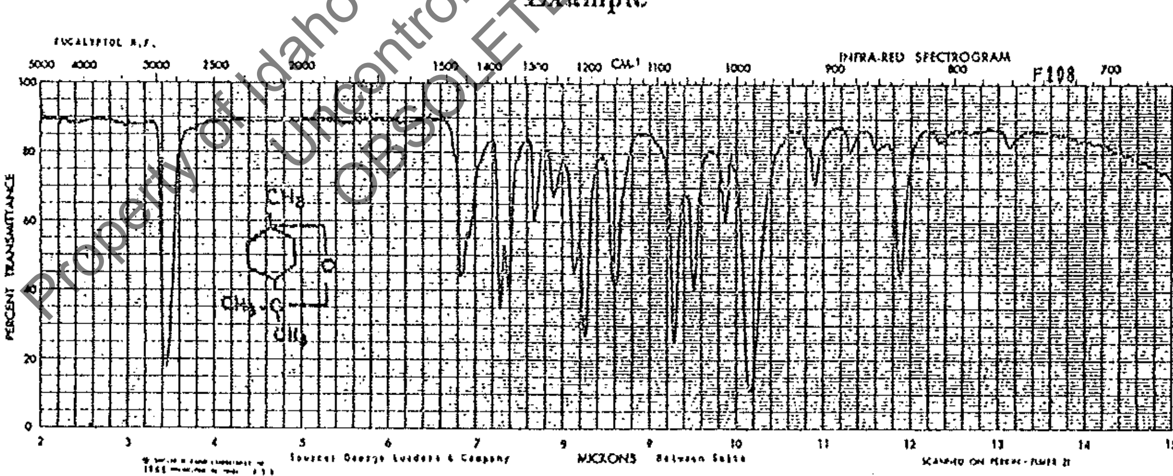
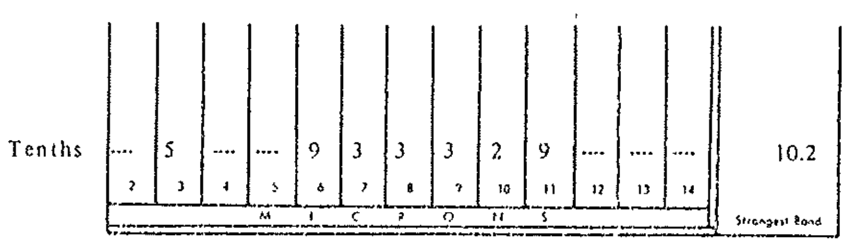
## Example



| Tenths | .... | 5 | .... | .... | 9 | 3 | 3 | 3 | 2 | 9 | .... | .... | .... | 10.2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | |
| | MICRONS | | | | | | | | | | | | | Strongest Band |

SADTLER CODING SLIP

## Sadtler Commercial Spec-Finder Illustration

281

| Microns 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | Strongest Band | Serial Number |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 6 | | 3 | 3 | 1 | | 1 | | 4 | | | 101 | D8464 |
| | 3 | | | 3 | 4 | 5 | 7 | 1 | | 1 | | 1 | 101 | X2814 |
| | 3 | | | 3 | 4 | 5 | 9 | 1 | | 1 | | 1 | 101 | X2814 |
| | 4 | | | 6 | 7 | 3 | 8 | 1 | 8 | 2 | | | 101 | E1823 |
| | 4 | | | 6 | 7 | 3 | 8 | 1 | 9 | 2 | | | 101 | E1823 |
| | 8 | | | 7 | | | 2 | 1 | | 4 | 1 | 5 | 101 | R 584 |
| | 1 | | | 7 | | 0 | | 1 | | 5 | 4 | | 101 | T 40 |
| | 0 | | | 7 | | 1 | | 1 | | | 2 | | 101 | W 572 |
| | 3 | | | 7 | 1 | | 3 | 1 | 1 | 9 | | 4 | 101 | D8111 |
| | 0 | | | 8 | 0 | 1 | | 1 | 3 | 3 | 2 | 8 | 101 | D4801 |
| | 0 | | | 8 | 0 | 1 | 7 | 1 | | | | | 101 | A4231 |
| | 3 | 1 | | 8 | 2 | 0 | 5 | 1 | 3 | 2 | 2 | 2 | 101 | R 252 |
| | 4 | | | 8 | 2 | 2 | 2 | 1 | 8 | | | | 101 | R 284 |
| | 4 | 5 | | 9 | 3 | | | 1 | | | | | 101 | D1879 |
| | 5 | | | 9 | 3 | | | 1 | 0 | 1 | | | 101 | P 187 |
| | 5 | | | 9 | 3 | | | 1 | 3 | 6 | 6 | | 101 | P 186 |
| | 2 | | | 9 | 4 | 8 | | 1 | 9 | 5 | | | 101 | J 247 |
| | 4 | 5 | | 9 | 6 | | | 1 | | | | | 101 | D1879 |
| | 0 | | | 9 | 8 | 1 | | 1 | 5 | | 3 | 7 | 101 | P 61 |
| | 4 | | 7 | 3 | 1 | 2 | 2 | 1 | 8 | 0 | 5 | 9 | 101 | D1330 |
| | 4 | | 8 | 0 | 8 | 9 | 9 | 1 | 9 | | 3 | | 101 | W 81 |
| | 0 | | 8 | 1 | 0 | 2 | 8 | 1 | | | | | 101 | D1227 |
| | 0 | | 8 | 1 | 1 | 2 | 8 | 1 | | | | | 101 | M 20 |
| | 5 | | 8 | 7 | 0 | 3 | 3 | 1 | 0 | 6 | 5 | 1 | 101 | F 67 |
| | 5 | | 8 | 7 | 4 | 3 | 3 | 1 | 0 | 6 | 5 | 1 | 101 | F 67 |
| 9 | | | | 2 | 7 | 5 | 4 | 1 | | | | 5 | 101 | A4327 |
| 9 | 4 | | | 3 | 5 | 3 | | 1 | 9 | 2 | 7 | 8 | 101 | M 61 |
| 9 | 4 | | 8 | 9 | 8 | 5 | 9 | 1 | | | | | 101 | X4445 |
| | 1 | | | | 1 | 2 | 8 | 2 | 0 | | | | 102 | L 608 |
| | 4 | | | 2 | 9 | 4 | 9 | 2 | 4 | 6 | 3 | 9 | 102 | A4112 |
| | 4 | | | 2 | 9 | 4 | 9 | 2 | 7 | 6 | 3 | 9 | 102 | A4112 |
| | 1 | | | 3 | 7 | 1 | 6 | 2 | | | | 2 | 102 | D1238 |
| | 1 | | | 4 | 7 | 1 | 6 | 2 | | | | 2 | 102 | M 78 |
| | | | | 5 | 3 | 2 | | 2 | 5 | 3 | | | 102 | W 429 |
| | | | | 5 | 3 | 2 | 3 | 2 | 5 | 3 | | | 102 | W 535 |
| | 4 | | | 7 | 3 | | | 2 | 0 | 3 | | | 102 | W 555 |
| | 4 | | | 8 | 2 | 1 | 6 | 2 | | | 3 | | 102 | P 163 |
| | 4 | | | 8 | 2 | 1 | 6 | 2 | 1 | | 3 | | 102 | P 164 |
| | 4 | | | 8 | 2 | 4 | 2 | 2 | | | 1 | 2 | 102 | D2084 |
| | 4 | 4 | | 8 | 3 | 3 | | 2 | | | 1 | 2 | 102 | D1827 |
| | 1 | | | 9 | | | 9 | 2 | 6 | 0 | | 1 | 102 | C 882 |
| | 0 | 1 | | 9 | 2 | 5 | 2 | 2 | 6 | 4 | 2 | 3 | 102 | R 129 |
| | 4 | | | 9 | 2 | 6 | 9 | 2 | 2 | 5 | | | 102 | A4117 |
| | 0 | 1 | | 9 | 2 | 9 | 2 | 2 | 6 | 4 | 2 | 3 | 102 | R 129 |
| | 5 | | | 9 | 3 | 3 | 3 | 2 | 9 | | | | 102 | F 198 ← |
| | 2 | | 7 | 8 | 3 | 6 | 5 | 2 | 6 | | 6 | | 102 | L 263 |
| | 4 | | 8 | 8 | 2 | | 2 | 2 | | | 1 | 2 | 102 | D2037 |
| | 4 | | 8 | 9 | 4 | 0 | 3 | 2 | | | 1 | 3 | 102 | D2035 |
| | 4 | | 8 | 9 | 6 | 0 | 3 | 2 | | | 1 | 3 | 102 | D2035 |
| 8 | | | | 2 | 3 | | 7 | 2 | | | | | 102 | A4244 |
| 9 | | | | | 8 | 3 | | 2 | | | | | 102 | G3522 |
| 9 | | | | | 8 | 8 | | 2 | | | | | 102 | G3522 |
| 9 | 4 | | | 7 | 2 | 0 | 6 | 2 | 0 | 7 | 4 | | 102 | R 247 |
| 9 | 4 | | | 7 | 2 | 1 | 6 | 2 | 0 | 7 | 4 | | 102 | R 247 |
| 9 | 4 | | | 9 | 6 | 1 | 1 | 2 | 9 | 8 | 8 | | 102 | R 320 |

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# THE IDENTIFICATION OF DRUGS FROM THEIR INFRARED SPECTRA

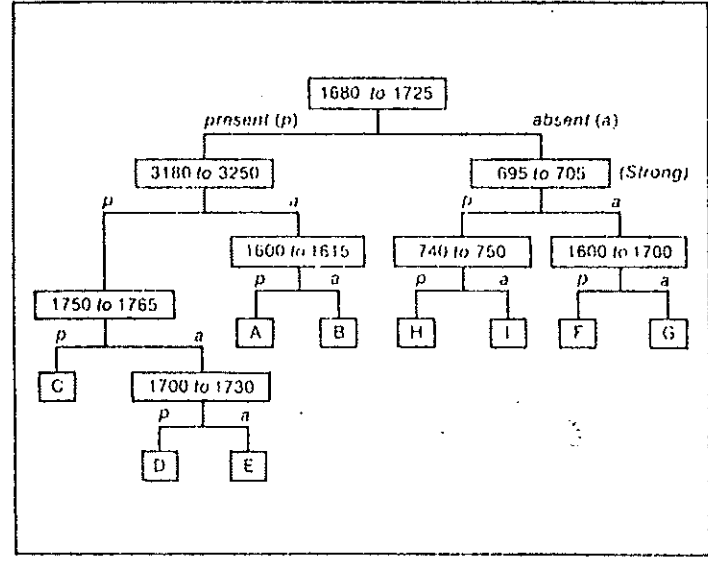



**Figure 1. Flow Diagram for Routine Drug Identification Using Infrared Spectra**

## DRUGS FLOW DIAGRAM GROUPS

**GROUP A**
Cocaine
Valium (Diazepam)
Acetylsalicylic Acid
Librium
Chlorpheniramine
Flurazepam
Caffeine

**GROUP B**
Compazine (Prochlorperazine)
Reserpine
Brevital – cast film
LSD – after solvent extraction
Methadone • HCl
Noludar (Methyprylon)

**GROUP C**
Amobarbital
Butethal
Barbital
Mephobarbital
Allylisobutyl barbituric acid
Diethyl barbituric acid
Allobarbital
Pentobarbital
Aprobarbital
Butabarbital
Secobarbital

**GROUP D**
Hexobarbital
Pentobarbital Sodium
Sedulon (Dihyprylone)
Serax (Oxazepam)
Dilantin (Diphenylhydantoin)
Doriden (Glutethimide)
Phenobarbital
Brevital

**GROUP E**
Meprobamate
Sodium Barbital
APC
Benzocaine
Procaine • HCl

**GROUP F**
Heroin • HCl
Morphine
Phenacetin
Quinine Sulfate
Codeine Alkaloid
Quinine • HCl
Codeine Sulfate
LSD – after solvent extraction

**GROUP G**
Elavil (Amitriptyline • HCl)
Trinaril
Stelazine (Trifluorperizine • HCl)
Thorazine (Chlorpromazine • HCl)
Tripelennamine
Methapyrilene

**GROUP H**
Dexedrine (D-amphetamine)
D-amphetamine sulfate
Methamphetamine • HCl

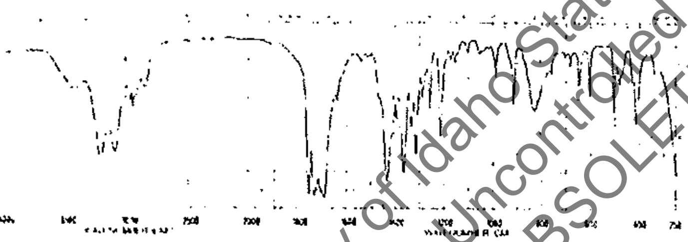
**GROUP I**
Darvon (Propoxyphene • HCl)
Phenazocaine

**GROUP J**
Lactose
Sucrose
Mannitol
$NaHCO_3$

**Table 1.**

## EXAMPLES OF USE

Examples follow to show how the diagram might be used. The samples and discussion are presented in such a way as to make it easy for the reader to follow the discussion and see the spectra and diagram at the same time.



The flow diagram asks us to look for a band in the 1680 to 1725 cm$^{-1}$ region. Such a band exists in this spectrum. We then look for a band in the 3180 to 3250 cm$^{-1}$ interval; it is present, so we proceed down the left branch once again. A band is present in the 1750 to 1765 cm$^{-1}$ region and this puts the compound into Group C which contains all the barbituates. The analyst looks at each of the spectra in Group C and by a band for band comparison picks out the exact match, in this case aprobarbital.

## WHY THE DIAGRAM WORKS

There is, of course, a very good spectral reason why such an approach as the flow diagram generated here works. A band between 1680 and 1725 cm$^{-1}$ may be assigned to a carbonyl. The frequency is somewhat low for an ester carbonyl and it is suggested that this is an aldehyde, ketone or, more likely an amide carbonyl. The second band in the left leg lies between 3180 and 3250 cm$^{-1}$ and it is most probably assigned to an NH stretch. Thus, in two of the groups at the end of a search down the left side are backbone structures such as those shown in Figures 2 and 3. Figure 2 shows the basic barbiturate nucleus which contains the amide carbonyl and the NH group. Similarly glutethimide, Figure 3, which falls in another group, also has some of these same basic structural features, and occurs, therefore, in the left side of the flow diagram, but in a different group from most of the barbiturates.

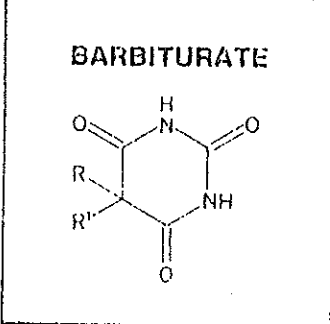



Figure 2

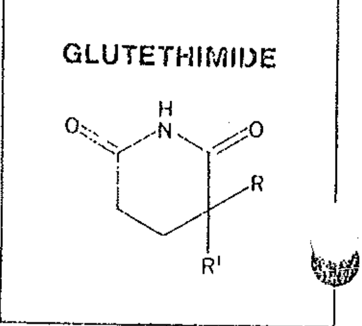



Figure 3

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## CRITERIA TO BE SATISFIED

To use an approach such as this, a number of important criteria, some instrumental, some sampling, must be satisfied. Instrumentally, the spectrophotometer must be in good abscissa calibration, within about ±5 $cm^{-1}$, and it must be operated properly. In particular, the dynamic response must be properly set. If the reference beam has been attenuated for any reason, the gain or slits or both must be properly adjusted. All of the reference spectra were obtained under grating resolution, and it is doubtful that data obtained on a prism instrument would be directly usable with the flow diagram described here.

With regard to sampling, all samples were prepared as KBr discs with the exception of Brevital in Group B, Dexedrine and Darvon. Unknowns must also be pre[illegible] this way. Good technique such as that described by Bradley and Potts (2) in *Applied Spectroscopy* in 19[illegible] for the grinding of samples for mulling should also b[illegible] observed here. In all cases possible, the sample sho[illegible] be preground or at least known to be finely divided – for example, a trapped GC fraction – before mixing KBr powder.

Offered in this paper is a simplified approach to th[illegible] interpretation of infrared spectra as applied to the identification of controlled drugs. The flow diagram described and presented in Figure 1 is by no means t[illegible] only one which might be generated. It is one which ha[illegible] been found useful and which fits not only the authors data but spectral data from other collections as well.

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(17)

This review covers publications cited in Chemical Abstracts (CA), volumes 80–83 (1974–75), through the December 29, 1975 issue.

**Selection of References.** The initial selection was based on a computer search of Chemical Abstracts Condensates (CAC) on magnetic tape.

The bibliography was managed throughout in computer readable form by means of an experimental computer program called LISE (LIterature Search and Edit), which was described briefly in the reviews for 1970–71 and 1972–73. This review has served as a vehicle for the development of LISE as an automated bibliographic tool.

This year, the bibliography is being submitted to Analytical Chemistry in computer readable form on magnetic tape which was generated via LISE from the CAC database. Thus, aside from selection, formatting, capitalization of titles, and minor corrections, the bibliography was effectively keyed by CA.

The general procedure was similar to that used for the 1972–73 review. The full CAC database for 1974–75 (725,000 citations) was first scanned with a broad profile to isolate a spectral database. From this database, three smaller databases were selected containing respectively books, reviews, and all infrared papers except books and reviews. The latter infrared database was sorted to bring all items from the same journal together.

Listings of these databases were scanned by eye to select a kernel database of relevant items. These were all printed on cards which were then annotated both from CA and the original papers. The number was reduced by a variety of criteria to reach a size acceptable to Analytical Chemistry. The cards were organized in the order in which the items were to appear, and the final manuscript and magnetic tape were generated from the kernel database via CA citations punched into the cards.

The total number of papers which are relevant to infrared spectrometry for the two years is about 8000. Obviously, many arbitrary choices were necessary to select the approximately 800 items which make up the bibliography.

Books and reviews were selected to give broad coverage, not only of infrared analysis, but of other topics which provide support for infrared analytical work. The remaining papers were selected with two points in mind: 1. to cover areas where new developments are underway, and 2. to give a broad view of applications of infrared spectrometry to chemical analysis.

It has been necessary to exclude most journal articles which describe application of infrared to structural problems, study of unstable species, adsorption and catalysis, and other topics which lie more in the field of physical than analytical chemistry. These papers are quite well indexed in the General Subject Indices of CA under infrared spectra. It is useful for active infrared spectrometrists to maintain a file of these indices close at hand.

The bibliography is mainly limited to work which has been cited in CA. For journals which submit directly to CA before publication, the coverage may include the December 1975 journal issue. Books and conference proceedings sometimes lag. A number of these items from 1970–1972 were eliminated because of their age.

**Organization of the Bibliography.** The bibliography is divided into twelve sections with separate numbering for each. Books (B) and Reviews (R) appear first because of their general interest. The remaining sections were selected by subject matter: Analytical Applications (A), Computer Applications (C), Environmental Applications (E), Fourier Spectrometry (F), General Interest (G), Hardware (H), Laser Spectrometry (L), Medical/Biological Applications (M), Polymer Applications (P), and IR Combined with Separati[illegible]s (S).

Each citation in the bibliography contains the complete title in English as given in CA. This follows a strong personal conviction of the reviewer that the title is an integral part of any bibliographic citation. The abbreviation, ibid., has not been used. Each citation is complete and can be clipped and pasted on a card if desired.

Translated titles provided by CAC are used for papers in languages other than English. For some Russian language journals which are available as English translations (i. e., Opt. Spektrosk.), the pages of the translated journal are provided as well as the original pages. The language is given for all types of citations except books.

**Table I. Sub-Search Hits on Infrared Database**

| Topic | Hits |
|---|---|
| Adsorption and Catalysis | 183 |
| Biological Applications | * |
| Carbonyl Groups and Metal Carbonyls | 206 |
| Coatings | 72 |
| Computer Applications | 47 |
| Chromatography | 118 |
| Detectors | 138 |
| Drugs | 18 |
| Forensic Applications | 8 |
| Fourier Spectroscopy | 53 |
| Gas and Nondispersion Analyzers | * |
| Gel Permeation Chromatography | 4 |
| Infrared Intensities | 188 |
| Infrared Lasers | 355 |
| Lignin | 2[illegible] |
| Matrix Isolation | |
| Minerals | [illegible] |
| Polarized Infrared | 81 |
| Pollution | 42 |
| Polymers | * |
| Quantitative Analyses | * |
| Tunable Lasers | 41 |

* Not feasible with inverted data base.

To compensate for space required by titles, the text has been kept brief. Titles are part of the text, and must be scanned in order to get the full message of this review. Comments are omitted from the text where the reviewer feels that the title suffices.

Within each section of the bibliography, references are listed in order of their CA citations to facilitate scanning of CA. It was originally planned to collect all citations for a given journal together to facilitate looking up original papers. A bibliography of this type was actually prepared, but so many journals had only one or two citations, that the order seemed purely random.

Table I indicates roughly the distribution of papers according to subject matter. Previously, this information was obtained by sub-searches on the infrared database. This year, these numbers were obtained from a commercial time-sharing computer search system which uses an inverted database. Some items which appeared in the corresponding table for 1972–73 could not be evaluated because of restrictions on the search of the inverted database.

## GENERAL TRENDS IN ANALYTICAL INFRARED SPECTROMETRY

**Instrumentation.** The pace is quickening in the development of new instruments and techniques for meas[illegible]ing infrared spectra.

Th[illegible] number and variety of new developments requires

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considerable effort on the part of the analytical infrared spectrometrist to maintain perspective between the old and the new.

Fourier spectrometers with self-contained data processing systems are showing tremendous versatility in many areas. The capability of the data system for storing, retrieving, and comparing spectra is at least as important as the special capabilities of the Fourier spectrometer, itself. The fact that more than 100 of these expensive systems are employed in industry and universities underscores their capabilities.

Conventional dispersion spectrometers, and tunable filter spectrometers continue to improve steadily. Several manufacturers produce systems for well under $5000 which can perform most routine analytical applications creditably.

The first micro-processor controlled dispersion spectrometer (Perkin-Elmer Model 283) was shown at the FACSS meeting in Indianapolis in November 1975. The preliminary program for the March 1976 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy brings notice of a microprocessor controlled infrared analyzer (Wilks Miran Analyzer). These are forerunners of instruments which will allow the spectrometrist to program special conditions for routine execution of elaborate analyses.

At the same time, developments in the field of tunable lasers threaten to surpass both dispersion spectrometers and interferometers in many respects. The tuning range of lasers has been increased dramatically. A recent review (R99) has defined a tunable laser as one which can be tuned over at least 100 $cm^{-1}$. A tuning range of 2 to 26 microns has already been reported (L31). It seems only a matter of time until a laser spectrometer for routine scanning of a wide spectral range is developed.

Lasers are also being used for detecting radiation by heterodyne techniques which are much more sensitive than thermal detectors (L11, L19, L27).

Non-tunable infrared lasers are finding applications in remote sensing via fluorescence and acoustic detection systems for atmospheric pollutants (B17, Bibliography Section L).

Techniques for use of infrared radiation for ellipsometry (G38, G68, G73) and circular dichroism (G6, G7, G23, G67) are becoming more practicable.

**Maturity of Infrared Spectrometry.** New editions of well known books (B12, B43, B45) and the number of other general texts (B4, B7, B10, B11, B14, B35, B37, B48) emphasize the growing maturity of infrared spectrometry.

Bellamy (B43) states in the introduction to his 3rd edition, "The basic data have changed little since the second edition, although they have been much extended in depth and detail. ... It remains the case that very few new group frequencies have emerged over the past 15 years."

Controversy has developed over an editorial by Laitinen (1) in Analytical Chemistry which likened the seven ages of an analytical method to Shakespeare's seven ages of man (2). The controversy arose because of the suggestion that infrared spectroscopy be kept in mind while contemplating the seven stages.

This was widely interpreted as suggesting that infrared spectroscopy has reached the seventh stage: "A period of senescence ... as other methods of greater speed, economy, convenience, sensitivity, selectivity, etc. surpass the method under consideration." The analogy with Shakespeare's final stage does seem somewhat strained: "Last scene of all, ... Is second childishness, and mere oblivion, Sans teeth, sans eyes, sans taste, sans everything."

A few spectroscopists were aroused sufficiently to reply in print, both in a review article (R62), and in paid advertising in Analytical Chemistry (3).

To this reviewer, who has been involved in all of Laitinen's stages except the first and the last, his description seems accurate. He indicates that the stages do not necessarily follow progressively, one after the other, and this has certainly been the case. Repeatedly, infrare spectrometry has been brought to bear rapidly on analytical problems in a new area of chemistry while mor direct, and more sensitive methods have been developed to displace it. This will continue, and it is highly desirable.

This reviewer wonders about use of senescence which implies a fading of capabilities. Knowledge, techniques, and capabilities will continue to develop indefinitely, assisted by competing techniques. It is hard to see how they can be lost, barring an atomic holocaust.

**Literature.** The total number of citations to infrared papers found by the computer search of CAC increased only by 1-2 percent over the corresponding number for the 1972-73 review (7800 vs 7700), while the total number of citations in CAC increased by nearly 15 percent (725,000 vs. 650,000). The period seems too short for any valid conclusion as to a tendency toward tapering off of infrared developments. In terms of actual employment of infrared techniques, this reviewer feels that there has been a significant increase, and that the quality of the literature cited in this review is higher than for the two preceding reviews.

Papers on infrared spectrometry are widely spread through the scientific literature. No less than fifty-seven journals contained at least 24 papers each during the present two year period. These are listed in Table II for those who may wish to follow developments by scanning title pages.

**Analytical Applications.** It is difficult to estimate the total activity in analytical infrared spectrometry from the literature. Most analytical applications either are not published, or are buried in descriptions of the work they supported.

Most papers on infrared analyses have been included in the bibliography in order to give a broad view of infrared activities throughout the world. These papers are mainly in sections (A), (M), and (P) of the bibliography. In most cases, these papers report the application of well known principles.

Governmental regulations are having a significant impact on infrared analytical applications, such as monitoring plant atmospheres and effluents, global monitoring of the atmosphere, and testing of internal compustion engines. This has stimulated work on remote sensing via absorption and fluorescence, heterodyne detection, semi-conductor detectors, diode laser spectrometers, non-dispersive and dispersive portable analyzers, etc.

## COMMENTS ON SELECTED AREAS

**Laser Spectrometry.** Several review articles and books have discussed tunable infrared lasers. Kuhl and Schmidt (R36) have surveyed basic principles and existing devices. Colles and Pidgeon (R99) have reviewed the physical principles and state-of-the-art devices in detail. Several books contain collections of papers on tunable lasers (B34, B36, L15).

The diode laser is possibly the simplest scanning laser. The problems of scanning an extended frequency range, and the strengths and weaknesses of diode lasers in high resolution molecular spectrometry have been discussed by Montgomery and Hill (L24). Most analytical spectrometrists will be dissatisfied with the tuning range of 1.0 $cm^{-1}$ or less, but there are, no doubt, applications in monitoring gas concentrations where this range suffices to sweep on and off a sharp analytical line. The present requirement of accurately controlled cryogenic temperatures below 10°K, and problems of reliability and lifetime tend to rule against routine applications temporarily. While the laser is, in principle, its own monochromator, users of diode lasers seem to be using very good monochromators, such as the Perkin Elmer E1, to isolate the laser line from satellites, etc.

The best current prospect for wide range IR scanning spectrometry employs a mixer for combining the output of

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**Table II. Journals with More than 24 Citations to Infrared in CAC, Vol. 80–83**

| Journal | Citations |
|---|---|
| *Anal. Chem.* | 37 |
| *Appl. Opt.* | 34 |
| *Appl. Phys. Lett.* | 44 |
| *Appl. Spectrosc.* | 46 |
| *Astrophys. J.* | 29 |
| *Aust. J. Chem.* | 27 |
| *Bull. Chem. Soc. Jpn.* | 50 |
| *Bull. Soc. Chim. Fr.* | 29 |
| *Can. J. Chem.* | 55 |
| *Chem. Phys. Lett.* | 69 |
| *Chem. Zvesti* | 30 |
| *Collect. Czech. Chem. Commun.* | 31 |
| *Dokl. Akad. Nauk. SSSR* | 49 |
| *Fiz. Tekh. Poluprov.* | 37 |
| *Fiz. Tverd. Tela (Leningrad)* | 35 |
| *Indian J. Chem.* | 27 |
| *Indian J. Pure Appl. Phys.* | 30 |
| *Infrared Phys.* | 30 |
| *Inorg. Chem.* | 92 |
| *Inorg. Chim. Acta* | 25 |
| *Izv. Akad. Nauk. SSSR, Ser. Khim.* | 58 |
| *J. Am. Chem. Soc.* | 45 |
| *J. Appl. Phys.* | 34 |
| *J. Catal.* | 27 |
| *J. Chem. Phys.* | 172 |
| *J. Chem. Soc., Dalton Trans.* | 55 |
| *J. Chem. Soc., Faraday Trans.* | 62 |
| *J. Chem. Soc., Perkin Trans.* | 38 |
| *J. Chim. Phys. Physicochim. Biol.* | 35 |
| *J. Inorg. Nucl. Chem.* | 70 |
| *J. Mol. Spectrosc.* | 119 |
| *J. Mol. Struct.* | 147 |
| *J. Organomet. Chem.* | 88 |
| *J. Phys. C* | 80 |
| *J. Phys. Chem.* | 80 |
| *J. Phys. Chem. Solids* | 28 |
| *J. Quant. Spectrosc. Radiat. Transfer* | 39 |
| *J. Raman Spectrosc.* | 24 |
| *Khim. Geterotsikl. Soedin.* | 24 |
| *Kvantovaya, Elektron. (Moscow)* | 24 |
| *Opt. Commun.* | 30 |
| *Opt. Spektrosk.* | 82 |
| *Phys. Rev. B* | 59 |
| *Phys. Status Solidi B* | 52 |
| *Rocz. Chem.* | 25 |
| *Solid State Commun.* | 51 |
| *Spectrochim. Acta., Part A* | 304 |
| *Spectrosc. Lett.* | 30 |
| *Ukr. Khim. Zh. (Russ. Ed.)* | 40 |
| *Z. Anorg. Allg. Chem.* | 50 |
| *Z. Naturforsch. B* | 35 |
| *Zh. Fiz. Khim.* | 80 |
| *Zh. Neorg. Khim.* | 95 |
| *Zh. Obshch. Khim.* | 67 |
| *Zh. Prikl. Khim. (Leningrad)* | 24 |
| *Zh. Prikl. Spektrosk.* | 182 |
| *Zh. Strukt. Khim.* | 24 |

two tunable dye lasers (which may be excited by a single laser). At least one of the dye lasers must be tunable over the required range in $cm^{-1}$.

Mixing devices are commonly non–centro–symmetric solids. Symmetric materials can be used as mixers by employing non–linearities related to dispersion, etc., to obtain the required phase matching between input and output beams. It is relatively easy to tune the visible dye laser over the 4000 $cm^{-1}$ required for a complete infrared scan of the fundamental vibration–rotation region, but the mixing crystal must be transparent not only i[illegible] the infrared, but in the visible a[illegible]ell. There has been a wide search for crystals with the [illegible]site characteristics.

Wynne, Sorokin, and La[illegible]31) utilized third order non–linear response of alkali metal vapors which are transparent throughout the infrared. Employing two tunable dye lasers excited by a single pulsed $N_2$ laser, the were able to scan from 2 to 26 microns by tuning one of the dye lasers. The other was continuously fine–tuned to maintain phase–matching. The mixer was a mixture of sodium and potassium vapors whose concentrations were also varied.

The technical problems associated with scanning a wide spectral range are formidable. Clearly, a routine 5–10 minute scan of the whole range would be difficult at present.

The pulsed output should present no problems with a microprocessor controlled photometer system. Line width is sufficiently small (0.2 $cm^{-1}$) to contribute negligibly to the absorption band width of most condensed phases. The energy seems sufficient for S/N superior to that of present dispersion or interferometric instruments.

This is not the only system which covers a major part of the IR region (B36).

**Step–Tuned Laser Spectrometers.** Continuous tuning is not needed for many analytical problems. Patty et al (E50) described a $CO_2$ laser system for determining ambient levels of ozone, ammonia, and ethylene in open air paths. Their procedure was to measure a number of the 60–odd $CO_2$ laser lines between 9.2 and 10.8 microns. The required concentrations were calculated from previously determined absorption coefficients. This system has potential for liquids, as well.

**Fixed Frequency Laser Measurements.** When the system to be measured has only one variable component which attenuates the laser beam, that component can be measured continuously even when the matrix is relatively opaque. Kraus and Maur (L16) reported continuous measurement of sulfate ion in water with a sensitivity of 10 ppm. They proposed monitoring sulfate in the effluent of a sewage plant by this means. Freeman and Upham (S2) used a fixed frequency laser as a detector in liquid chromatography.

**Laser Induced Fluorescence.** The sensitivity of infrared absorption measurements is limited because absorption is measured as the difference between two sizable signals. One way around this is to excite vibrational fluorescence of the species which is to be measured. Under suitable conditions, the fluorescence lies in a transmission band of the matrix, and can be measured against a very low background. This technique is receiving attention, for detection of atmospheric contaminants (R55, R65, R121, R136, E36, E49, L4–5, L18, L25).

**Opto–Acoustic Detection.** An extraordinarliy sensitive technique for measuring pollutants in gaseous systems employs a modulated laser to excite acoustic vibrations in a cell which is monitored by a sensitive microphone (R98, E6). Absorption of laser radiation by the gas causes a change in pressure due to heating. When the laser line coincides with a line of the component to be detected, and the matrix gas is transparent at that wavelength, the sensitivity is probably higher than can be attained by any other infrared technique, i. e., a few ppb. The detector is inherently small. If the laser is not too large, there is hope for portability.

**Multiphonon Absorption of Laser Radiation.** When radiation from a high power laser is focussed into a narrow beam, it can damage windows and mirrors (R76, G57, L13). In the search for higher and higher power densities, the optical properties of IR transparent materials are receiving considerable attention (G46–7, G57, G60, L29).

When gaseous substances are exposed to very high intensity radiation fields in a vibrational absorption band, appreciable numbers of molecules are excited to very high vibrational levels, and even dissociated or ionized (R123, L10, [illegible]21, L23, L32) This is not yet understood in detail, but [illegible] of extreme impor[illegible]ance in laser isotope separation bec[illegible] the selectivity seems to be determined by the width of the fundamental transition (L32). Stepwise

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climbing of the vibrational energy level ladder by absorption of successive photons does not explain the results. A molecule which has been raised to the first excited vibrational state by a photon can not absorb an identical photon because of mechanical anharmonicity. Electrical anharmonicity may be responsible for producing the highly excited states in one jump. These phenomena may have analytical applications.

**Backscatter of Laser Radiation.** Some infrared lasers are so powerful that they can be employed for long range open-air measurements in which the laser radiation is scattered back to the detector by a distant object, or by aerosols or dust in the air. The laser is scanned back and forth across an absorption line to establish a baseline for measuring peak absorption. This reviewer has little enthusiasm over the prospect of sending out high energy, invisible laser pulses wholesale for this purpose. Clearly, this technique should be employed only after considerable thought, and consultation with regulatory agencies.

**Heterodyne Detection of Laser Radiation.** Heterodyne detection has been employed to detect weak infrared signals (L11, L19, L27). The radiation to be measured is mixed with that of a local oscillator whose frequency is chosen to produce a microwave difference frequency which can be detected electrically. Such detectors are far more sensitive and faster than conventional infrared detectors. When it is possible to use such a detector with a laser spectrometer, S/N will be dramatically improved over that of either grating or Fourier spectrometers.

**Non-linear Spectroscopy.** The ability to excite a large fraction of the molecules in a gas is permitting a variety of new techniques of non-linear spectroscopy (R114, R143, L20), and double resonance experiments (L2-3, L6, L9, L28). It seems that most of the related techniques which have been so powerful in microwave and nuclear resonance spectroscopy can be performed with infrared radiation.

**Fourier Spectrometers.** Koenig (R133, P96) has reported work on polymer systems which provides a fine illustration of the power of the Fourier spectrometer with built-in data system. The characteristics which make the system powerful are discussed (R133). Hirschfeld and Kizer (F16) have emphasized features which permit novel procedures for handling spectroscopic data.

Many advantages commonly associated with the Fourier spectrometer are due to the self-contained data system. Since data processing is feasible for other types of spectrometers, it is discussed under Computer Applications. It should be pointed out here that data processing which intermixes spectra measured at different times requires extraordinary frequency reproducibility. Proponents of Fourier spectrometers claim that the requisite accuracy is inherent in the laser controlled readout mechanism. Comparable reproducibility for dispersion instruments also requires digital recording.

**Comparison of Fourier and Dispersion Spectrometers.** This reviewer has not yet seen a comprehensive comparison of the capabilities of high performance Fourier and dispersion spectrometers. This is a difficult comparison to make without involving the manufacturers, and it is awkward for them because strong business interests are involved.

This reviewer sees the following clear advantages of the Fourier spectrometer: 1. Long term frequency accuracy, 2. ability to make use of longer effective integration times, 3. ability to record data over the full spectral range much faster than most conventional instruments, 4. absence of artifacts caused by changing gratings and filters, 5. better S/N at low signal levels, and 6. all of these characteristics are combined into one general purpose system which is integrated with a powerful data processor.

On the other hand, there are the following clear advantages of dispersion spectrometers: 1. low cost, 2. shorter elapsed time from introduction of the sample to completion of a hard-copy spectrum, 3. true double beam operation, and 4. conditions can be chosen such that the noise period is much shorter than the narrowest spectral feature that can be resolved. For the Fourier spectrometer, the apparent noise period is equal to the resolving power of the spectrometer. Thus, comparison of several spectra may be required to establish confidence in a small spectral feature.

For the present, this reviewer is reserving judgment on the throughput and multiplex advantages which are claimed for the Fourier spectrometer. Koenig (R133) has optimistically set these advantages at 80-200 times, and 80 times, respectively. The combination of these two factors gives more than 6000. Why is little of this realized in practice, except for very short and very long scan times, or at low light levels? Even a factor of ten would be obvious by inspection of standard spectra.

In a recent book on Fourier transform spectroscopy, Griffiths (B49) has indicated that the throughput factor favors the interferometer by only a modest amount. Comparing the Digilab FTS14™ and the Perkin Elmer 621, using more or less standard conditions, of 2 $cm^{-1}$ resolving power, he states, "..for these two instruments at least, through-put considerations appear to favor neither the interferometer or the grating spectrometer to any great extent."

Fourier spectrometers can scan spectra faster than conventional spectrometers, but so far, they require somewhat longer elapsed time from introduction of the sample to completion of the plotted spectrum. For standard laboratory samples, the rate limiting steps are in the settling time for sweeping out water vapor after introduction of the sample, in performing the Fourier transform, and in plotting the spectrum. Oertel and Fehl (F15) reported a glove box arrangement for reducing ingress of water vapor while changing a sample. An equivalent arrangement is indispensable for utilizing the speed of the spectrometer for routine samples. The calculations are rapid when the number of points permits the transform to be carried out in core memory, and slow when the disk must be used. For this reason, the purchaser of a Fourier transform machine is well advised to buy as much core as possible.

A number of terms which are foreign to the dispersion spectrometrist are employed by the Fourier spectrometrist, such as: zero-filling, A-D noise, apodization, etc. These have been explained by Griffiths (B49). This reviewer has just come across chirping (F4, F19, F20), an important technique which spreads the central peak of an interferogram by means of a dispersive material in one arm of the interferometer. The calculations are more complicated, but this technique may improve the performance of Fourier spectrometers markedly.

**Infrared Imagery.** Development of techniques for obtaining images by long wavelength radiation (R91, R106, G22, G51, L8) may before long make infrared microscopy feasible in the fundamental vibration region.

**Computer Applications.** A number of papers on learning-machines and pattern recognition techniques have appeared (C1, C9, C10, C19-20, C22-3). One reason for selection of infrared data for these studies is that a relatively large database is readily available, i. e., the ASTM infrared index. It is unfortunate that it does not contain intensities as well as more accurate wavelength data. It would be interesting to know how much human beings could have accomplished in assigning group frequencies if they had been limited to the 180 bits of spectral information per compound which is represented in the ASTM data.

Erly (R127) has made a number of constructive suggestions with regard to coding, storage, and retrieval of infrared and related chemical information.

Other computer activities have involved search and retrieval (C2, C7, C11-13, C16, C21), and progress is being made on structural interpretation of spectra (C5, C6, C8, C17, C18, C24-5).

**Application of Data Processing to Chemical Spectroscopy.** As mentioned previously, data processing can be employed with dispersion spectrometers as well as Fourier spectrometers. However, use of dedicated data systems for such conventional spectrometers has lagged.

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More conventional spectrometers are being equipped with digital readout systems, and it is just a question of time before dedicated data processing systems will be employed widely with such systems.

It takes an appreciable amount of time to carry out data processing and plotting (B49). To avoid tying up the spectrometer, the data system should be capable of acquiring spectra while data processing and display are in progress. The features which are needed for infrared data processing are also applicable to other laboratory instruments, such as visible and ultraviolet spectrometers, scanning calorimeters, etc.

As mentioned above, superior frequency accuracy is required for wavelength registration of spectra for computer data processing. Hirschfeld and Kizer (F16) have indicated that, "The tolerances ... are quite frightening" Long term maintenance of registration between current and archival spectra will be difficult. It is required that the spectrometer calibration should not drift, and that it should not change during service adjustments. The Fourier spectrometer with its laser calibration system seems to have an advantage. However, microprocessor controlled dispersion instruments permit check-out and adjustment of calibration much more readily than at present.

Digital recording emphasizes intensity errors, also. These have been discussed by Jones (R26). Digital measurements are desirable for work on band contours (G4, G8, G18, G69, P53, P95). One application area where this will be of importance is in the effect of mechanical strain on vibration frequencies (P7, P64, P69).

**Infrared Reference Data.** A good library of reference spectra is as important as the infrared spectrometer itself. Organization of spectra according to functional class for ready access and browsing is nearly as important as indexing.

The 2nd edition of the Aldrich Library of Infrared Spectra by Pouchert (B40) has appeared. Evidently this publishing venture has been a success for the publisher as well as the user. It is hoped that other manufacturers of fine chemicals will follow this lead. Approximately 2000 spectra have been added to the 9000 spectra of the 1st edition, including 150 polymers. This collection makes a wide variety of spectra available to the average organic chemist, and even to the smallest technical school. Browsing is feasible because many spectra appear on each page, and spectra are organized according to chemical class. In addition to the book s own indices, the spectra are indexed in the Aldrich Catalog Handbook of Organic and Biochemicals.

Colthup (B45) in his 2nd edition has added a few Raman spectra to his fine collection of 600 infrared spectra. The compact, but readable format of the spectra, and representation of most classes of compound make this collection almost as effective as the well known Colthup Chart of group frequencies. This reviewer has found it useful to combine the spectra into a wall chart which occupies only about 12 square feet.

An excellent book has appeared showing the spectra of a number of toxic gases at the 8 hour exposure limit superimposed on atmospheric absorption in a 20 meter gas cell (B26).

Other collections of spectra have appeared (B1-2, B19, B29, G10, G43, G72, M17). In addition, there are a number of tables of absorption bands and indices to published spectra (B9, B20, B22, B32, B46, B52, B53, B54, G31). Many reviews of spectra of specific classes of compound have been published (B3, B15, B16, B21, B25, B30, B38, B42, B46, B50, B51, R7-11, R13, R18, R35, R46-8, R73, R101-3, G10, G20).

Three ASTM volumes (B52, B53, B54) index the final supplement to the ASTM infrared index on magnetic tape. These cover the 14th supplement as well as the 15th including 43,000 compounds. This brings the total for the ASTM infrared index to 135,000.

ASTM has terminated the project which generated the data in these books and on magnetic tape, and has sold the data to Sadtler Laboratories. For the present, the data are available from Sadtler on the same basis as from ASTM.

The Coblentz Society has prepared a comprehensive list of collections of infrared spectra which is available on request from the secretary (4).

**Publication of Infrared Data.** The Coblentz Society has revised its specifications for Class II infrared spectra (G64), and criteria for spectra submitted to journals have been given by members of the Joint Committee on Atomic and Molecular Physical Data (G66). The latter note has emphasized the need for published spectra to serve, not only as documentation of the work in which they appear, but also as reference data as well. Both publications emphasize the use of photographs of original spectra to avoid inaccuracies of tracing and reformatting.

It is this reviewer's observation that the format generated by most infrared spectrometers is wasteful of journal space because the ordinate is more expanded than necessary. To keep space requirements within bounds, spectra are often much reduced for printing, adversely affecting the abscissa accuracy. The format employed by Colthup (B45) in which the ordinate is reduced by a factor of 4 provides sufficient ordinate information for most purposes, and improves abscissa accuracy.

In view of the increasing numbers of spectrometers which can generate digital spectra, it is not too soon to consider submission of spectra in computer readable form, possibly to a special journal, which would convert them to a standard format for publication. It is time to start work on specifications for Coblentz Type I spectra which can only be represented in computer readable form.

**Analytical Applications.** A number of papers have discussed general trace techniques (B18, B23, R39, E39, E42, G5, G19, G25, A29, A35, A37-8, S10).

Methods have been reported for determining $CO_2$ in wine by ATR (A6), and for determining alcohol in breath using a He/Ne laser (L22). Some analytical problems have received considerable attention: determination of water content (A68, A76, A107, A109, A124, A141, P24, P36), determination of penicillin (A111, M7, M11, M33-4), kidney stones (M2, M8, M13, M20, M23-4), wood and lignin (A67, A84, A89, A103, A104, A108, A118, A131), airborne quartz dust (E12, E23, E29, E47, E51). In the area of petroleum analyses, work has been reported on identification of petroleum in waste water (A133, E66, E71), identification of the source of oil spills from spectral features (A123, E5, E7-8, E21), and analysis of petroleum itself (A5, A7, A86, A100, A105, A114, A119, A127-8, A132).

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It was not possible to reproduce all the inorganic spectra from this article. I did feel, however, that the text was worth copying. The reference should be available and has been reprinted in the State DOJ IR Manual.

# Infrared Spectra and Characteristic Frequencies of Inorganic Ions

## *Their Use in Qualitative Analysis*


FOIL A. MILLER AND CHARLES H. WILKINS

*Department of Research in Chemical Physics, Mellon Institute, Pittsburgh 13, Pa.*



**Polyatomic ions exhibit characteristic infrared spectra. Although such spectra are potentially useful, there is very little reference to them in the recent literature. In particular, the literature contains no extensive collection of infrared spectra of pure inorganic salts obtained with a modern spectrometer. In order to investigate the possible utility of such data, the infrared spectra of 159 pure inorganic compounds (principally salts of polyatomic ions) have been obtained and are presented here in both graphical and tabular form. A table of characteristic frequencies for 33 polyatomic ions is given. These characteristic frequencies are shown to be useful in the qualitative analysis of inorganic unknowns. Still more fruitful is a combination of emission analysis, infrared examination, and x-ray diffraction, in that order. Several actual examples are given. It is evident that a number of problems involving inorganic salts containing polyatomic ions will benefit by infrared study. The chief limitation at present is the practical necessity of working with powders, which makes it difficult to put the spectra on a quantitative basis.**



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ALTHOUGH there has been a vast amount of work on the Raman spectra of inorganic salts (2, 4), the study of them in the infrared has been relatively neglected. Schaefer and Matossi (10) have reviewed work done up to 1930, most of which deals with reflection spectra. The most extensive surveys of infrared absorption spectra have been made by Lecomte and his coworkers (6, 7), but unfortunately many of their data are somewhat out of date and are not always presented in the most useful form. References to studies on a few ions are given in the books by Wu (12) and by Herzberg (3). There has recently been renewed interest in the detailed study of the infrared spectra of selected salts, as exemplified by the papers of Halford (8), Hornig (11), and their coworkers. The well known Colthup chart (1) contains characteristic frequencies for nitrate, sulfate, carbonate, phosphate, and ammonium ions. An excellent recent paper by Hunt, Wisherd, and Bonham (5) contains the spectra of 64 naturally occurring minerals and related inorganic compounds.

Aside from sixteen spectra in this latter paper, there is in the literature no compilation of infrared spectra of inorganic salts obtained with a modern spectrometer. It therefore seemed worth while to make a fairly extensive survey to seek answers to the following questions: Is it generally possible to obtain good spectra? Do the ions possess frequencies which are sufficiently characteristic to be useful for analytical purposes? What is the effect on the vibrational frequencies of varying the positive ion? Is infrared spectroscopy useful in the analysis of salts?

This paper presents the spectra from 2 to 16 microns of 159 pure inorganic compounds, most of which are salts containing polyatomic ions. A chart of characteristic frequencies for 33 such ions is given. The use of these data for the qualitative analysis of inorganic mixtures is demonstrated. Finally, a number of interesting or puzzling features of the spectra are described.

A brief classification of the various types of vibrations in crystals may be appropriate. Ionic solids are considered first. In a crystal composed solely of monatomic ions, such as sodium chloride, potassium bromide, and calcium fluoride, the only vibrations are "lattice" vibrations, in which the individual ions undergo translatory oscillations. The resulting spectral bands are broad and are responsible for the long wave-length cutoff in transmission. In a crystal containing polyatomic ions, such as calcium carbonate or ammonium chloride, the lattice vibrations also include rotatory oscillations. Of greater interest in this case, however, is the existence of "internal" vibrations. These are essentially the distortions of molecules whose centers of mass and principal axes of rotation are at rest. The internal vibrations are characteristic of each particular kind of ion.

In molecular solids, such as benzene, phosphorus, and ice, the units are uncharged molecules held in the lattice by weak forces of the van der Waals type, and often also by hydrogen bonds. The same classification into internal and lattice modes can be made. A few examples of such solids are represented in this paper (boric acid, and possibly the oxides of arsenic and antimony).

Finally there are the covalent solids, such as diamond and quartz, in which the entire lattice is held together by covalent bonds. Here the distinction between lattice and internal vibrations disappears. One might at first expect an ill-defined and featureless spectrum, but such is not the case. Actually there are bands that are very characteristic. The situation is in some ways analogous to that in a polymer, which in spite of its size and complexity possesses a remarkably discrete spectrum. Silica gel is the only representative of this type included here.

### EXPERIMENTAL

**Origin and Preparation of Samples.** Practically all the samples were commercial products of C.P. or analytical reagent grade. The samples were gound to a fine powder to minimize the scattering of light, and were examined as Nujol mulls. When there were spectral features that were obscured by the Nujol bands, the samples were either run as a dry powder or mulled in fluorolube (a mixture of completely fluorinated hydrocarbons. Fluorolube is a product of the Hooker Electrochemical Co., perfluoro lube oil of E. I. du Pont de Nemours & Co.). Some compounds, such as ferric nitrate nonahydrate (No. 49) and calcium permanganate tetrahydrate (No. 150), seemed to mull up in their own water of hydration. When the fine powder was rubbed between salt plates, it acquired the appearance and feel of a typical mull, but no appreciable fogging of the salt plates resulted. For other compounds, such as potassium carbonate, breathing on the sample achieved the same result. This is not recommended, however, for it varies the water content unnecessarily, and with potassium carbonate some of the bands are shifted.

Although these techniques are satisfactory for qualitative examination, it may be of interest to list some other methods which have been mentioned in the literature for handling inorganic solids. Lecomte, who introduced most of them, has pointed out that a finely ground dry powder scatters very little radiation of wave length greater than 6 microns and consequently it may be used directly in that region (6, 7). He also suggests coating

one of the salt plates with a very thin layer of solid paraffin to hold the particles in place (*6, 7*). The fine powder may be prepared by grinding, by evaporation of a suitable solvent (*6, 7*), or by sedimentation (*5*). Vacuum evaporation which has been used for preparing films of ammonium halides (*11*), may be useful for other relatively volatile inorganic materials.

Spectroscopic Procedures. All samples were examined from 2 to 16 microns with a Baird Model A infrared spectrophotometer. Wave lengths are accurate to about ±0.03 micron, although for broad bands the error of judging the center may exceed this. It was sometimes found that duplicate spectra for the same compound differed by more than this amount. Some possible reasons are mentioned below.

Representative examples of several ions were examined in the potassium bromide region with a Perkin-Elmer 12B spectrometer. Likewise, a series of ten nitrates was examined in the rock salt region with this same instrument in order to fix the wave lengths of absorption more accurately.

No attempt was made to put the spectra on a quantitative basis.

The purities of the samples are indicated in the legends for the curves.

Some idiosyncrasies of the curves warrant mention. Many them show weak remnants of the carbon dioxide bands near ( and 14.8 microns. The latter always appears as a sharp upwa pip. Many of the curves exhibit a drop in transmission near 15 microns and then a small increase beginning at 15.5 microns. The initial decrease is due to the absorption by the sodium chloride plates, which was not compensated in the reference beam. The reason for the later increase is not known, but it is not real. It has the effect of suggesting an incorrect position for bands near 16 microns. For example, in ferrous sulfate heptahydrate (No. 91) the curve indicates a band at 650 cm.$^{-1}$ (15.5 microns), but actually it is at 611 cm.$^{-1}$ (16.5 microns).

**Table III. Infrared Bands of Various Nitrates (Cm.$^{-1}$)**

| Intensity | m, sp[a] | w | m, sp | w | vs | s | s | vs | vw |
|---|---|---|---|---|---|---|---|---|---|
| $NaNO_3$ | .. | .. | 830 | .. | 1358 | .. | .. | 1700 | 2428 |
| $KNO_3$ | .. | .. | 824 | .. | 1380 | .. | .. | 1767 | .. |
| $AgNO_3$ | 733 | 603 | 835 | .. | 1348 | .. | .. | .. | .. |
| $Ca(NO_3)_2.xH_2O$ | .. | .. | 820 | 1044 | (1350) | (1430) | (1640) | .. | .. |
| $Sr(NO_3)_2$ | 737 | .. | 815 | .. | 1387 | 1441 | .. | 1795 | 2420 |
| $Ba(NO_3)_2$ | 729 | .. | 817 | .. | 1352 | 1418 | .. | 1774 | (2410) |
| $Fe(NO_3)_3.9H_2O$ | .. | .. | 835 | .. | 1361 | .... | 1615 | (1785) | .. |
| $Co(NO_3)_2.6H_2O$ | .. | (807) | 836 | .. | 1372 | .. | (1640) | .. | .. |
| $Cu(NO_3)_2.3H_2O$ | .. | .. | 830 | .. | 1378 | .... | 1587 | 1790 | 2431 |
| $Pb(NO_3)_2$ | 726 | 807 | 836 | .. | 1373 | .. | .. | .. | .. |

Bands > 3000 cm.$^{-1}$ are omitted.
( ) Baird values, less accurate.

[a] w, m, s = weak, medium, strong. sp = sharp. v = very.

### RESULTS

The spectra are presented at the end of this paper. Table I lists the compounds examined and gives the numbers of the corresponding spectral curves. Table II summarizes the positions of the bands in wave numbers and in microns, and gives estimated peak intensities. If more precise wave numbers have been determined with the Perkin Elmer spectrometer, they are used. Asterisks indicate those compounds examined in the potassium bromide region.

The spectra themselves are shown in graphical form. Nujol bands are marked with asterisks; portions of curves run in fluorolube are indicated by an F. The spectra of Nujol and fluorolube are included for comparison (No. 160). In a few cases the powder was used without a mulling agent; these are indicated by P.



Figure 1. Comparison of Infrared Spectra of Ten Sulfates

W. Water
vb. Very broad
sh. Shoulder

### DISCUSSION OF RESULTS

The spectra range in quality from surprisingly good ones, wi sharp, intense bands (see curves for barium thiocyanate dil drate, No. 28; strontium nitrate, No. 44; and ammoni hexanitratocerate, No. 157,) to very poorly defined ones such as those for potassium silicate, No. 32; monobasic magnesium phosphate, No. 69; and monobasic potassium orthoarsenate, No. 75. It seems to be characteristic of the phosphates, and especially of their monobasic and dibasic modifications, to have ill-defined spectra. The reason for this is not clear, but it may be due to lack of a single, well-ordered crystal structure.

Effect of Varying Positive Ion. One of the purposes of this study was to ascertain whether the various ions have useful characteristic frequencies. It was therefore of interest to know the effect of altering the positive ion. The spectra of ten sulfates are shown in Figure 1 in the form of a line graph. It is seen that two characteristic frequencies occur, one at 610 to 680 cm.$^{-1}$ (m) and the other at 1080 to 1130 cm.$^{-1}$ (s). There is enough variation between the individual sulfates so that it is often possible to distinguish between them from the exact positions of the bands. Table III presents similar data for ten nitrates. Again there are characteristic frequencies, at 815 to 840 cm.$^{-1}$ (m) and 1350 to 1380 (vs). The authors have been unable to find any orderly relation between the positions of these nitrate bands and a property of the positive ion, such as its charge or mass. This is not surprising, for there are at least three reasons why a frequency may shift slightly as the kind of positive ion is changed.

The different charges and radii of the vari positive ions produce different electrical fields i the various salts. These doubtless affect the vibrational frequencies of the negative ions.

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Changing the positive ion may produce a different crystalline arrangement, resulting in a different symmetry or intensity of the electrical field around a negative ion.

A difference in the type or extent of hydration probably alters some of the frequencies.

On the other hand Hunt, Wisherd, and Bonham (*5*) found that for anhydrous carbonates there is an approximately linear relationship between the wave length of the 11- to 12-micron band and the logarithm of the mass of the positive ion(s). Hunt has kindly pointed out that the authors' data fit his curve, with the exception of lithium carbonate.

**Characteristic Frequencies of Inorganic Ions.** Just as with sulfates and nitrates, most other polyatomic ions exhibit characteristic frequencies. These are summarized in Figure 2. It is evident that they are distinctive and that they do not have a great spread in wave numbers.

**Qualitative Analysis.** The usefulness of these characteristic frequencies in qualitative analysis is obvious. It appeared that the infrared spectrum might give, rapidly and easily, some information about the polyatomic ions that are present in an unknown inorganic mixture. If only one or two compounds are involved, it might even be possible to narrow the possibilities to a few specific salts. It also seemed that a combination of infrared, emission, and x-ray analysis might be very effective. Presumably emission analysis would determine the metals, infrared would say something about the polyatomic ions, and x-ray analysis might give their combination into specific salts.

To explore this possibility, a series of eight synthetic mixtures was prepared and analyzed independently by the three techniques. (A photographic x-ray procedure was used.) This information was then pooled, the data were re-examined, and a combined analysis was obtained. The test was not completely fair because it was known that infrared spectra have been obtained for nearly all the inorganic salts in the laboratory, and that these same salts would be used in making up the mixtures. In addition, the components were mixed in roughly equal amounts by bulk.

The results are shown in Table IV. The analysis of mixture 3 is discussed in greater detail below. It is apparent that no one of the techniques by itself is powerful enough to give a complete analysis of even these idealized unknowns. This is partly because there was no prior information about the content of the samples, and therefore every possibility had to be considered. As with any other analysis, much more detailed and reliable

**Table IV. Use of Infrared Spectra in Qualitative Analysis**

| No. | Independent Analyses: Emission | Independent Analyses: Infrared | Independent Analyses: X-ray | Final Combined Analysis | Actual Composition |
|---|---|---|---|---|---|
| 1 | Na<br>K<br>Ca | $NaHCO_3$ | $NaHCO_3$<br>$CaCO_3$ ?<br>KNO ? | $NaHCO_3$<br>$CaCO_3$<br>$KNO_3$ | $NaHCO_3$<br>$CaCO_3$<br>$KNO_3$ |
| 2 | Si<br>B<br>Pb<br>Al | $NH_4^+$<br>$NO_3^-$<br>$SO_4^{--}$<br>Silica gel | $NH_4NO_3$<br>Very poor pattern | $NH_4NO_3$<br>$SO_4^{--}$<br>Silica gel<br>B<br>Pb<br>Al | $NH_4NO_3$<br>$CaSO_4$<br>85% $SiO_2$ - 15% $Al_2O_3$<br>$Pb(BO_2)_2$ |
| 3 Yellow | Na<br>K<br>Cr<br>Sulfide odor<br>Pb ? | $K_2Cr_2O_7$<br>NaSCN or $NH_4SCN$<br>$Mg(ClO_4)_2$ | Nothing<br>Very poor pattern | $K_2Cr_2O_7$<br>NaSCN<br>$Mg(ClO_4)_2$ ?<br>Pb ? | $K_2Cr_2O_7$<br>NaSCN<br>KSCN<br>$CaSO_4 \cdot 2H_2O$ |
| 4 Yellow | Cd<br>Bi<br>B<br>Na ?<br>C ?? | $Na_2B_4O_7$ | $Na_2B_4O_7 \cdot 10H_2O$<br>CdS | $Na_2B_4O_7 \cdot 10H_2O$<br>CdS<br>$NaBiO_3$ ? | $Na_2B_4O_7 \cdot 10H_2O$<br>CdS<br>$NaBiO_3$ |
| 5 | As<br>Pb<br>Na<br>B ? | $Na_2SO_4$<br>$NaAsO_2$ | $Na_2SO_4$<br>$PbCO_3$ ?<br>$BaCO_3$ ?<br>$MgSO_4$ ? | $Na_2SO_4$<br>$NaAsO_2$<br>$PbCO_3$ | $Na_2SO_4$<br>$NaAsO_2$<br>$Pb(BO_2)_2 \cdot H_2O$ |
| 6 | Mo<br>Ca<br>K<br>Na ?<br>Sr ?? | $K_2S_2O_8$<br>$CaCO_3$<br>$PbCrO_4$<br>$Na_2SO_4$ ? | $K_2S_2O_8$<br>$CaCO_3$<br>$Na_3PO_4$ ?<br>$Na_2SO_4$ ? | $K_2S_2O_8$<br>$CaCO_3$<br>$Na_2SO_4$<br>Mo in some form | $K_2S_2O_8$<br>$CaCO_3$<br>$Na_2MoO_4 \cdot 2H_2O$ |
| 7 | Sb<br>Si<br>P<br>Ca<br>C ?? | $Ca_3(PO_4)_2$ | $Ca_{10}O(PO_4)_6$<br>$CaSiO_3$ ?? | $Ca_3(PO_4)_2$<br>$CaSiO_3$<br>Sb | $Ca_3(PO_4)_2$<br>$Sb_2O_3$ |
| 8 | Ba<br>Na<br>V<br>Al ?<br>Sr ?? | $NaVO_3$<br>A nitrate; probably $Ba(NO_3)_2$, possibly $NaNO_3$<br>Other component(s)<br>Possibilities: Another nitrate, $NaBrO_3$, $Na_2WO_4$ or $K_2WO_4$, $NaMoO_4$ or $KMoO_4$ | $Ba(NO_3)_2$<br>$PbSO_4$ ? | $NaVO_3$<br>$Ba(NO_3)_2$<br>Another component | $NaVO_3 \cdot 4H_2O$<br>$Ba(NO_3)_2$<br>$Na_2WO_4 \cdot 4H_2O$ |

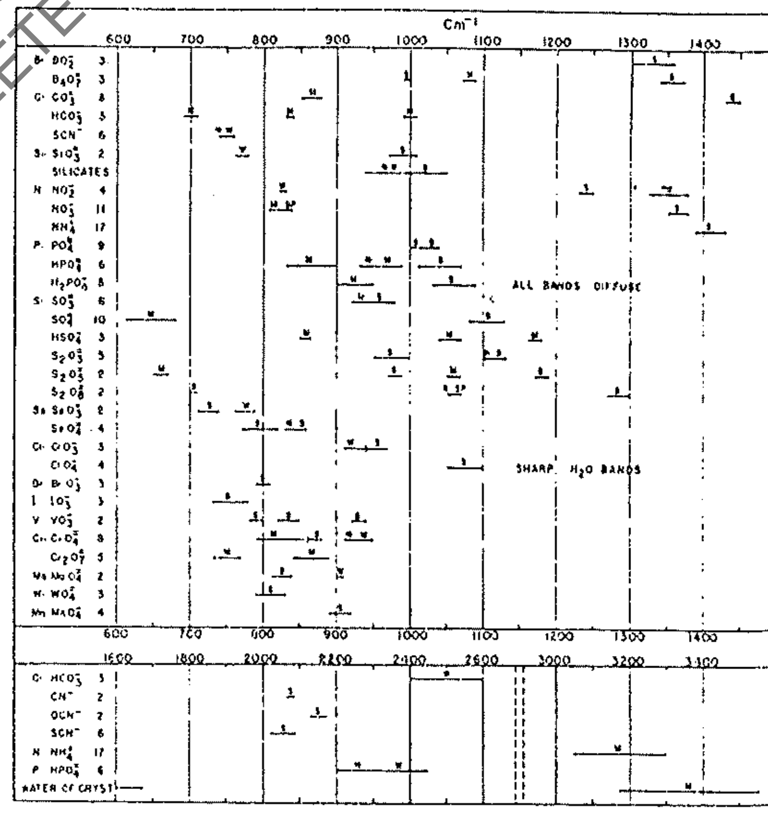


Figure 2. Characteristic Frequencies of Polyatomic Inorganic Ions

s, m, w. Strong, medium, weak
sp. Sharp
* In most, but not all, examples
** Literature value

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results can be obtained if there is some advance information about the nature of the unknowns. However, Table IV also shows that the three techniques are nicely complementary, and that together they are capable of providing a considerable amount of information even when such prior knowledge is lacking. Although there are two or three surprising errors in the combined analyses, the over-all results are very encouraging. It is especially noteworthy that the actual chemical compounds are given in many cases.

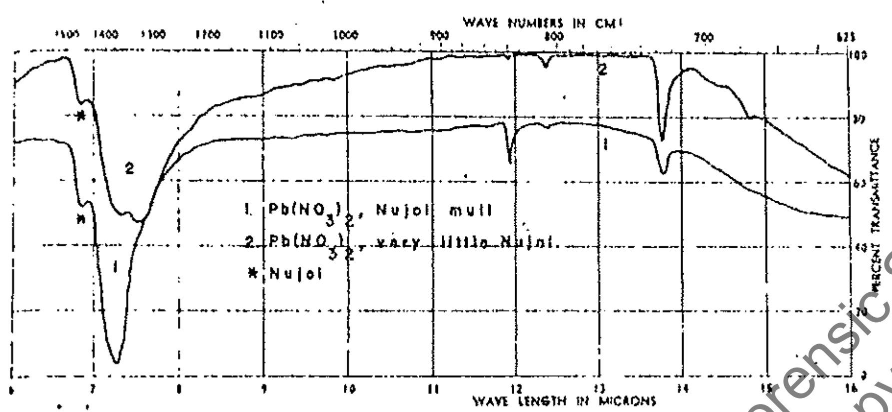


Figure 3. Portion of Infrared Spectrum of Lead Nitrate

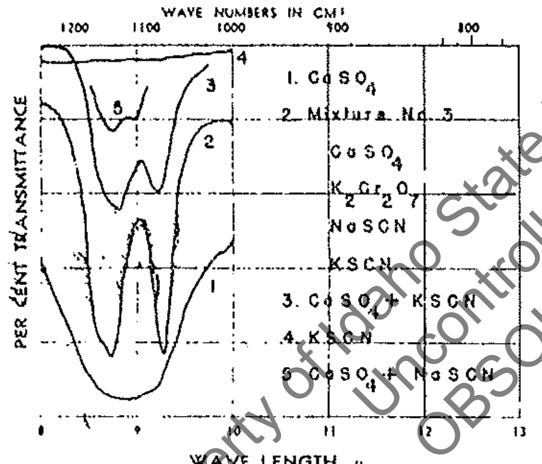


Figure 4. Anomalous Band in Unknown 3

In every case $CaSO_4$ should be written $CaSO_4.2H_2O$

INDIVIDUAL TECHNIQUES. X-ray analysis of a completely unknown sample becomes difficult when there are more than two components. It is not applicable to noncrystalline materials (cf. unknown 2) and runs into trouble with substances that gain or lose water of hydration readily. In both cases infrared is often a reliable tool.

Substances like metal oxides, hydroxides, and sulfides generally have no sharply defined infrared absorption from 2 to 16 microns aside from possible water and O—H bands. On the other hand, they are often good samples for x-ray analysis (cf. cadmium sulfide in unknown 4).

The principal fault with emission analysis is its great sensitivity; it is frequently difficult to distinguish between major components and impurities. This fact accounts for the surprising oversight of calcium in unknown 2, and tungsten in 8.

Infrared examination has advantages over wet chemistry for detecting the more unusual ions, such as $BO_2^-$, $B_4O_7^{--}$, $S_2O_4^{--}$, and $S_2O_5^{--}$, since these are not included in the usual schemes of analysis.

The proper sequence in using these techniques is the order emission, infrared, and then x-ray. The first two present certain possibilities for the x-ray analysis which greatly simplify its interpretation.

The advantages of this physical analysis include small sample requirement, reasonable time, and the ability to determine the actual compounds in many cases. It is evident, too, that any or all of these three techniques are valuable preliminaries to a chemical analysis on an unknown material, especially a quantitative one.

**Variability of Spectra.** It is not uncommon to find that the spectra of two samples of the same compound are somewhat different. There are several possible reasons for this.

IMPURITIES. In the spectra of sodium cyanide, potassium cyanide, and potassium cyanate (Nos. 21, 22, 23) bands have been marked that are plainly due to the corresponding carbonates and bicarbonates.

CRYSTAL ORIENTATION. It is well known that the spectra of anisotropic crystals depend on the orientation of the sample. Consequently it is desirable to have completely random orientation of the crystallites to avoid such effects. This is an additional reason for grinding the sample very finely.

POLYMORPHISM. Different crystalline forms of the same compound are often capable of exhibiting slightly different infrared spectra (*11*).

VARYING DEGREES OF HYDRATION.

Several examples of variable spectra have been observed, for which the cause is not definitely known. Two different samples of potassium metabisulfite, $K_2S_2O_5$, were examined, and proved to have different patterns of band intensities in one region (see curve 104). In potassium carbonate there is a band at 880 cm.$^{-1}$ or at 865 cm.$^{-1}$, and in one spectrogram out of a total of ten both bands appear. There is no clear correlation between position and water content.

Figure 3 shows that the mode of preparation is important. It compares the spectra of two lead nitrate samples, one prepared normally with Nujol and one with very little Nujol. Differences near 1300 cm.$^{-1}$ and 850 to 700 cm.$^{-1}$ are striking. This may be an orientation effect.

A more baffling case of unexpected variation was observed with unknown No. 3. In analyzing this by infrared, calcium sulfate dihydrate was missed completely and magnesium perchlorate was reported in its place. The reason is brought out in Figure 4. Pure calcium sulfate dihydrate has a single broad band centered near 1140 cm.$^{-1}$ (8.8 microns), whereas in mixture 3 a strong doublet was observed at 1080 and 1140 cm.$^{-1}$ The origin of the doublet was puzzling because no other component of 3 but calcium sulfate has a band near here. Calcium sulfate dihydrate had been run as a Nujol mull and mixture 3 as a dry powder. Reversing each did not change their spectra. Then calcium sulfate dihydrate was mixed with each of the other components in turn in the dry state, and the mixtures were examined as Nujol mulls. It was found that the mixture with potassium thiocyanate gave a doublet. With sodium thiocyanate there was also a doublet, but it was much less pronounced.

It seems unlikely that a chemical reaction between calcium sulfate dihydrate and potassium thiocyanate could account for these peculiar results, because the materials are in the solid state. Two other possible causes are changes in crystal structure, presumably caused by changing the hydrate, and an orientation effect. The following observations seem to rule out variable water content as a cause, and suggest the orientation effect.

A calcium sulfate dihydrate-potassium thiocyanate mixture heated at 170° C. for 3 days gave the two bands near 1100 cm.$^{-1}$ Only one band was found after the salt plates were separated and the mull exposed to air for an hour.

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Another portion of the same heated mixture was exposed to air under more humid conditions for an hour and then mulled in Nujol; two bands again resulted.

This mull was opened to the air for an additional half hour, and only one band was found.

When calcium sulfate dihydrate alone was heated overnight at 170° C., three bands were found. The sulfate vibration absorbing near 1100 cm.$^{-1}$ is triply degenerate (*12*), and this may be a case of splitting of the degeneracy as a result of altering the crystal symmetry. Finally, potassium sulfate has exhibited a similar variability in this same band.

**Table V. Characteristic Frequencies in Complex Ions**

| Complex Ion | Cm.$^{-1}$ | Simple Ion | Cm.$^{-1}$ |
|---|---|---|---|
| $Fe(CN)_6^{---}$ | 2100 | $CN^-$ | 2070-80 |
| $Fe(CN)_6^{----}$ | ~2010 | $CN^-$ | 2070-80 |
| $Fe(CN)_5NO^{--}$ | 2140 | $CN^-$ | 2070-80 |
| | 1925 | NO (gas) | 1878 |
| $Co(NO_2)_6^{---}$ | 847 | $NO_2^-$ | 820-35 |
| | 1335 | | 1235-50 |
| | 1430 | | 1328-80 |
| $Ce(NO_3)_6^{--}$ | 745 | $NO_3^-$ | 725-40 |
| | 807 | | 815-35 |
| | 1030 | | ... |
| | 1260 | | ... |
| | 1420 | | 1350-80 |
| | 1530 | | ... |

It is much safer to base arguments on the identity of spectra than on their nonidentity. More empirical experience with the spectra of salts from many different sources should improve this situation.

**Miscellaneous Observations.** Anomalous Dispersion and Christiansen Filter Effects. These have been adequately described in the literature (*5, 9*). Examples will be seen in the steep-sided band of magnesium carbonate at 3 microns (No. 13), of sodium thiocyanate near 5 microns (No. 26), and of potassium ferricyanide near 5 microns (No. 155).

Water and Hydroxyl Bands. The sharpness of the water bands near 3 and 6 microns in sodium and magnesium perchlorate (Nos. 117, 119), and the high value of their O—H stretching frequency (>3500 cm.$^{-1}$), are striking. Apparently there is very little hydrogen bonding in these salts. It is interesting to note that ammonium perchlorate (No. 116), which forms no hydrate, has a high N—H stretching frequency. Other compounds with sharp water bands are barium chlorate (No. 115) and barium chloride (No. 159).

In bicarbonate there is a band at 2500 to 2600 cm.$^{-1}$, in bisulfate at 2300 to 2600 cm.$^{-1}$ (very broad), and in $HPO_4^{--}$, $H_2PO_4^-$, $HAsO_4^{--}$, and $H_2AsO_4^-$ at about 2300 cm.$^{-1}$ (very broad). There is evidence that these are O—H stretching frequencies of the hydroxyl groups attached to the central atom.

Barium Chloride. Several chlorides of the purely ionic type were examined to observe how the bands due to water of hydration varied. Among these was barium chloride dihydrate (No. 159). Surprisingly it has a strong band at 700 cm.$^{-1}$, which was totally unexpected but was confirmed on a second sample. It is not attributable to carbonate or bicarbonate, but may be due to a torsional motion of the water molecules in the lattice.

Complex Ions. The characteristic frequencies carry over moderately well into complex ions—for example, potassium ferricyanide has a band at 2100 cm.$^{-1}$, and each of the three ferrocyanides has one near 2010 cm.$^{-1}$. This is obviously the stretching frequency of the $CN^-$ group, which in simple cyanides is 2070 to 2080 cm.$^{-1}$ Other examples are shown in Table V.

Compounds with No Absorption. Nickel hydroxide, ferric oxide, cadmium sulfide, and mercuric sulfide have no absorption in the rock salt region aside from water and hydroxyl bands.


## ACKNOWLEDGMENT

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## LITERATURE CITED


(1) Colthup, N. B., *J. Optical Soc. Am.*, 40, 397 (1950).
(2) Glockler, G., *Rev. Modern Phys.*, 15, 111 (1943).
(3) Herzberg, G., "Infrared and Raman Spectra of Polyatomic Molecules," New York, D. Van Nostrand Co., 1945.
(4) Hibben, J. H., "The Raman Effect and Its Chemical Applications," New York, Reinhold Publishing Corp., 1939.
(5) Hunt, J. M., Wisherd, M. P., and Bonham, L. C., Anal. Chem., 22, 1478 (1950).
(6) Lecomte, J., *Anal. Chim. Acta*, 2, 727 (1948).
(7) Lecomte, J., *Cahiers phys.*, 17, 1 (1943), and references cited therein.
(8) Newman, R., and Halford, R. S., *J. Chem. Phys.*, 18, 1276, 1291 (1950).
(9) Price, W. C., and Tetlow, K. S., *Ibid.*, 16, 1157 (1948).
(10) Schaefer, C., and Matossi, F., "Das ultrarote Spektrum," Berlin, Julius Springer, 1930; reprinted by Edwards Bros., Inc., Ann Arbor, Mich.
(11) Wagner, E. L., and Hornig, D. F., *J. Chem. Phys.*, 18, 296, 305 (1950).
(12) Wu, Ta-You, "Vibrational Spectra and Structure of Polyatomic Molecules," 2nd ed., Ann Arbor, Mich., J. W. Edwards, 1946.



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## REFERENCES

Interpretation of Infrared Spectra, N.B. Colthop, A.C.S. Audio Course, A.C.S., Washington, D.C., 1971.

Chemical Instrumentation, 2nd Ed., H.A. Strobel, Addison-Wesley Publ. Co., Reading, Mass., 1973.

Instrumental Methods of Analysis, 6th Ed., H.H. Willard, et al, Van Nostrand Co., New York, 1981.

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INTRODUCTION

This is the Qualitative Organic Analysis portion of the instrument course. It is of necessity a very restricted look at a very complex area.

This course is only intended to illustrate an approach to the identification of unknown organic compounds. It shows how the use of some simple physical and chemical property tests can lead to an unknown identification when used in conjunction with instrumental analysis and certain specialized literature references.

The main emphasis is on pure compounds. This is done primarily due to lack of space. Short sections on purifications and separations of mixture are included.

The primary intent is to encourage the development of a systematic approach to this type of identification problem. The shotgun approach is not very efficient. Hopefully the "do every test you can think of" approach will give way to the procedure of doing the test that will answer a specific question or supply a needed bit of information.

This is a brief overview of the organization of this course. These are the steps a person can take to effect an identification of a total unknown. Each of these steps will be covered in detail in the text.

1. Preliminary examination - looking at physical form, color, odor and a few simple tests such as a flame test or microscopic exam.

   Questions: Is it organic, a salt of an organic, or is it inorganic? Is it homogenous or is it a mixture?

2. Measure physical properties -

   Solids - melting point range (if wide purify or separate. Beware of eutectic mixtures)
   Liquids - Boiling point range can be done during a distillation which also may separate or purify
   Refractive index -
   Solids & Liquids - Elemental analysis Sodium fusion test - tests for N, S, Halides.
   -Instrumental methods such as EDX or Emission Spec - tests for S, some Halides & metals.

3. Solubility properties - simple scheme of solubility checks places the unknown in a group of possible types of compounds.

4. Functional group determination - Two main approaches instrumental i.e. IR or UV spectra are checked for clues as to possible functional groups.
   -classical approach using wet chemical spot tests to test for presence of certain functional groups.

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5. Literature search - using tables of organic compounds, a list of possible compounds is made based on all the data collected.

6. Identification

   a. Classical method - derivatives are prepared and their physical properties compared to literature tables.
   b. Instrumental-IR or Mass Sp ctra of possible compounds are obtained and spectra of the unknown is compared to them.
      Derivatives may be confirmed by IR.
   c. Techniques such as mixed melting point may also be used to confirm an identification.

Since much of the material which follows depends heavily on basic knowledge of organic chemistry - primarily functional group names and structures. A short review of these functional groups follows. Anyone wanting a more indepth review should consult any current organic chemistry text.

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# FUNCTIONAL GROUPS AND CARBON SKELETON

## X=Y Type Bonds

C=O

| | |
|---|---|
| Carboxylic acid anhydrides | $-C(=O)-O-C(=O)-$ |
| Carboxylic acid halides | $-C(=O)-X$ |
| Aldehydes | $-C(=O)-H$ |
| Carboxylic acids | $-C(=O)-OH$ |
| Carboxylic acid amides (prim) | $-C(=O)-NH_2$ |
| (sec) | $-C(=O)-NH-$ |
| (tert) | $-C(=O)-N<$ |
| cyclic (lactams) | $(CH_2)_n$ ring with $C=O$ and $NH$ |
| cyclic (imides) | $(CH_2)_n$ ring with $C(=O)-NH-C(=O)$ |
| Carboxylic acid salts | $-C(=O)-O-Metal$ |

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| | |
|---|---|
| Ketones | $-\overset{O}{\overset{\parallel}{C}}-$ |
| Carboxylic acid esters | $-\overset{O}{\overset{\parallel}{C}}-O-C-$ |
| cyclic (lactones) | $(CH_2)_n$ ring: $\overset{O}{\overset{\parallel}{C}}$–O |

<u>C=C</u>

| | |
|---|---|
| Vinyl | $CH_2=CH$ |
| Vinylidene | $CH_2=C-$ |
| Trans | $HC=CH$ |
| Cis | $HC=CH$ |
| Trisubstituted | $HC=C-$ |
| Tetrasubstituted | $-C=C-$ |
| Benzene ring | |

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N=O

| | |
|---|---|
| Nitroso | $-N=O$ |
| Nitrite | $-O-N=O$ |
| Nitro | $-\overset{O}{\overset{\|}{N}}\overset{+}{-}O^{-}$ |
| Nitrate | $-O-\overset{O}{\overset{\|}{N}}\overset{+}{-}O^{-}$ |

S=O

| | |
|---|---|
| Sulfoxide | $:\overset{\mid}{\underset{\mid}{S}}=O$ |
| Sulfone | $-\overset{O}{\overset{\|}{\underset{O}{\underset{\|}{S}}}}-$ |
| Sulfinic acid | $-\overset{O}{\overset{\|}{\underset{\cdot\cdot}{S}}}-OH$ |
| Sulfonic acid | $-\overset{O}{\overset{\|}{\underset{O}{\underset{\|}{S}}}}-OH$ |
| Sulfonamide | $-\overset{O}{\overset{\|}{\underset{O}{\underset{\|}{S}}}}-NH_2$ |
| Sulfate | $-O-\overset{O}{\overset{\|}{\underset{O}{\underset{\|}{S}}}}-O-$ |

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## X=Y=Z Type Bonds

| | |
|---|---|
| Allenes | $-C=C=CH_2$ |
| Isocyanates | $-N=C=O$ |
| Isothiocyanates | $-N=C=S$ |
| Carbodiamides | $-N=C=N-$ |

## X≡Y Type Bonds

| | |
|---|---|
| Alkynes | $-C\equiv C-$ |

C≡N

| | |
|---|---|
| Nitriles | $-C-C\equiv N$ |
| Cyanamides | $-N-C\equiv N$ |
| Thiocyanic acid esters | $-S-C\equiv N$ |

## H-Y Type Bonds

| | | |
|---|---|---|
| Hydrocarbons | | H-C(H)(H)-, -C(H)(H)-, -C(-)(-)-H |
| Alcohols, phenols, and other hydroxy compounds | (prim) | $-CH_2-O-H$ |
| | (sec) | -CH(-)-O-H |
| | ( ert) | -C(-)(-)- -H |

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Amines

```
(prim)   -N-H
            |
            H

(sec)    -N-H
            |
```

Thiols

```
-S-H
```

<u>P-H</u>

Phosphines

```
  H        H
  |        |
 -P:  ,   -P:
  |        |
  H
```

Phosphinous acids and phosphinites

```
  OH       O-
  |        |
 -P:  ,   -P:
  |        |
  H        H
```

Phosphine oxides

```
   |
 H-P=O
   |
```

Phosphinic acids and phosphinates

```
   OH         O-
   |          |
 H-P=O  ,   H-P=O
   |          |
```

Phosphonic acids and phosphonates

```
   OH         O-
   |          |
 H-P=O  ,   H-P=O
   |          |
   O-         O-
```

<u>Si-H</u>

Silanes

```
   H
   |              |
 -Si-H   →     -Si-H
   |              |
   H
```

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## X-Y Type Bonds

### C-O

Ethers

```
-C-O-C-
```

### P-O

Phosphonous acids and phosphonites

```
  OH        OH        O-
  |         |         |
 -P:   ,   -P:   ,   -P:
  |         |         |
  OH        O-        O-
```

Phosphorous acids and phosphites

```
    OH          OH          O-
    |           |           |
 -O-P:   ,   -O-P:   ,   -O-P:
    |           |           |
    OH          O-          O-
```

Phosphinic acids and phosphinates

```
  OH        O-
  |         |
 -P=O  ,   -P=O
  |         |
```

Phosphonic acids and phosphonates

```
  OH        OH        O-
  |         |         |
 -P=O  ,   -P=O  ,   -P=O
  |         |         |
  OH        O-        O-
```

Phosphoric acids and phosphates

```
    OH            O-            O-
    |             |             |
 -O-P=O   ,   -O-P=O   ,   -O-P=O
    |             |             |
    OH            OH            O-
```

Phosphoramides

```
    O-
    |
 -O-P=O
    |
    N-H
    |
```

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Si-O

| | |
|---|---|
| Silanols | $-Si(OH)_2-OH \rightarrow -Si-OH$ |
| Siloxanes | $-Si(O-)_2-O- \rightarrow -Si-O-$ |
| Silyl esters | $-Si-O-C(=O)-$ |
| Silicates | $-Si-O- \rightarrow -O-Si(O-)_2-O-$ |

C-X

| | |
|---|---|
| Fluoro hydrocarbons | $-C-F$ |
| Chloro hydrocarbons | $-C-Cl$ |
| Bromo hydrocarbons | $-C-Br$ |
| Iodo hydrocarbons | $-C-I$ |

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## LIST OF FUNCTIONAL GROUPS CHARACTERIZED BY SPECIFIC CLASS TESTS

| | |
|---|---|
| ACIDS (CARBOXYLIC) | $R-C(=O)-OH$ |
| ACID ANHYDRIDES | $R-C(=O)-O-C(=O)-R$ |
| ACID HALIDES | $R-C(=O)-X$ |
| ALCOHOLS | $R-OH$ |
| AMIDES | $R-C(=O)-NH_2$, $R-C(=O)-NH-R_1$, and $R-C(=O)-N-R_1R_2$ |
| AMINES | $R-NH_2$, $R_2-NH$, and $R_3-N$ |
| CARBOHYDRATES | $C_x(H_2O)_y$ |
| ALDEHYDES | $R-C(=O)-H$ |
| ESTERS | $R-C(=O)-O-R$ |
| ETHERS | $R-O-R$ |
| HYDROCARBONS | CH |
| KETONES | $R-C(=O)-R$ |
| NITRO COMPOUNDS | $R-N(=O)=O$ |
| SALTS (CARBOXYLATES | $R-C(=O)-O-M$ |
| THIOLS (MERCAPTANS & THIOPHENOLS) | $R-SH$ |



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## RE-ARRANGEMENT OF CHEMICAL CLASSES IN RESPECT TO SPECIFIC LINKAGES

### C=O GROUP:

| | |
|---|---|
| ACIDS (CARBOXYLIC) | $R-C(=O)-OH$ |
| ACID ANHYDRIDES | $R-C(=O)-O-C(=O)-R$ |
| ACID HALIDES | $R-C(=O)-X$ |
| AMIDES | $R-C(=O)-NH_2$, $R-C(=O)-NH-R_1$, and $R-C(=O)-N-R_1R_2$ |
| ALDEHYDES | $R-C(=O)-H$ |
| KETONES | $R-C(=O)-R$ |
| ESTERS | $R-C(=O)-O-R$ |
| SALTS (CARBOXYLATES) | $R-C(=O)-OM$ |

### OTHER DOUBLE BONDED GROUPS:

| | |
|---|---|
| HYDRAZONES | $R_2-C=N-NH_2$ |
| NITRO COMPOUNDS | $R-N(=O)=O$ |
| NITRATES AND NITRITES | $R-O-N(=O)=O$ and $R-O-N=O$ |
| HYDROCARBONS, ALKENE | $C=C$ |
| HYDROCARBONS, PHENYL RING | $C=C$ |

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## H-Y BOND GROUPS:

| | |
|---|---|
| ALCOHOLS | R-OH |
| AMIDES | $R-\overset{O}{\overset{\|\|}{C}}-NH_2$ and $R-\overset{O}{\overset{\|\|}{C}}-NH-R_1$ |
| AMINES | $R-NH_2$ and $R_2NH$ |
| HYDROCARBONS | CH |
| THIOLS | R-SH |

## TRIPLE BONDED GROUPS:

| | |
|---|---|
| NITRILES | R-C≡N |
| ALKYNES | R-C≡C-R |

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# Preliminary Examination

***Physical State.*** A note is made as to whether the unknown substance is a liquid or solid. This information is useful in consulting tables of compounds, which are subdivided on this basis.

***Color.*** The color of the original sample is noted as well as any change in color which may occur during the determination of the boiling point (p. 35).

The color of some compounds is due to impurities; frequently these are produced by slow oxidation of the compound by oxygen in the air. Aniline, for example, usually is reddish brown, but a freshly distilled sample is nearly colorless.

Many liquids and solids are definitely colored because of the presence of chromophoric groups in the molecule. Many nitro compounds, quinones, azo compounds, carbonium salts (triphenylmethane dyes), and compounds with extended conjugated systems are colored. If an unknown compound is a stable, colorless liquid or a white crystalline solid, this information is valuable because it excludes chromophoric functional groups as well as many groups which by oxidation would become chromophores.

***Odor.*** Many types of organic compounds have characteristic odors. It is not possible to describe odors in a precise manner, but the student should become familiar with the odors of common compounds. Alcohols have odors different from those of esters; phenols from amines; aldehydes from ketones. Mercaptans, isonitriles, and pentamethylenediamine usually are described as possessing disagreeable odors; however, they differ from each other. Moreover, the odor is most pronounced in the lower-molecular-weight members of a class since these are more volatile. Benzaldehyde, nitrobenzene, and benzonitrile all have the odor of bitter almond oil. Eugenol, coumarin, vanillin, methyl salicylate, and isoamyl acetate have characteristic odors which are easily remembered. Hydrocarbons also differ in their odors—toluene, hexane, isoprene, indene, pinene, and naphthalene possess distinctive odors.

The student should note *cautiously* the odors of the common organic compounds used in the tests and compare them with the odors of his unknowns.

***The Ignition Test.*** *Procedure.* A sample of 0.1 g. of the substance is placed in a porcelain crucible cover and brought to the edge of a flame to determine flammability. It is then heated gently over a low flame and finally ignited strongly. A note is made of: (1) flammability and nature of the flame (is the compound explosive?); (2) if the compound is a solid, whether it melted and the manner of its melting; (3) odor of gases or vapors evolved (*caution!*); (4) residue left after ignition. Will it fuse? If a residue is left, the lid is allowed to cool, a drop of distilled water is added, and the solution tested with litmus. A drop of dilute hydrochloric acid is added. Is a gas evolved? A flame test with a platinum wire is made on the hydrochloric acid solution to determine the metal present.

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*Discussion.* Many liquids burn with a characteristic flame that assists in determining the nature of the compound. Thus, an aromatic hydrocarbon (which has a relatively high carbon content) burns with a yellow, sooty flame. Aliphatic hydrocarbons burn with flames which are yellow but much less sooty. As the oxygen content of the compound increases the flame becomes more and more clear (blue). If the substance is flammable the usual precautions must be taken in subsequent manipulation of the compound. This test also shows whether a melting point of a solid should be taken and indicates whether the solid is explosive.

**Flame Test**

| Flame* | Compound class | |
|---|---|---|
| | Liquids | Solids (all melt and burn) |
| Sooty | Aromatic hydrocarbon | Aromatic hydrocarbon |
| Yellow | Aliphatic hydrocarbon | Aliphatic hydrocarbon |
| Whitish | Ether | Solid ether |
| Clear blue | Alcohol, ketone, acid, or ester† | Solid alcohol, ketone, acid, or ester† |

If an inorganic residue is left after ignition it should be examined for the metallic elements. Since the number of metallic elements commonly found in organic compounds is not very large, a few simple tests will usually determine the nature of the metal present. If the flame test indicates sodium, a sample of the compound should be ignited on a platinum foil instead of a porcelain crucible cover.

Many times the nature of the metal present is more easily determined instrumentaly. Energy dispersive x-ray or emission spec would be logical choices. EDX is especially good because of its non-destructive nature. The metal determination may be preformed on a portion of the original unknown with no loss in the amount of material for further testing. Also remember that with non-volatile unknowns, other elements such as S, P, Cl, and Si can also be detected with EDX.

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# The Determination of Physical Properties

Physical properties, such as the melting point, boiling point, refractive index, density, and molecular weight, are important in establishing the identity of an organic compound. These constants alone will often indicate a great deal about the structure of a compound and, at least, will narrow the search for identification purposes to a few compounds in a class or group.

Physical properties of organic compounds may be classified in several ways, depending on the use to be made of them. For our purpose, the method of classification used by Ostwald is very useful. He described two classes of physical properties: (a) additive and (b) constitutive. Additive properties are those that depend only on the numbers and kinds of atoms in the molecule; molecular weight and vapor density are examples of this class. Constitutive properties depend not only on the numbers and kinds of atoms in the molecule, but also on their arrangement in the molecule. Two kinds of constitutive properties are recognized. Constitutive properties of the first kind are independent of molecular association, and are represented by such properties as optical rotation and molar refraction. Constitutive properties of the second kind are dependent on molecular association; properties such as melting points, boiling points, and density are representative of this class.

One other type of physical property should be mentioned in this connection. Colligative properties are those that are dependent only on the number of particles present. The lowering of the freezing point by a solute, the elevation of the boiling point by a solute, and the osmotic pressure of a solution are colligative properties. It should be noted, however, that these are properties of solutions, not of pure substances.

Several important physical properties will be discussed in the following sections. Procedures will be given for determining properties with small amounts of material, and these properties will be related to the chemical structures of the molecules.

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## Melting Point

The melting point is the most important single physical property of solids for qualitative analysis. Usually, the first physical property determined by the chemist, after he prepares a new solid compound or isolates a solid from a natural mixture, is the melting point. If the compound has been prepared before, he can compare the melting point of his solid with published values and, very quickly, determine if he has the desired compound. If the compound has not been previously prepared, the melting point will immediately establish one basis for comparison in case the compound is

ever made again. Furthermore, the melting range of the substance is an indication of its purity. Even if the identity of the compound is unknown, the fact that the melting occurs over a range of 0.5° shows that the compound is fairly pure. If the range of melting is greater, we shall probably wish to recrystallize the compound (as described later ), and redetermine the melting point. It should be noted that purifying the compound will not only shorten the melting range, but also raise the whole range to a higher value.

The melting point is an important physical property for still other reasons. (1) Very little material is required for a melting point determination--from 1 mg down to a single crystal will suffice. Thus, 5 to 20 mg of solid can be crystallized and the melting point determined several times, if necessary, in order to obtain the desired purity. (2) The apparatus required for determination of the melting point is quite simple and inexpensive. A beaker with a liquid, which can be heated, a capillary tube, and a thermometer are the minimum essentials. Melting points taken carefully with this equipment are as good as those taken with much more expensive apparatus. (3) Conditions such as the atmospheric pressure do not affect the melting point. Although application of extreme pressures will change melting points, there is no indication that small variations in pressure have a measurable effect on the melting points of solids. (4) The melting process is not subject to superheating. Although liquids may be cooled below their melting points without solidifying, the reverse is not true. Crystals, evidently, cannot be heated above their melting points without melting.

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It is helpful to visualize the process of melting in order to understand the factors that influence it. Let us imagine an orderly arrangement of molecules, which we call a crystal. This arrangement is stable over a relatively wide range of conditions of temperature and mechanical stress. As the temperature is raised, the molecules must absorb energy; the higher the temperature, the more energy that must be absorbed. Some of this energy goes into the increasing of the vibrations of the molecule and some of it may go into an increase in the rotational energy of the molecule. Eventually, if the temperature continues to increase, these modes of energy absorption will be inadequate, and the molecule will burst from its lattice and acquire translational energy. At this point, it becomes a liquid in which the molecules are still close to one another but have no regular arrangement. Let us examine the factors that affect the temperature of melting.

1. The forces that operate between molecules play an important role; the stronger these forces, the more energy must be applied to break down the orderly arrangement and, consequently, the higher the temperature of melting. These forces have been classified in various ways but, for our purposes, we can list them as ionic attraction, hydrogen bonding, dipole-dipole interaction, and van der Waal forces. Some organic compounds have a great deal of ionic character, and may exist as ions; salts of amines and salts of carboxyl acids are examples. These have a great deal of attraction between particles. Molecules such as glycerol show a great attractive force between molecules, due to hydrogen bonding. A molecule

such as acetone cannot form hydrogen bonds with other molecules of acetone. However, it is slightly bound to another molecule of acetone by

$$O \leftarrow C \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}$$

the mutual attraction of the dipole, which exists in each molecule. This dipole is created by the greater attraction of electrons by the oxygen atom than by the carbon atom. It should be pointed out, however, that some acetone molecules exist in the enol form, $CH_3-\underset{\displaystyle OH}{\underset{|}{C}}=CH_2$, which can form hydrogen bonds with other molecules. Even in hydrocarbons, weak forces, called van der Waal forces, operate between molecules. The nature of these forces is poorly understood, but they operate only when the molecules are very close to one another; the greater the opportunity for contact between the molecules, the greater the van der Waal forces.

2. Symmetry is one of the powerful factors governing the melting process. If a molecule is symmetrical, it can absorb more energy without disrupting the crystal lattice. Many examples of the operation of this factor can be found, and some will be discussed later.

3. Size of the molecule affects the melting point, if other factors are equal. Larger molecules, in general, melt higher, if they are otherwise similar.

4. Polymorphism is responsible for some difference in melting points. Sometimes a compound will crystallize in more than one type of lattice, and each of these two crystals will have its own melting point. It would be difficult to compare the melting points of two similar compounds, which existed in two different crystal structures. Fortunately, the phenomenon of polymorphism is not common.

*Melting Points of Mixtures*

If a pure liquid *A* is cooled, it will produce, with time, a cooling curve shown at (*1*) in Figure 5.02(*A*). If however a liquid solution of *X* and *Y* is cooled, a cooling curve like that at (*2*), (*3*), or (*4*) in Figure 5.02(*A*) will be obtained. From these cooling curves, the phase diagram shown in Figure 5.02(*B*) can be derived.

From the diagram in Figure 5.02(*B*), it is clear that small amounts of *Y* in *X* will lower the melting point, even though *Y* has a higher melting point than *X*. A small percentage of *X* in *Y* will also lower the melting point of *Y*. In each case, the melting range is widened as it is lowered. Only in rare cases will a mixture of two organic compounds have a melting point no lower than those for the two pure compounds. This is the principle by which so-called "mixed" melting points are used to determine

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whether two substances are identical.

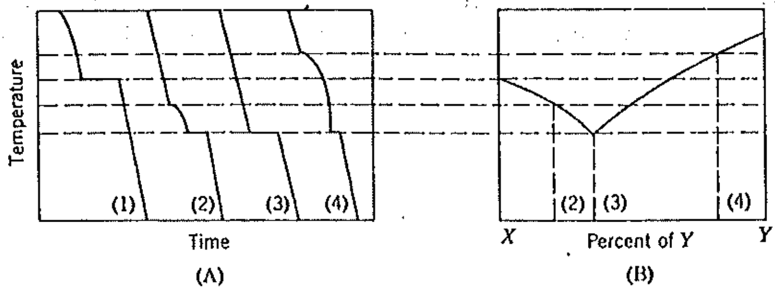


FIGURE 5.02
(*A*) Cooling curves. (*B*) Phase diagram.

Note that at the eutectic point (the lowest point on the melting point curve for the two compounds) the melting range is narrow. This mixture of substances could easily be mistaken for a pure compound. If, however, the mixture were recrystallized once more, the composition would change, and the range of melting would increase.

*Apparatus*

The *capillary-tube* method is commonly used for the determination of melting points. About a milligram (or less) of the solid is placed in a thin-wall glass capillary tube having a diameter close to 1 mm. The capillary tube is attached to a thermometer, then placed in a liquid bath, and heated slowly. The temperature at which the solid within the capillary tube begins to liquefy and the temperature at which the liquid is clear is recorded as the *observed melting range*. When the values are corrected they are called *corrected melting points*. It should be noted that melting points determined by this method are not *true melting points* but *capillary melting points*; the latter are slightly higher than the true melting points, which are determined by cooling or heating curves;[2] these require larger samples but give more exact information as to the purity of the compound. For most ordinary purposes, the capillary-tube method may be used. The amount of substance required for a single determination by the capillary-tube method is usually 1 to 2 mg, although a fraction of this amount may be used.

Other methods for the determination of melting temperatures of solids are *heating bars* and *heating stages*. In the former, the crystals are heated on a metal bar whose temperature is determined either by a thermometer or thermocouple; in the latter, a few crystals weighing a fraction of a microgram are placed on an electrically heated stage, and the temperature at which the crystals melt is observed. With this method, it is possible, in most cases, to observe the temperature at which liquid and fragments of crystals coexist and, for this reason, *corrected micromelting points* should be differentiated from corrected *capillary melting points*.

Several factors determine the precision and accuracy of the measurement of melting temperatures. Although it is possible to obtain a precision of 0.5° with ordinary thermometers, and with specially constructed thermometers a precision of 0.1°, the accuracy depends primarily on the calibration of the device by which the temperature is measured at the region where the crystals are situated.



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Glass capillaries of uniform diameter (1 to 1.2 mm) and 70 to 75 or 100 mm length are commercially available, packed in vials; we prefer capillary tubes of about 100 to 120 mm length, since they are more suitable for most melting-point apparatus.

To load the capillary tube, a few milligrams of the crystalline materiai are placed on a watch glass, a piece of clean paper, or a porous plate, and crushed to a fine powder by drawing the spatula over them. The open end of the capillary tube is pressed into the fine powder; then the closed end is tapped on the desk, or the tube is lightly scratched with the flat part of a file, in order to force the sample to the bottom. The tube is filled to a height of about 1 to 2 mm, and is then attached to the thermometer so that the end of the capillary tube reaches the middle of the mercury bulb. If oil is used as a bath, the capillary tube is attached to the thermometer by means of a small rubber band cut from ordinary 3/16-in. tubing. The rubber band is placed near the top of the capillary tube well above the liquid bath. If the rubber band comes in contact with the hot bath liquid, the latter will be discolored; therefore, it is advisable to use new rubber bands for every determination. Silicone fluids do not affect rubber or metal bands. A fine copper wire, wound several times around the thermometer and capillary, has been successfully used by us. Another method, suggested in the literature, is to omit the rubber band and attach the capillary by placing a glass rod against the entire length of the thermometer above the bulb; the capillary is fitted in the groove, formed by the rod and thermometer. If sulfuric acid is used as a bath, the rubber band is unnecessary, as capillary attraction will hold the melting-point tube to the thermometer.

In some cases it is desirable to seal the capillary after filling, and even to displace the air above the crystals with an inert gas. In such cases, the capillary is filled either through a small capillary funnel or, better, by the following method. Two capillaries are constructed so that one exactly fits into the other. The thinner capillary is made 10 mm longer than the wider one. Each capillary is sealed at one end, and the thinner one is filled in the usual manner, but without packing the sample tightly at the bottom of the tube. The filled capillary is carefully wiped off to remove any adhering powdered substances from the sides and open end. The larger capillary is fitted over the filled capillary, which is held upright and allowed to descend slowly until its closed end reaches the open end of the filled tube. Then the two capillaries are inverted rapidly, and the filled tube is raised about 10 mm above the larger tube and emptied slowly by rasping it with a file. The thinner tube is now raised another 10 mm, and its open end is slowly rubbed about the walls of the outer tube to remove any adhering particles; the capillaries are held at an angle, and the inner capillary is raised slightly and, after the process has been repeated, is finally withdrawn. In this manner it is possible to fill capillaries without any crystals adhering to the neck of the tube where they might decompose and contaminate the sample when the open end is sealed.

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Figure 5.03(*A*) shows the well-known Thiele tube, and Figure 5.03(*B*) shows a modification to improve uniform heat transfer by stirring with air. The Thiele tube, even with the modification, must be heated very slowly to insure an even temperature about the sample and thermometer bulb.

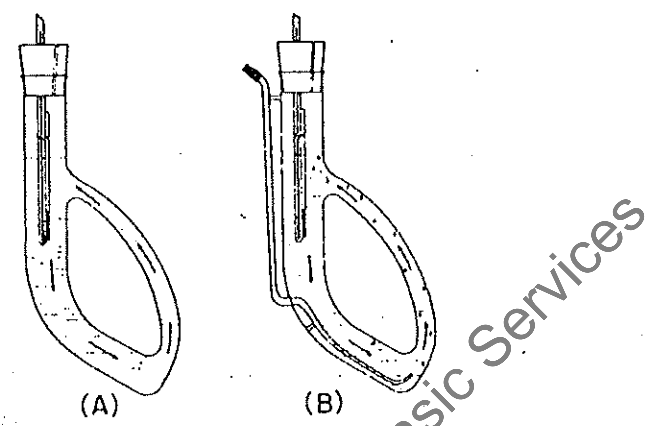


FIGURE 5.03
(*A*) Thiele tube for determination of melting points. (*B*) Modified Thiele tube for melting-point determination.

A beaker filled with a suitable liquid, with a magnetic stirring bar, placed on a stirring hot plate can also be used as a heating bath.

For the determination of melting points up to 200°, a high grade of heavy petroleum (mineral) oil may be used as the liquid for the melting-point apparatus. Concentrated sulfuric acid may be used for determinations up to 300°. A mixture of six parts of acid and four parts of potassium sulfate, which is solid at ordinary temperatures, may be heated up to 365°. There is *great danger* involved in heating sulfuric acid and mixtures of the acid and potassium sulfate; in addition to the danger resulting from breakage, the mixture of acid and salt may separate into two layers and, when heated, mix with explosive violence. In laboratories in which the use of sulfuric acid for heating baths is not permitted, a petroleum wax melting at 60 to 70° is used for temperatures of 250 to 350°.

Silicone fluids, although expensive, have been found to be among the better thermal conducting media for melting-point apparatus. We recommend silicone fluid 9981-LTNV-40[9] and Type-550-100 cst. These organic polysiloxanes are colorless, clear, stable to heat, and resistant to most chemical reagents; in addition, they exhibit a low rate of viscosity change over a wide temperature range, and have higher flash points than petroleum oils of equivalent viscosity. They can be used without appreciable discoloration in a Thiele tube apparatus for one year or more. Turbidity and darkening can be eliminated by filtration after shaking with a mixture of Filter-cel and charcoal.

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### *Procedure Using Liquid-Bath Apparatus*

The thermometer is arranged in the apparatus so that the lower end of the capillary is clearly visible. If a rubber band is used to hold the capillary to the thermometer, it is so adjusted that it is out of the liquid. In the Thiele apparatus, the oil level is about 10 to 15 mm above the circular side tube and the thermometer 15 mm below it, so that the latter is near the mid-point between the upper and lower side arms.

When the thermometer bearing the capillary tube has been adjusted, the tube or flask is heated rapidly to about 10 to 15° below the known melting point of the substance. If the substance is an unknown, the approximate melting point is first determined by heating fairly rapidly until the substance has melted. The bath is then allowed to cool to about 20° below the observed melting point; the thermometer is carefully removed and held until it has acquired the temperaure of the room; then a new loaded capillary tube is inserted. The thermometer is replaced and the bath heated until the temperature rises to within 10 to 15° of the melting point. The flame is removed until the temperature begins to drop. The heating is then resumed at such a rate that the temperature rises 2 to 3° per minute, the liquid bath being stirred so that the temperature in the various parts of the apparatus will be as uniform as possible. When the temperature comes to within 2 to 4° of the melting point, a rise of 1° per minute is desirable. It should be stressed that the slower the rate of heating the greater is the precision and the rate for the last 1° should be 1° each 2 to 3 minutes. The temperature at which the substance begins to liquefy and the temperature at which the liquid is clear are noted. The interval of temperature is recorded as the melting-point range of the substance. If the compound melts without decomposition, it is suggested that a second and a third observation be made by removing the thermometer from the bath, holding it in air until the liquid in the capillary solidifies, then repeating the melting-point determination.

### *Procedure Using Heating Bars or Blocks*

Two types of heating bars or blocks are described here in detail. A more comprehensive review will be found in the literature.[10]

The Fisher-Johns melting point apparatus,[11] shown in Figure 5.05A, consists of a small aluminum block which is electrically heated. The

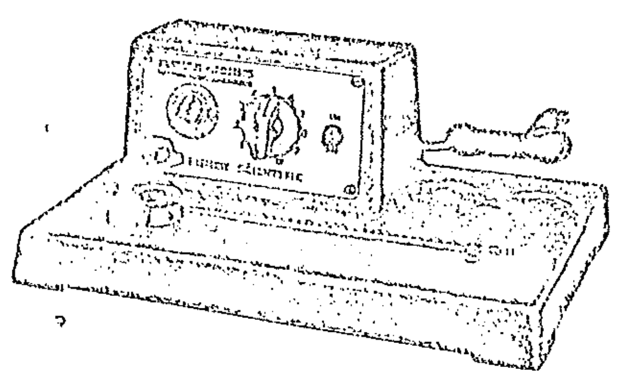

FIGURE 5.05A
Fisher-Johns melting-point apparatus. (Courtesy Fisher Scientific Co.)

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resistance box is equipped with a knob for temperature control. The temperature measurement is made through a direct reading thermometer graduated from 20 to 300°, imbedded immediately below the stage on which the sample is placed. To determine the melting point of a substance, a few crystals are placed between cover glasses on the well of the aluminum stage and the magnifier is adjusted over the sample. The temperature is raised rapidly at first and then at a rate of 1° per minute until the crystals coalesce and form droplets. The temperature control of the instrument is good for temperatures below 200° but somewhat difficult above this range.

There are two sources of error in the use of the instrument, both of which can be eliminated. Obviously, variations will occur unless the thermometer bulb is in contact with the hot stage, and this contact is the same at all times. To eliminate this difficulty the manufacturer furnishes a small amount of silver thread, which is wound around the thermometer bulb to give uniform contact with the stage. The second, more serious difficulty arises because the thermometer is not calibrated by the manufacturer. Since the error due to this variable may be as high as 5 to 8°, it is necessary even for routine work to calibrate the thermometer,[12] as outlined in a later section (p. ). When this is done, the variations become comparable to those obtained with a liquid bath apparatus provided with a calibrated thermometer.

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*Procedure for Solids which Decompose when Heated*

Some organic compounds, such as amino acids, osazones, and many of the quaternary salts and, generally, compounds that have bonds of partial ionic character, melt with decomposition. As the sample is heated, decomposition begins, and a lowering of the melting point of the substance occurs. Thus, D-glutamic acid has been reported to melt with decomposition at temperatures varying from 198 to 225°. Similarly, DL-tyrosine has been reported to decompose at 295°, 318°, and 340°; the melting point of a sample of phenyl-D-glucosazone was 210° when the liquid bath was heated at the rate of 40 to 60° per minute, and 194 to 198° when the rate of heating was 8 to 10° per minute.

The temperature range at which decomposition takes place depends on the rate of heating. It is erroneous to consider organic substances that decompose slowly on heating, such as the classes of compounds cited, as having true melting points. On the other hand, a distinction should be made for compounds that melt sharply with decomposition, such as some of the substituted malonic acids; these decompose on melting with the evolution of carbon dioxide.

The determination of melting points of compounds that decompose presents more difficulties. One method is to preheat the bath to within 10° of the expected melting point and, then, to insert the thermometer with the capillary and heat as rapidly as possible, noting the rate of the increase in temperature. The initial temperature of the bath and the rate of temperature rise should always be reported.

*Procedure for Melting Points of Mixtures*

The determination of the melting point of a mixture plays an important role in organic qualitative analysis; this process is often referred to as the determination of a "mixed" melting point. In an earlier discussion it was

pointed out that a mixture of two substances usually has a wide range and a lower melting point than either of the two pure solids. If, therefore, an unknown material is mixed with a known solid, and the mixture melts at the same temperature as the known and the unknown substance, the two solids are probably the same chemical.

It should be remembered, however, that there are cases of unlike crystalline substances that show a higher melting point than either of the two components because of the formation of a new compound. In a number of instances, two different compounds, melting a few degrees apart, may show no depression in melting point when mixed. Thus, naphthalene picrate, mp 151°, and benzothiophene picrate, mp 149°, melt when mixed at 149°,[24] and D-dimethyl tartrate, mp 48°, and L-dimethyl tartrate, mp 43.3°, melt when mixed in equal proportions at 89.4°.[25] It is also well known that two different organic substances, when mixed in different ratios, may form two or more eutectics, which melt considerably below either component, and one or more molecular compounds, which may melt higher than either component. Therefore, the "mixed-melting-point method" should only be used in conjunction with other pertinent data. On the other hand, the use of fusion techniques eliminates most of these errors since the formation of eutectics is clearly visible under the microscope.

*Calibration of Thermometers*

The accuracy of melting-point determinations by the capillary tube method, and by practically every method in which a thermometer is used, depends to a large extent on the calibration of the instrument by which the temperature is measured. Calibration by the National Bureau of Standards by total immersion eliminates the errors inherent in the thermometer if it is used in a total-immersion apparatus. In most apparatus the thermometer is only partially immersed and corrections must be made for the exposed stem. Aside from the inconvenience of such a procedure, the errors introduced offset the benefits of the calibration. The Anschütz thermometers that are used by total immersion are difficult to calibrate. Most of these thermometers are duplicates of the original German models and are not supplied with an ice point, which is a prerequisite for certification by the National Bureau of Standards. However, the Bureau will submit a report on each thermometer that indicates the amount of variation at certain points. If each thermometer is checked at three points, the cost of such a report for a set of Anschütz thermometers will exceed $300.00. This is extremely high when we consider that after one year a recalibration is necessary for thermometers that have been used extensively. This is true for all thermometers calibrated either by the Bureau of Standards or by direct comparison with a standard thermometer,[28] and arises from the fact that when thermometers are used at high temperatures, they undergo gradual irreversible changes in bulb volume. Since the bulb of an average thermometer contains mercury corresponding to about 6000° of scale length,[29] it is obvious that small changes in bulb volume will have a relatively large effect upon the reading of the thermometer.

The older methods for making corrections on observed melting temperatures involved essentially a calibration of the thermometer and applying a correction for the stem immersed in the liquid. The common thermometer has been calibrated while being totally immersed in a bath. In the melting-

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point apparatus described, only a part of the stem is immersed. The column of mercury above the liquid in the bath will show a lower temperature than that for which the thermometer was calibrated. Therefore, either a thermometer calibrated by partial immersion should be used, or a correction must be made for the unequal heating of the mercury in the stem of the thermometer. The correction for unequal heating of the thermometer is given by the formula

$$\textit{Stem correction (degrees)} = 0.000154(t_o - t_s)N,$$

where the fraction 0.000154 represents the difference in the coefficients of expansion of glass and mercury, $t_o$ is the temperature read, and $t_s$ is the average temperature of the column of mercury not immersed in the substance; $t_s$ is determined (approximately) by reading a second thermometer whose bulb is held at the midpoint of that part of the column of mercury not immersed in the substance. $N$ is the length in degrees of the portion of the column that is not immersed. The error due to this variable is small at temperatures below 100°, but may amount to 3–6° at 200° and above.

On the basis of an extensive investigation by one of us[30] the procedure recommended is to employ a thermometer calibrated by the manufacturer by partial immersion and then calibrate the thermometer by means of reference standards *in situ*, that is, in the apparatus that is to be employed.

A thermometer is selected in which 1° is equivalent to 1 to 1.2 mm and calibrated by partial immersion. If greater precision is desired, two thermometers, one reading from 0 to 180° and the other from 150 to 320°, with subdivision in 0.5°, are employed.

The thermometers to be calibrated are first heated for 6 to 8 hours at about 300°, which is higher than the temperatures to which they are commonly exposed. The thermometers are allowed to stand at room temperature for 2 to 3 days, and are then calibrated by the reference standards listed in Table 5.03, *in the same apparatus and with the same technique that is employed for the melting point determinations.* Three determinations or more are made for each fixed point; the deviation from the average value should not exceed 0.5°. Average values are used to plot the calibration curve from which the correction to be applied to observed melting points may be read directly. Figure 5.10 shows calibration curves for three thermometers.

Since there is no great differential in the price of the thermometers

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**TABLE 5.03**
**PRIMARY STANDARDS FOR THERMOMETER CALIBRATION BY MELTING POINT***

| *Substance* | *Melting Point, °C* | *Substance* | *Melting Point, °C* |
|---|---|---|---|
| Water-ice | 0.0 | Urea | 132.8 |
| Cyclohexanol | 25.4 | Salicylic acid | 158.3 |
| Menthol | 42.5 | Succinic acid | 182.8 |
| Benzophenone | 48.1 | Anthracene | 216.2 |
| *p*-Nitrotoluene | 51.6 | Phthalimide | 233.5 |
| Naphthalene | 80.2 | *p*-Nitrobenzoic acid | 241.0 |
| Acetanilide | 114.2 | Phenolphthalein | 265.0 |
| Benzoic acid | 122.4 | Anthraquinone | 286.0 |

* *Source.* N. D. Cheronis, "Micro and Semimicro Methods" in *Technique of Organic Chemistry*, Vol. 6, A. Weissberger, ed., Interscience, New York, 1954, p. 156.

calibrated by partial immersion, they are recommended with the provision that they should be calibrated. Such thermometers are marked with an etched ring at the 76-mm mark and, above it, bear the inscription "76-mm immersion." To use such thermometers, the stem is immersed so that the mark is even with the surface of the liquid bath. These thermometers are recommended in place of the common thermometers. The length of the capillary to be used with such thermometers is 100 to 120 mm.

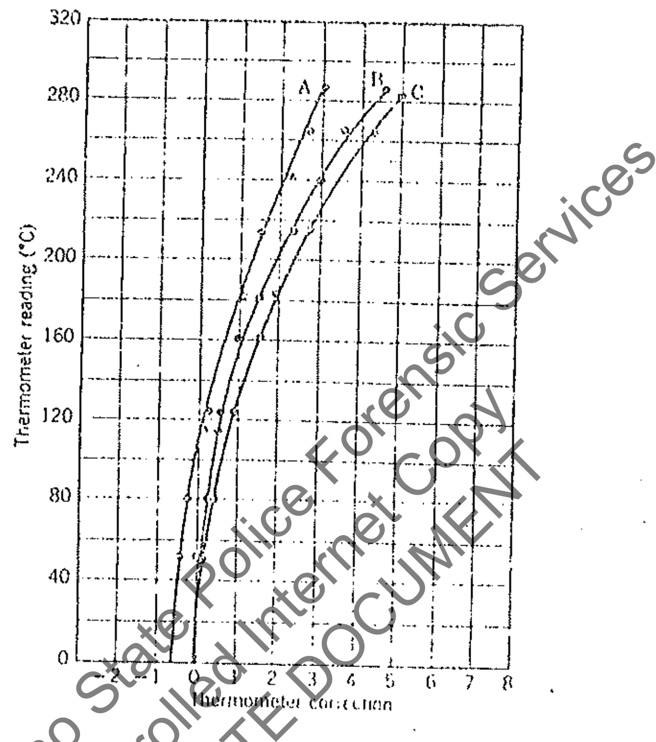


FIGURE 5.10
Calibration curves of three thermometers.

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## FREEZING POINTS

*Procedure.* A few milliliters of the liquid is placed in an ordinary test tube fitted with a thermometer and a wire stirrer (made of copper, nickel, or platinum). The tube is fastened in a slightly larger test tube by means of a cork and cooled in an ice or ice-salt bath or an acetone-Dry Ice mixture, and the liquid is stirred vigorously (Fig. 6). As soon as crystals start to form, the tube is removed from the bath, and vigorous stirring is continued while the temperature on the thermometer is being read. The freezing point is the temperature reached after the initial supercooling effect has disappeared.

*Discussion.* The temperature of the cooling bath should not be too far below the freezing point of the compound.

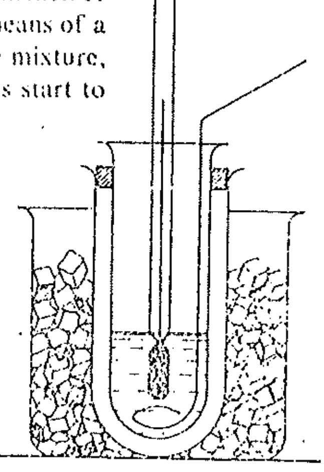

Figure 6. Simple freezing-point apparatus.

*Procedure A.* A small-scale distillation apparatus similar to that shown in Fig. 7 is set up. It consists of a 25-ml. distilling flask placed on an asbestos board with a 2-cm. hole in the center. Test tubes immersed in a beaker of ice are used to condense the vapors and act as receivers. A piece of clay plate is placed in the flask, and 10 ml. of the liquid whose boiling point is to be determined is added. The thermometer is inserted so that the top of the mercury bulb is just below the side arm. The liquid is heated to boiling by means of a low flame which should be protected from drafts by a properly placed shield. The liquid is distilled at as uniform a rate as possible. After the first 2 to 3 ml. of distillate has collected, the receiver is changed without interruption of the distillation, and the next 5 to 6 ml. is collected in a clean dry test tube. There will be a considerable lag of the thermometer reading, but usually the boiling-point range can be determined during the collection of the second portion of distillate. This boiling-point range should be recorded.

The boiling-point range is a useful index of purity of the sample. Many organic liquids are hygroscopic, and some decompose on standing. Generally the first few milliliters of distillate will contain any water or more volatile impurities, and the second fraction will consist of the pure

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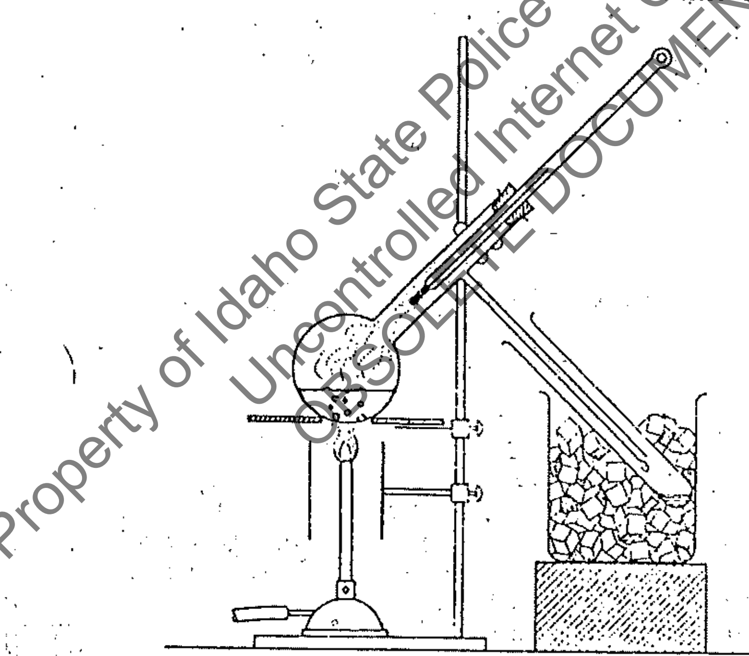

Figure 7. Simple distillation apparatus.

substance. If the boiling-point range is large, the liquid should be refractionated through a suitable column.

The boiling point determined by the distillation of a small amount of liquid as described above is frequently in error. Unless special care is taken, the vapor may be superheated; also the boiling points observed for high-boiling liquids may be too low because of the time required for the mercury in the thermometer bulb to reach the temperature of the vapor. The second fraction collected above should be used for a more accurate boiling-point

determination by Procedure *B* or *C*. Portions of this same fraction should also be used for the determination of specific gravity, refractive index, or optical rotation.

*Procedure B.* When as much as 1 ml. of the pure anhydrous liquid is available its boiling point should be determined by means of the apparatus shown in Fig. 8. It consists of an ordinary Pyrex test tube, 16 by 150 mm. (*A*), fitted with a thermometer ($T_1$) by means of a cork (*B*) which has a slot to provide access to the air. The percolator cup (*C*) is made of glass tubing of such diameter that there will be about 1-mm. clearance between the bulb of the thermometer and the walls of the cup. Usually 8-mm. tubing will be suitable. The bottom of the cup flares outward so that it covers most of the bottom of the test tube, in which a few boiling stones (*E*) are placed. The test tube is insulated by two layers of asbestos paper (*F*) which extend 5 mm. above the height of the cup. The apparatus is mounted in a vertical position, the test tube being placed on an asbestos board (*D*) over a hole about 8 mm. in diameter. A second thermometer ($T_2$) is placed beside the first in order to get the temperature of the exposed mercury thread so that the stem correction may be calculated.

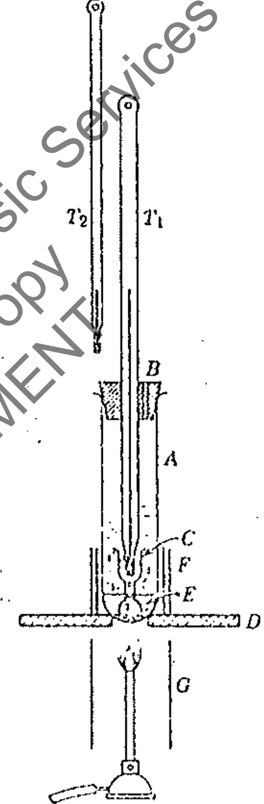


Figure 8. Percolator boiling-point apparatus.

One milliliter of liquid is placed in the test tube, and the flame is adjusted so as to produce gentle boiling. The shield (*G*) protects the flame from drafts. The vapor should rise through the percolator cup carrying some liquid with it so that the mercury bulb is in contact with both liquid and vapor. The liquid should boil vigorously enough so that the vapors rise about 1.0 cm. above the asbestos wrapping (*F*); the upper part of the test tube acts as a reflux condenser. The temperature ($T_1$) is read after equilibrium is reached (3 to 5 minutes). The auxiliary thermometer ($T_2$) is also read, and the stem correction is calculated and added to the reading on $T_1$. The corrected boiling point is given by the following equation:

$$\text{B.P.} = T_1 + N(T_1 - T_2)0.000154$$

*Procedure C.* A micro boiling-point tube is made as shown in Fig. 9. The outer tube is made by sealing one end of a 5-mm. glass tube and cutting off a piece 5 cm. long. A capillary tube is sealed about 3 to 4 mm. from one end and placed in the larger tube (Fig. 9). Two drops of the liquid whose boiling point is to be determined is added, and the tube is fastened to the thermometer in the apparatus used for the determination of melting points (see Fig. 1). The temperature is raised until a rapid and continuous stream of bubbles comes out of the small capillary and passes through the liquid.

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The flame is then removed and the bath allowed to cool, while being stirred continuously. The temperature is noted at the instant bubbles cease to come out of the capillary and just before the liquid enters it. This temperature is the boiling point.

Figure 9. Micro boiling-point tube.

### Effect of Pressure on Boiling Point

At the time the boiling point is being determined the atmospheric pressure should be recorded. When the pressure is near 760 mm., the correction caused by a 10-mm. difference in pressure may be found by dividing the absolute boiling point by 850 for non-associated liquids (hydrocarbons, alkyl halides, ethers, esters) and by 1020 for associated liquids[a] (alcohols, acids). Table I illustrates the magnitude of such barometric corrections

TABLE I

| B.P., °C. | B.P., Absolute | Correction in °C. for 10-mm. Difference in Pressure: Non-associated Liquids | Associated Liquids |
|---|---|---|---|
| 50 | 323° | 0.38 | 0.32 |
| 100 | 373 | .44 | .37 |
| 150 | 423 | .50 | .42 |
| 200 | 473 | .56 | .46 |
| 300 | 573 | .68 | .56 |
| 400 | 673 | .79 | .66 |
| 500 | 773 | .91 | .76 |

for pressures that do not differ from 760 mm. by more than about 30 mm.

It is evident that small deviations in pressure from 760 mm., such as 5 mm., may be neglected in ordinary work.

Many different equations have been proposed for calculating the boiling points of organic liquids at reduced pressures. The integrated form of the Clausius-Clapeyron equation has been used along with Trouton's rule. This method has not proved to be generally satisfactory because Trouton's constant is not the same for associated and non-associated liquids; moreover, the degree of association varies with the temperature. This fact has heavier than water. Introduction of the aromatic ring also may cause esters to be heavier than water. Examples of esters of these types which are heavier than water are phenyl acetate, methyl benzoate, benzyl acetate, ethyl salicylate, *n*-butyl oxalate, triacetin, isopropyl tartrate, and ethyl citrate. Since the hydrocarbons are lighter than water, it is to be expected that esters containing long hydrocarbon chains will show a correspondingly diminished specific gravity.

In general, compounds containing several functional groups—especially those groups that promote association—will have a specific gravity greater than 1.0. Merely noting whether a compound is lighter or heavier than water gives some idea of its complexity. This is of considerable value in the case of neutral liquids. If the compound contains no halogen and has a specific gravity less than 1.0 it probably does not contain more than a single functional group in addition to the hydrocarbon or ether portion. If the compound is heavier than water it is probably polyfunctional.

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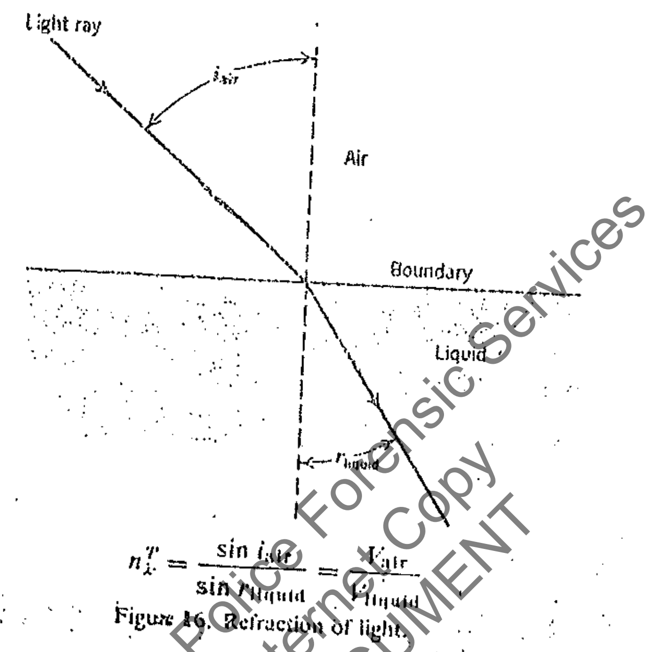
## THE INDEX OF REFRACTION OF LIQUIDS

The refractive index of a liquid is equal to the ratio of the sine of the angle of incidence of a ray of light in air to the sine of the angle of refraction in the liquid (Fig. 16). The ray of light undergoes changes in wave velocity




$$n_D^T = \frac{\sin i_{air}}{\sin r_{liquid}} = \frac{V_{air}}{V_{liquid}}$$

Figure 16. Refraction of light.

($V_{air} \rightarrow V_{liquid}$) and in direction at the boundary interface, and these changes are dependent on temperature and wavelength of light. Direct measurements of the angles of incidence and refraction are not feasible; hence specialized optical systems have been devised dependent on the critical angle of reflection at the boundary of the liquid with a glass prism of known refractive index. The Abbe type of refractometer operates on this principle, and a number of instruments are available commercially.[10] The advantages of the Abbe type of refractometer are (*a*) a white-light source of illumination may be used, but the prism system gives indices of refraction for the sodium D line; (*b*) only a few drops of liquid are needed; (*c*) provision for temperature control of prisms and sample is incorporated; and (*d*) the compensating Amici prisms permit the determination of specific dispersion. The manuals for operating the various instruments should always be consulted since there are variations in the exact operating procedures. A complete discussion of the principles of refractometry and refractometers is given by Bauer, Fajans, and Lewin.[11]

[11] Bauer, Fajans, and Lewin, Refractometry, Chapter 18, pp. 1140-1281, in *Physical Methods of Organic Chemistry*, Vol. I, Part II, 3rd ed., edited by Weissberger, Interscience Publishers, New York, 1960.

*Discussion.* The values for density and refractive index are useful in excluding certain compounds from consideration in the identification of an unknown. Care must be taken, however, that the sample is pure. It is best to determine these physical constants on a portion of the distillate resulting from the determination of the boiling point.

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Density has not been discussed as it is difficult to do on small samples without special equitment. Many times a rough measure of density may be helpful. This can be done by putting a small drop of the unknown in an immisible liquid of which the density is known and noting whether it floats or if it sinks, how fast it sinks. Water is the most likely liquid for crude tests on most organic liquids.

## OPTICAL ROTATION

The optical rotation is determined only if the list of possible compounds contains optically active substances.

## MOLECULAR WEIGHT

Normally this is not needed or if it is, it is done using Mass spec. However the following is included to show that it is possible to approximate the MW .

The usual methods of determining molecular weights accurately are too cumbersome and time-consuming for rapid identification work. For compounds that have been described previously, molecular-weight determinations are unnecessary. In certain special cases where a derivative cannot be prepared or is unknown, the molecular weight may be determined conveniently by measuring the depression of the melting point of natural *d*-camphor (Rast[18] method).

***Procedure.*** The weight of a small, clean, dry test tube (8 by 50 mm.) is determined. Approximately 50 mg. of the compound is placed in the tube and weighed accurately. Then about 0.5 g. of camphor is added and the tube again weighed. The contents of the tube are quickly melted by a very low flame to a clear liquid. (*Caution:* Do not heat too long.) After being cooled the contents of the tube are removed to a clean watch glass. The material is powdered and its melting point determined by the capillary-tube method. A thermometer graduated to tenths of a degree with a range of 130° to 180° should be used. The capillary tube in which the melting point is determined should contain a column of the material only 1 mm. high; the column must be tightly packed by means of a smaller capillary tube. That temperature is taken as the melting point at which the *solution* becomes entirely clear of solid. The melting point of the original camphor is determined. The difference in these melting points gives the depression in melting point of the camphor caused by the compound. The molecular weight is calculated by the formula

$$M = \frac{39.7 \times w \times 1000}{\Delta \times W}$$

where $w$ = weight of compound.

$W$ = weight of camphor.

$\Delta$ = depression in the melting point.

***Discussion.*** The above method depends on the fact that the molar lowering in the melting point of camphor is very large. A study[19] has

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shown that the value of 39.7 for the molar depression constant $K$ for *d*-camphor holds for solute concentrations above 0.2 molar. More dilute solutions cause an increase in the constant to about 50. Consequently it is necessary to run a preliminary molecular-weight determination on an unknown compound and calculate an approximate molecular weight. Then a second determination is carried out, using an amount of the solute such that a 0.2 to 0.5 molar solution of the solute in camphor results.

This Rast camphor method sometimes yields incorrect molecular weights because of association of solute molecules or decomposition at the temperatures of *d*-camphor solutions (140 to 176°). In such cases other solvents having a high molar depression of the freezing point should be selected. Some examples are as follows.

| | M.P., °C. | Molar Depression ($K$) |
|---|---|---|
| Camphene | 49.0 | 31.08 |
| Cyclohexane | 6.5 | 20.0 |
| Cyclopentadecanone | 65.6 | 21.3 |
| Tetrahydrothiophene-1,1-dioxide | 27.0 | 66.0 |
| Tribromophenol | 96.0 | 20.4 |

More reliable results may be obtained by determining the ratio of the freezing-point depression of the unknown to that of a known compound in the same solvent. The reference compound should have the same general structure and functional groups, and approximately the same molecular weight. See Reference D, p. 228, for molecular-weight procedures and discussion.

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# Qualitative Analysis for the Elements

The elements commonly occurring along with carbon, hydrogen, and oxygen are nitrogen, sulfur, fluorine, chlorine, bromine, and iodine.

The detection of these elements depends on converting them to water-soluble ionic compounds and applying specific tests.

## FUSION OF ORGANIC COMPOUNDS WITH METALS

$$C, H, O, N, S, X \xrightarrow{Na} \begin{cases} NaX \\ NaCN \\ Na_2S \\ NaCNS \end{cases}$$

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***Caution.*** A few classes of organic compounds such as nitroalkanes, organic azides, diazo esters, diazonium salts, and some aliphatic polyhalides (chloroform, carbon tetrachloride) react explosively with hot sodium or magnesium. *Safety goggles always should be worn when these decompositions are being carried out.*

If a sharp report or explosion occurs when the first portion of the unknown is heated with the sodium, the procedure is interrupted and about 0.5 g. of the unknown is reduced by boiling gently with 5 ml. of glacial acetic acid and 0.5 g. of zinc dust. After most of the zinc has dissolved, the mixture is evaporated to dryness and the entire residue is then decomposed by Procedure *A* or *B* below.

***Procedure A.*** *Sodium.*[1] A small test tube (50 by 8 mm.) is fastened in a vertical position in a Bunsen clamp from which the rubber has been removed. A piece of clean sodium metal about 4 mm. on an edge is placed in the test tube. The lower part of the tube is heated until the sodium melts and the sodium vapors rise in the tube. Then 5 mg. of the compound, mixed with an equal weight of powdered sucrose, is added, and heat is again applied. The addition and heating are repeated a second time, and then the bottom of the tube is heated to a dull red. The tube is allowed to cool, and 1 ml. of ethanol is added to dissolve any unchanged sodium. The tube is heated again and while still hot is dropped into a small beaker containing 20 ml. of distilled water (*caution!*). The tube is broken up with a stirring rod, and the solution is heated to boiling and filtered. The filtrate, which should be colorless, is used for the specific tests described below.

***Procedure B.*** *Magnesium and Potassium Carbonate.*[2] The fusion mixture is prepared by grinding together 2 parts of anhydrous potassium carbonate and 1 part of magnesium powder. About 0.1 g. of the compound to be tested is placed in the bottom of a small (8 by 50 mm.) dry test tube. If the substance is a liquid it is best added by means of a pipet, so as not to wet the walls of the test tube. The tube is clamped at an angle of about 30° from the horizontal, and 0.2 g. of the magnesium-potassium carbonate mixture is allowed to slide down the side of the test tube. This mixture should extend about 3 cm. up the tube and come within 1 cm. of the sample. Two drops of ether is allowed to run down on the mixture, and the mouth of the tube is plugged with glass wool.

Heating of the mixture is started with the Bunsen burner near the mouth of the tube. When the mass begins to glow, the lower end of the tube is brought into the flame so as to distil the compound over the glowing mass.

The compound in the test tube must not be heated until the reaction between the carbonate and magnesium has started. The magnesium-potassium carbonate mixture must be a glowing red.

Heating is continued, and finally the whole tube is brought to a dull redness. Then the hot tube is dropped into 20 ml. of distilled water in a beaker. The tube is broken up; the mixture is thoroughly stirred and then filtered. The filtrate is used for the subsequent tests.

*Specific Tests For Elements. Sulfur.* A few milliliters of the above solution is acidified with acetic acid, and a few drops of lead acetate solution is added. A black precipitate of lead sulfide indicates sulfur.

To another milliliter of the solution 2 drops of a solution of sodium nitroprusside is added. A deep reddish violet coloration indicates sulfur.

*Nitrogen.* (*a*) About 3 ml. of the stock solution is acidified with acetic acid; 2 drops of a freshly prepared 1% solution of benzidine in 50% acetic acid is added, and the mixture is stirred. The addition of 1 drop of a 1% copper sulfate solution produces a blue color or a blue precipitate if nitrogen is present. Chlorides and bromides do not yield colors, but iodides give a greenish precipitate. If both nitrogen (as cyanide) and iodides are present the precipitate is blue.

(*b*) The pH of 1 ml. of the filtrate is adjusted to 13 with Hydrion E paper. Two drops each of saturated ferrous ammonium sulfate solution and 30% potassium fluoride solution are added, and the resulting solution is boiled gently for about 30 seconds. The hot solution is acidified carefully by adding 30% sulfuric acid dropwise until the iron hydroxide just dissolves. Excess acid may be harmful. The appearance of the characteristic precipitate of Prussian blue indicates the presence of nitrogen.

This precipitate may best be observed if it is collected and washed on white filter paper. If no precipitate is observed but a blue or greenish blue solution is obtained, probably the original sodium decomposition was not complete.

(*c*) About 2 drops of ammonium polysulfide solution is added to 2 ml. of the stock solution, and the mixture is evaporated to dryness on the steam bath. Dilute hydrochloric acid (5 ml.) is added, and the solution is warmed and filtered. A few drops of ferric chloride solution is added to the filtrate. A red coloration indicates nitrogen.

(*d*) A few crystals of sodium nitrite are dissolved in 3 ml. of the stock solution, 2 drops of ferric chloride solution is added, and the resulting solution is acidified with dilute sulfuric acid. The mixture is heated to boiling, made alkaline with ammonia, and filtered. The addition to the filtrate of 1 drop of hydrogen sulfide water or alkali sulfide produces a violet color in the presence of nitrogen.

The test for nitrogen is sometimes unsatisfactory, but indications of its presence may frequently be obtained as a result of solubility determinations or classification reactions. It is especially difficult to obtain a test for nitrogen from a nitro compound. The nitro group may be detected by the fact that most nitro compounds give a deep red color with alkalies. Many organic compounds containing nitrogen liberate ammonia when heated in a small test tube with soda-lime. The ammonia may be detected by its odor or by means of a piece of moist red litmus paper held in the mouth of the tube.

*The Halogens.* (*a*) About 2 ml. of the solution is acidified with dilute

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nitric acid and boiled gently for a few minutes to expel any hydrogen cyanide or hydrogen sulfide that may be present. A few drops of silver nitrate solution is added. A heavy precipitate indicates the presence of chlorine, bromine, or iodine. Silver chloride is white, silver bromide is pale yellow, and silver iodide is yellow. If only a faint turbidity or opalescence is produced it is probably due to the presence of impurities in the reagents or in the glass of the test tube used in the original sodium decomposition.

(*b*) *Beilstein's Test.* A small loop is made in the end of a copper wire and is heated in the Bunsen flame until the flame is no longer colored. The wire is cooled; the loop is dipped in a little of the original compound and heated in the edge of the Bunsen flame. A green flame indicates halogen.

This test is extremely sensitive and should always be confirmed by the silver nitrate test since minute traces of impurities containing halogen suffice to produce a green flame. Very volatile liquids may evaporate completely before the wire can be heated sufficiently to cause decomposition, thus causing failure of the test.

It has been stated that certain compounds not containing halogen cause a green color to be imparted to the flame. Among the compounds listed are quinoline and pyridine derivatives, organic acids,[3] urea, and copper cyanide.[1]

(*c*) *Bromine and Iodine.* About 3 ml. of the solution from the sodium decomposition is acidified with dilute sulfuric acid and boiled for a few minutes. The solution is cooled, and 1 ml. of carbon tetrachloride is introduced; then a drop of freshly prepared chlorine water[5] is added. The production of a purple color in the carbon tetrachloride indicates iodine. The addition of chlorine water is continued drop by drop, the solution being shaken after each addition. The purple will gradually disappear and will be replaced by a reddish brown color if bromine is present.

(*d*) *Bromine.* To 3 ml. of the stock solution in a test tube are added 3 ml. of glacial acetic acid and 0.1 g. of lead dioxide. A piece of filter paper, moistened with the fuchsin-aldehyde reagent (p. 129), is placed over the mouth of the test tube, and the contents of the tube are heated to boiling. If bromide is present in the solution, the vapors of bromine turn the paper violet. Chlorides and cyanides do not produce any color. Iodides give a blue color.

A piece of filter paper dipped in a 1% alcoholic solution of fluorescein may be used in place of the fuchsin-aldehyde reagent. The bromine vapors cause the yellow fluorescein to turn pink owing to the formation of eosin. Chlorides and cyanides do not interfere with this test. Iodides give a brown color.

(*e*) *Chlorine.* If the above tests for bromine and iodine are negative and a good precipitate was produced by silver nitrate, the presence of chlorine is indicated. If bromine and iodine have been found to be present, one of the following procedures should be used to detect the presence of chlorine.

(*f*) *Chlorine, Bromine, and Iodine.* About 10 ml. of the stock solution is acidified with dilute sulfuric acid and boiled for a few minutes. The solution is cooled and tested for iodine by adding 0.5 ml. of carbon tetrachloride to 1 ml. of the solution and adding a few drops of sodium nitrite solution. A purple color indicates iodine. If iodine is present the remainder of the solution is treated with sodium nitrite and the iodine is extracted



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with carbon tetrachloride. The solution is finally boiled for a minute and then cooled. To 1 ml. of this solution, 0.5 ml. of carbon tetrachloride and 2 drops of chlorine water[5] are added. A brown color indicates bromine. The remaining solution is diluted to 60 ml., 2 ml. of concentrated sulfuric acid and then 0.5 g. of potassium persulfate are added, and the solution is boiled for 5 minutes. After the mixture has been cooled, silver nitrate solution is added; a white precipitate indicates chlorine.

A similar procedure for the detection of chlorine, in which lead peroxide and acetic acid replace potassium persulfate and sulfuric acid as the oxidizing agent, may also be used.

(g) *Chlorine in the Presence of Nitrogen, Sulfur, Bromine, and Iodine.* About 10 ml. of the original stock solution is acidified with dilute nitric acid and boiled to expel hydrogen cyanide and hydrogen sulfide. Sufficient silver nitrate is added to precipitate completely all the halogens as silver halides, and the precipitate is removed. If both nitrogen and sulfur are present, the precipitate is boiled for 10 mintues with 30 ml. of concentrated nitric acid to destroy any silver thiocyanate that may be present. The mixture is then diluted with 30 ml. of distilled water and filtered. The precipitate of silver halides is then boiled with 20 ml. of 0.1% sodium hydroxide for 2 minutes. The solution is filtered, the filtrate is acidified with nitric acid, and silver nitrate solution is added. A white precipitate indicates chlorine.

(h) *Fluorine.* About 2 ml. of the stock solution is acidified with acetic acid, and the solution is boiled and cooled. One drop of the solution is placed on a piece of zirconium-alizarin test paper. A yellow color on the red paper indicates the presence of fluoride. The test paper is prepared by dipping a piece of filter paper into a solution composed of 3 ml. of 1% ethanolic alizarin solution and 2 ml. of a 0.4% solution of zirconium chloride (or nitrate). The red filter paper is dried and, just before use, is moistened with a drop of 50% acetic acid.

## FUSION OF ORGANIC COMPOUNDS WITH ZINC-CALCIUM OXIDE

When a check on the sodium fusion is desired, an alternative method of carrying out a qualitative analysis for the elements depends on the fact that fusion of an organic compound containing nitrogen, halogen, and sulfur with a mixture of calcium oxide and zinc metal converts the nitrogen to ammonia, the halogen to halide ion, and the sulfur to sulfide ion. These are detected by the standard methods of analytical chemistry.[6]

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# Solubility Behavior

Three general kinds of information can be obtained about an unknown substance by a study of its solubility behavior in various liquids: namely, water, 5% sodium hydroxide solution, 5% sodium bicarbonate solution, 5% hydrochloric acid, and cold concentrated sulfuric acid. First, the presence of a functional group is often indicated. For instance, since hydrocarbons are insoluble in water, the mere fact that an unknown such as ethyl ether is partially soluble in water indicates that a functional group is present. Second, solubility in certain solvents often leads to more specific information about the functional group. For example, benzoic acid is insoluble in the polar solvent, water, but is converted by dilute sodium hydroxide to a salt, sodium benzoate, which is readily water soluble. In this case, then, the solubility in 5% sodium hydroxide solution of a water-insoluble unknown is a strong indication of an acidic functional group. Finally, certain deductions about molecular weight may sometimes be made. For example, in many homologous series of monofunctional compounds the members with less than about five carbon atoms are water soluble while the higher homologs are insoluble.

Compounds are first tested for solubility in water. The water-soluble compounds are divided into three main classes: (1) acidic compounds give solutions which turn litmus paper red, and (2) basic compounds blue; (3) sufficiently weak acids and bases and neutral compounds leave litmus paper unchanged. In considering solubility in water a substance is arbitrarily said to be "soluble" if it dissolves to the extent of 3 g. per 100 ml. of solvent. This standard is dictated by the limitations inherent in the method employed, which depends on rough semiquantitative visual observations, as will be seen. Care is needed in interpreting classifications of "soluble" and "insoluble" in other references since different definitions of these words are often used.

When solubility in dilute acid or base is being considered, the significant observation to be made is not whether the unknown is soluble to the extent of 3% or to any arbitrary extent, but, rather, whether it is significantly more soluble in aqueous acid or base than it is in water. Such increased solubility is the desired positive test for an acidic or basic functional group.

Acidic compounds are detected by their solubility in 5% sodium hydroxide. Strong and weak acids are differentiated by the solubility of the former but not the latter in the weakly basic solvent, 5% sodium bicarbonate. Compounds that behave as bases in aqueous solution are detected by their solubility in 5% hydrochloric acid, and no attempt is made in general to distinguish between strong and weak bases.[1]

Many compounds that are neutral even in strong aqueous acid solutions behave as bases in more acidic solvents such as concentrated sulfuric acid

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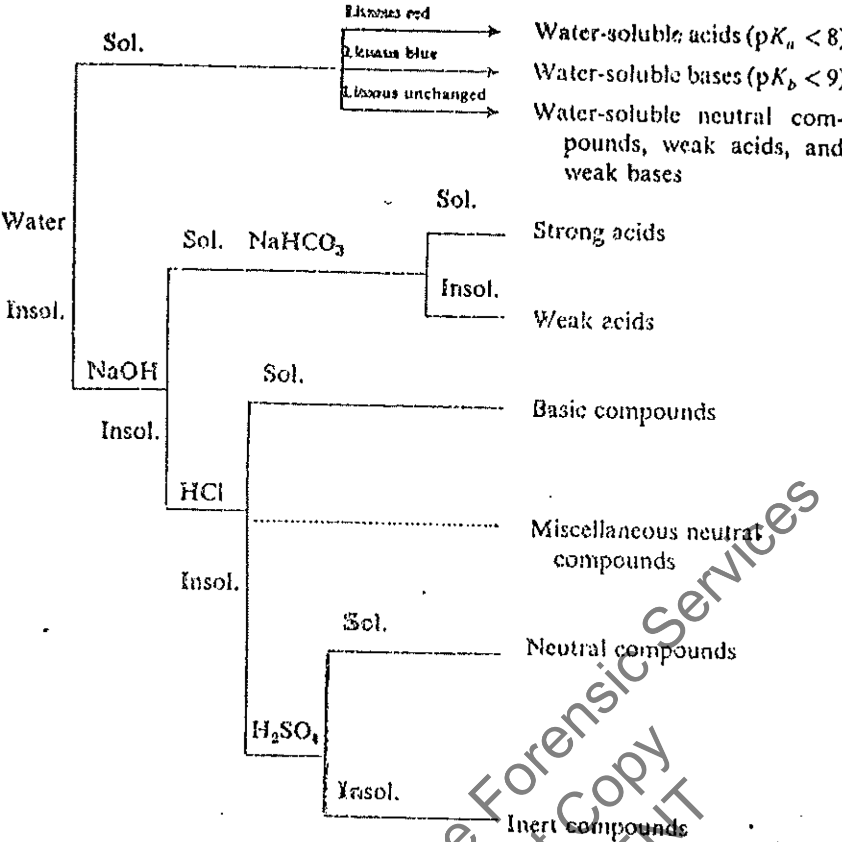


Figure 21. Summary of solubility tests.

In general, compounds containing sulfur or nitrogen have an atom with an unshared pair of electrons and would be expected to dissolve in such strongly acidic media. No additional information would be gained therefore by observing such solubility, and for this reason, when the elementary analysis has shown the presence of sulfur or nitrogen, no solubility tests beyond those for acidity and basicity in aqueous solution are carried out.

Most compounds neutral in water and containing oxygen in any form are reasonably strong bases in concentrated sulfuric acid. Solubility in, or any other evidence of a reaction with, this reagent is indicative of an oxygen atom or else of a reactive hydrocarbon function such as an olefinic bond or easily sulfonated aromatic ring.

Since the solubility behavior of water-soluble compounds gives no information about the presence of acidic or basic functional groups, this information must be obtained by testing their aqueous solutions with litmus paper. In general, the behavior of an acid in 5% hydrochloric acid and of a base in sodium hydroxide solution should be examined routinely since the molecule may have both acidic and basic functional groups.

A more detailed survey of solubility behavior is given on pp. 72-85.

## DETERMINATION OF SOLUBILITIES

### Experiment on Known Compounds

Place 0.2 ml. (0.1 g. of a solid) of the compound in a test tube, and add in portions 3 ml. of water. Shake vigorously after the addition of each portion of solvent, being careful to keep the mixture at room temperature. If the compound dissolves completely, record it as soluble.



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Since the molecular weights of many common organic compounds are in the range of 50 to 300, a concentration of 3 g. per 100 ml. is about 0.1 to 0.6$M$. It will be recalled that when an organic acid is dissolved in pure water the hydronium-ion concentration of the resulting solution is given approximately by the equation

$$[H_3O^+] = \sqrt{K_a C}$$

where $K_a$ is the acid dissociation constant and $C$ is the stoichiometric concentration of acid. The pH of a solution of a soluble acid prepared in the test for solubility in water is then approximately equal to $pK_a/2$. Consequently, phenols with $pK_a$ of about 10 give aqueous solutions too weakly acidic (pH about 5) to turn litmus paper red.[2] For similar reasons

TABLE 9

| Name | Structural Formula | Solubility in | | | | | Litmus Test |
|---|---|---|---|---|---|---|---|
| | | Water | NaOH | $NaHCO_3$ | HCl | $H_2SO_4$ | |
| Catechol | (ring with OH, OH) | + | | | | | Neutral |
| *o*-Cresol | (ring with OH, $CH_3$) | − | + | − | | | |
| Carbon disulfide | $CS_2$ | − | − | | − | | |
| *n*-Hexane | $CH_3CH_2CH_2CH_2CH_2CH_3$ | − | − | | − | − | |
| Acetophenone | (ring with $\overset{O}{\overset{\|}{C}}CH_3$) | − | − | | − | + | |
| Allyl acetate | $CH_3\overset{O}{\overset{\|}{C}}OCH_2CH=CH_2$ | ± | ± | | ± | + | |

an aromatic amine such as aniline is too weak a base ($pK_b$ 9.4) to turn litmus blue in aqueous solution. Although more refined procedures can be developed using a pH-indicating paper such as Hydrion paper, the problems of obtaining water of adequate purity and excluding carbon dioxide are sufficient to make it more convenient as a general rule, to rely on tests like the ferric chloride test, which will be discussed in Chapter 8.

To test for solubility in dilute sodium hydroxide, sodium bicarbonate, or hydrochloric acid solution, shake the mixture thoroughly, separate (filter if necessary) the aqueous solution from any undissolved unknown, and neutralize with acid or base. Examine the solution very carefully for any sign of the separation of the original unknown. Even a cloudy appearance of the neutralized filtrate is a positive test.

By means of the foregoing procedure, determine the solubility of each of the following compounds: (1) toluene, (2) benzyl alcohol, (3) ethyl acetate, (4) acetanilide, (5) sucrose, (6) ethyl benzoate, (7) dimethylaniline, (8) phthalimide, (9) benzonitrile, (10) anthranilic acid. Tabulate the results as shown in Table 9. In that table, + denotes solubility, − insolubility, and ± a doubtful or borderline case. All results of the last type should be checked by a second determination.

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*Discussion.* Solids should be finely powdered to increase the rate of solution. If the solid appears to be insoluble in water or ether it is sometimes advisable to heat the mixture gently. If solution is effected in this way the liquid again is cooled to room temperature and is shaken to prevent supersaturation. It is always well in such cases to "seed" the cooled solution by adding a crystal of the solid. Especial care should be taken in weighing the sample; it should weigh 0.10 g. within 0.01 g.

Liquids are handled most conveniently by means of a graduated pipet which permits accurate measurement of the amount added. When two colorless liquid phases lie one above the other, it is often possible to overlook the boundary between them and thus to see only one phase. This mistake can generally be avoided by shaking the test tube vigorously when a liquid unknown seems to have dissolved in the solvent. If two phases are present the solution will become cloudy. In the rare cases where two colorless phases have the same refractive index the presence of a second phase will escape detection even if this precaution is taken.

When solubility in acid or alkali is being determined heat should not be applied since it might cause hydrolysis to occur. If the mixture is shaken thoroughly the time required for solution to take place should not be more than 1 or 2 minutes.

In the case of reaction solvents, it frequently is more expeditious and more economical to place the 3 ml. of solvent in the test tube and add the solute portionwise. Thus if a compound is very insoluble the fact may be established by use of only a very little of the substance, and the amount will not need to be weighed or measured. In general, where rough tests of solubility are adequate, the prescribed procedure may be simplified greatly, but whenever any doubt exists the solubility determinations should be made accurately.

Often it is possible to utilize a single portion of solute for tests with several different solvents. Thus if the compound is found to be insoluble in water a fairly accurate measure of its solubility in dilute sodium hydroxide solution can be obtained by adding about 1 ml. of a 20% solution of sodium hydroxide. The resulting 4 ml. of solvent will contain about 5% of sodium hydroxide. If the substance is very insoluble it often may be recovered and used subsequently for the hydrochloric acid test.

When sulfuric acid is used it is more convenient to place the 3 ml. of solvent in the test tube and then add the solute. With this reagent significant reactions sometimes take place, and it is important to look for such manifestations as the production of heat, a change of color, the formation of a precipitate, or the evolution of a gas. Careful notes should be made of all such observations since they may be very useful at a later stage of the identification.

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## GENERALIZATIONS

By the study of solubility data it has been found possible to lay down certain generalizations which often enable one to predict the solubility behavior of a compound merely by inspection of its structural formula. As an introduction, it is perhaps worth while to discuss briefly the phenomenon of dissolution.

***Polarity and Solubility.*** When a solute dissolves, its molecules or ions become distributed more or less randomly among those of the solvent. In crystalline sodium chloride, for example, the average distance between sodium and chloride ions is 2.8 Å. In a 1 *M* solution the solvent has interspersed itself in such a way that sodium and chloride ions are about 10 Å

apart. The difficulty of separating such ions is indicated by the high melting point (800°) and boiling point (1413°) of pure sodium chloride.

It will be recalled from elementary physics that the work required to separate two oppositely charged plates is lowered by the introduction of matter between them by a factor which is called the dielectric constant of the medium. It is not surprising then that water, with the high dielectric constant of 80, facilitates the separation of sodium and chloride ions and, in fact, dissolves sodium chloride readily, whereas ether (dielectric constant 4.4) or hexane (dielectric constant 1.9) is an extremely poor solvent for salts of this type. Water molecules between two ions (or the charged plates of a condenser) are small dipoles, which orient themselves end to end in such a way as to neutralize partially the ionic charges and thus stabilize the system. It might be expected, therefore, that solvating ability and dielectric constant should be parallel. This is not entirely true, however. A high dielectric constant is necessary but not sufficient for an effective ion solvent. For example, hydrogen cyanide with a dielectric constant of 116 is a very poor solvent for salts like sodium chloride. Although the situation is complex, one major factor responsible for the efficiency of water and other hydroxylic solvents is their ability to form hydrogen bonds.[3]

The high dielectric constant and hydrogen-bonding ability of water, which make it a good solvent for salts, also make it a poor solvent for non-polar substances. In pure water, molecules are oriented in such a way that positive and negative centers are adjacent. To attempt to dissolve a non-polar substance such as benzene in water is to try to separate unlike charges in a medium of low dielectric constant. In general, then, a polar solvent may be expected to dissolve readily only polar solutes, and a non-polar solvent, only non-polar solutes. This generalization has been summarized briefly as "like dissolves like."

Since most organic molecules have both a polar and a non-polar part, it might be expected that the solubility would depend upon the balance between the two parts. As the hydrocarbon part of the molecule increases, the properties of the compounds approach those of the hydrocarbons from which the compounds may be considered to be derived. This means that water solubility decreases and ether solubility increases. A similar change in solubility occurs as the number of aromatic hydrocarbon residues in the molecule increases. Thus α-naphthol and *p*-hydroxybiphenyl are less soluble than phenol. The phenyl radical when present as a substituent in aliphatic acids, alcohols, aldehydes, and similar compounds has an effect on solubility approximately equivalent to a four-carbon-atom aliphatic radical. Benzyl alcohol, for example, is about as soluble as normal amyl alcohol, and hydrocinnamic acid exhibits a solubility similar to that of *n*-heptoic acid.

***Effect on Solubility of Intermolecular Forces in the Pure Solute.*** The solubility of a substance is a measure of an equilibrium between the pure substance and its solution. It is seen, then, that such an equilibrium is affected not only by the solvent-solute interactions already discussed but also by the intermolecular forces in the pure solute. These forces are independent of the polarity or other properties of the solvent, and their relative strengths may be estimated by a comparison of melting and boiling points, since the processes of the melting of a solid or the boiling of a liquid involve a separation of molecules that is somewhat related to the separation which occurs on solution.

The dicarboxylic acids illustrate the inverse relationship of melting

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point and solubility. The data in Table 10 show that each member with an

TABLE 10

| Even Number of Carbon Atoms | M.P., °C. | Solubility, g./100 g. Water at 20° | Odd Number of Carbon Atoms | M.P., °C. | Solubility, g./100 g. Water at 20° |
|---|---|---|---|---|---|
| Oxalic | 189 | 9.5 | Malonic | 135 | 73.5 |
| Succinic | 185 | 6.8 | Glutaric | 97 | 64 |
| Adipic | 153 | 2 | Pimelic | 103 | 5 |
| Suberic | 140 | 0.16 | Azelaic | 106 | 0.24 |
| Sebacic | 133 | 0.10 | | | |

even number of carbon atoms melts higher than either the immediately preceding or following acid (with an odd number of carbon atoms). The intracrystalline forces in the members with an even number of carbon atoms evidently are greater than in those with an odd number. Since the solubility limit for solids is set at 3.3 g. per 100 ml. of water, it is evident that adipic acid (six carbon atoms) is water insoluble but pimelic acid (seven carbon atoms) is water soluble.

The concomitance of high melting point and low solubility is further illustrated by the isomers maleic and fumaric acids.

HCCOOH ‖ HCCOOH — Maleic acid (*cis*)

HOOCCH ‖ HCCOOH — Fumaric acid (*trans*)

Fumaric acid sublimes at 200° and is insoluble in water. Maleic acid melts at 130° and is soluble in water. Among *cis-trans* isomers, the *cis* form generally is the more soluble. Similarly, with polymorphous substances such as benzophenone, the lower-melting form possesses the higher solubility.

The diamides of dicarboxylic acids constitute another group of compounds in which the melting point is a valuable index of the forces present in the crystals. Urea (m.p. 132°) is water soluble. On the other hand, oxamide has the very high melting point of 420° and consequently a low solubility in water. Substitution of methyl groups for the hydrogen atoms

| $C(=O)(NH_2)_2$ | $CONH_2$–$CONH_2$ | $CONHCH_3$–$CONHCH_3$ | $CON(CH_3)_2$–$CON(CH_3)_2$ |
|---|---|---|---|
| m.p. 132 | m.p. 420 | m.p. 217 | m.p. 80 |

in the amide group lowers the melting point and increases the solubility in water; *N,N'*-dimethyl- and *N,N,N',N'*-tetramethyloxamide are water soluble. Adipamide is water insoluble, whereas its *N,N,N',N'*-tetraethyl derivative is water soluble.

Amides of the type $RCONH_2$ and RCONHR obey the general rule that the borderline compounds contain about five carbon atoms (p. 80). However, *N,N*-dialkylamides ($RCONR_2$) melt lower than the corresponding unsubstituted amides and are much more soluble in water, the solubility limit being in the neighborhood of nine to ten carbon atoms. It has been suggested that amides having the group $-CONH_2$ are associated[4] owing to the fact that they may act both as acceptors and as donors in

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forming hydrogen bonds.

$$\begin{array}{l} R{-}C({=}O){-}N(H){-}H \cdots O{=}C(R){-}N(H){-}H \cdots O{=}C(R){-}N(H){-}H \\ \quad R{-}C({=}O){-}N(H){-}H \cdots O{=}C(R){-}N{-}H \end{array}$$

Such an association is not possible for the *N,N*-disubstituted amides ($RCONR_2$), and hence their state of molecular aggregation is low, as indicated by their low melting points and higher solubilities.

In general an increase in *molecular weight* leads to an increase in intermolecular forces in a solid. Polymers and other compounds of high molecular weight generally exhibit low solubilities in water and ether. Thus formaldehyde is readily soluble in water, whereas paraformaldehyde is insoluble.

$$\underset{\text{Water soluble}}{CH_2O} \rightarrow \underset{\text{Water insoluble}}{HO(CH_2O-)_xH}$$

Methyl acrylate is soluble in water, but its polymer is insoluble.

$$CH_2{=}CHCOOCH_3 \rightarrow (-CH_2\underset{COOCH_3}{\underset{|}{C}H}-)_x$$

Glucose is soluble in water, but its polymers—starch, glycogen, and cellulose—are insoluble. Many amino acids are soluble in water, but their condensation polymers, the proteins, are insoluble. The tendency of some types such as proteins, dextrins, and starches to form colloidal dispersions may be deceptive.

Another method of increasing the molecular weight of a molecule is by the introduction of halogens. Usually this introduction merely results in a decreased water solubility with the result that some water-soluble compounds when substituted by halogens then fall in the water-insoluble class.

***Effect of Chain Branching on Solubility.*** It might be anticipated from a consideration of the effect of branching of the hydrocarbon chain on boiling points of the lower homologous series, such as the hydrocarbons and alcohols, that branching lowers intermolecular forces and decreases intermolecular attraction. It is not surprising then that a compound having a branched chain is more soluble than the corresponding straight-chain compound. This is a very general rule and is particularly useful in connection with simple aliphatic compounds. For example, the solubility of an iso compound differs widely from that of its normal isomer and is close to that of the next lower normal member of the homologous series in question. Effects of chain branching are shown in Table 11. In general, the more highly branched of two isomeric compounds possesses the greater solubility.

TABLE 11

| Types of Compounds | Soluble | Borderline | Insoluble |
|---|---|---|---|
| Acids | Pivalic | Isovaleric | *n*-Valeric |
| Acid chlorides | Isobutyryl | *n*-Butyryl | |
| Alcohols | Neopentyl | 2-Methyl-3-butanol | *n*-Amyl |
| Amides | Isobutyramide | *n*-Butyramide | |
| Esters | Isopropyl acetate | *n*-Propyl acetate | |
| Ketones | Isopropyl methyl | Methyl *n*-propyl | |
| Nitriles | | Isobutyronitrile | *n*-Butyronitrile |



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The position of the functional group in the carbon chain also affects solubility. For example, 3-pentanol is more soluble that 2-pentanol, which in turn is more soluble than 1-pentanol. When the branching effect is combined with moving the functional group toward the center of the molecule, as illustrated by 2-methyl-2-butanol, a very marked increase in solubility is noted. In general, the more compact the structure the greater the solubility, provided that comparisons are made on alcohols of the same type.

*Effect of Structure on Acidity and Basicity.*[5] In general the problem of deciding whether a water-insoluble unknown should dissolve in dilute acid or base is primarily a matter of estimating its approximate acid or base strength. There are two principal effects of structure on acidity or basicity, and these will be discussed in turn.

*Electronic Effects on Acidity and Basicity.* Extensive studies have been made on the quantitative correlation of structure with acid or base strength of *meta-* and *para-*substituted aromatic substances.[6] These effects are primarily electronic, and important evidence has become available as to the nature of the transmission of such electrical effects to the site of a reaction from the *meta-* or *para-*positions of the benzene ring. A quantitative correlation has also been made of the dissociation constants of acids in the aliphatic series in which there are not only important polar effects but also steric effects that must be taken into account. Such detailed treatments are outside the scope of this discussion, however.

Most organic carboxylic acids have dissociation constants in water at 25° of $10^{-6}$ or greater and are therefore readily soluble in 5% sodium hydroxide. Phenols, on the other hand, since they are generally less acidic (the dissociation constant of phenol is about $10^{-10}$) although soluble in strongly basic sodium hydroxide solution, are insoluble in dilute sodium bicarbonate (carbonic acid has a first dissociation constant of $4 \times 10^{-7}$). The introduction of substituent groups, however, may have a profound effect on acidity. Thus *o-* and *p*-nitrophenol have dissociation constants of about $6 \times 10^{-8}$; in other words, the introduction of an *ortho* or *para* nitro group increases the acidity of phenol by a factor of about 600. As might be anticipated, the addition of two nitro groups, as in 2,4-dinitrophenol, increases the acidity to such an extent that the compound is soluble in dilute sodium bicarbonate solution. The acidity-increasing

$$\text{OH} \;\; (p\text{-}C_6H_4\text{-}NO_2) + H_2O \rightleftharpoons \left[ \text{O}^- \;\; \longleftrightarrow \;\; \text{O} \right] + H_3O^+$$

effect of the nitro group is due to stabilization of the phenoxide anion by partial distribution of the negative charge on the nitro group.

A similar acid-strengthening effect is observed when halogen is introduced into phenol. Thus an *ortho* bromine atom increases the acidity of phenol by a factor of about 30 and a *para* bromine atom by a factor of about 5. It is not surprising, then, that 2,4,6-tribromophenol is a sufficiently strong acid to dissolve in sodium bicarbonate solution.

The inductive effect of bromine is probably responsible to a large extent for stabilization of the tribromophenoxide anion. It is apparent that there is not the same possibility for resonance stabilization which exists in the nitrophenoxide anions.



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Similar electronic influences affect the basicity of amines. Thus the aliphatic amines in aqueous solution have hydrolysis constants of about $10^{-3}$ or $10^{-4}$, not very different from that of ammonia ($10^{-5}$). Introduction of the phenyl group, however, lowers the basicity by some 5 powers of 10. Thus aniline has a $K_b$ of $5 \times 10^{-10}$. The effect of the phenyl ring is to stabilize the free amine on the left side of the equilibrium by resonance, which is impossible with the conjugate base on the right.

$$H_2O + \left[ C_6H_5NH_2 \leftrightarrow \text{(quinoid form, } =NH_2^{+}\text{, } H^{-}\text{ on ring)} \right] \rightleftharpoons C_6H_5NH_3^{+} + OH^-$$

It is not surprising that a second phenyl substituent decreases the basicity to such an extent that the amine is no longer measurably basic in water. Thus, diphenylamine is insoluble in dilute hydrochloric acid. Substitution of a nitro group on the phenyl ring of aniline lowers the base strength because of stabilization of the nitroamine by the contribution of the structure below to the resonance hybrid amine but not to the conjugate acid.

$$H-\overset{+}{N}-H \quad (\text{quinoid ring}) \quad :\overset{-}{O}-N-\overset{-}{O}:$$

*Steric Effects on Acidity and Basicity.* It has been known for some time that *ortho*-substituted phenols have very much reduced solubility in aqueous alkali, and the term "cryptophenol" has been used to emphasize this behavior. Claisen's alkali (35% potassium hydroxide in methanol-water) has been used to dissolve such hindered phenols.[7] An extreme example is 2,4,6-tri-*t*-butylphenol, which fails to dissolve in aqueous sodium hydroxide or Claisen's alkali.[7] It can be converted to a sodium salt by treatment with sodium in liquid ammonia.

2,4,6-Tri-*t*-butylaniline shows similarly unusual behavior.[8] It is such a weak base that the $pK_a$ of the conjugate acid is too low to measure in aqueous solution.

2,6-Di-*t*-butylpyridine is also significantly weaker as a base than the corresponding dimethylpyridine.[9] It has been suggested that the weakening of the base strengths of the amines is due to steric strain introduced when a proton is added to the nitrogen atom, but it seems rather more likely that the instability of 2,6-di-*t*-butylphenoxide ion and of the hindered ammonium ions mentioned above is due to steric interference with solvation of ions.[8]

Steric strain may either increase or decrease the acidity of carboxylic acids. For example, substitution of alkyl groups on the α-carbon atom of acetic acid tends to decrease acidity. Methyl-*t*-butylneopentylacetic acid is only $\frac{1}{25}$ as strong as acetic acid, probably because of the destabilization of the conjugate base by steric inhibition of solvation.[10] *ortho*-Substituted benzoic acids, on the other hand, are very appreciably stronger than the corresponding *para*-isomers. It seems likely that the effect of substituents is to turn the carboxyl group out of the plane of the ring, with consequent greater destabilization of the acid than of the anion.[11] This latter case



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exemplifies a second kind of steric effect, which is frequently important and is referred to as steric inhibition of resonance.[12] As a second example, although *p*-nitrophenol is some 2.8 $pK_a$ units stronger than phenol, 3,5-dimethyl-4-nitrophenol is only about 1.6 $pK_a$ units stronger.[13]

| | Phenol (OH) | p-Nitrophenol (OH, $NO_2$) | 3,5-Dimethyl-4-nitrophenol (OH, $CH_3$, $CH_3$, $NO_2$) |
|---|---|---|---|
| $pK_a$ at 25°: | 9.99 | 7.21 | 8.24 |

A part of the effect of the two methyl groups in reducing the acidity of the nitrophenol is electronic, but a large part seems to be due to steric inhibition of resonance in the anion. Thus structure A below, in order to contribute significantly to the hybrid, requires coplanarity or near coplanarity of the nitro group and the aromatic ring. Such coplanarity is inhibited by the presence of the methyl groups in the ion (B).

A: O= ring =N(O⁻)(O⁻) B: O= ring ($CH_3$, $CH_3$) =N(O⁻)(O⁻)

## A SURVEY OF SOLUBILITY BEHAVIOR

***Solubility in Water.*** Since water is a polar compound it is a poor solvent for hydrocarbons. Olefinic and acetylenic linkages or benzenoid structures do not affect the polarity greatly. Hence, unsaturated or aromatic hydrocarbons are not very different from paraffins in their water solubility. The introduction of halogen atoms does not alter the polarity appreciably. It does increase the molecular weight, and for this reason the water solubility always falls off. On the other hand salts are extremely polar, the ones encountered in this work generally being water soluble.

Other compounds lie between these two extremes. Here are found the alcohols, esters, ethers, acids, amines, nitriles, amides, ketones, and aldehydes—to mention a few of the classes of frequent occurrence.

As might be expected, acids and amines generally are more soluble than neutral compounds. The amines probably owe their abnormally high solubility to their tendency to form hydrogen-bonded complexes with water molecules. This theory is in harmony with the fact that the solubility of amines diminishes as the basicity decreases. It also explains the observation that many tertiary amines are more soluble in cold than in hot water. Apparently at lower temperatures the solubility of the hydrate is involved, whereas at higher temperatures the hydrate is unstable and the solubility measured is that of the free amine.

Monofunctional ethers, esters, ketones, aldehydes, alcohols, nitriles, amides, acids, and amines may be considered together with respect to water solubility. *In most homologous series of this type the upper limit of water solubility will be found in the neighborhood of the member containing five carbon atoms.*

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This rule follows from a very general principle, that increased structural similarity between the solute and the solvent is accompanied by increased solubility. Because of the polar nature of water, compounds owe their solubility in it almost entirely to the polar groups which they may contain. As an homologous series is ascended, the hydrocarbon (non-polar) part of the molecule continually increases while the polar function remains essentially unchanged. There follows, then, a trend toward a decrease in the solubility in polar solvents such as water.

That the upper limits of water solubility for many series lie in the same neighborhood is due to the fact that the polarities of many functional groups are similar. The particular region (that of the member containing five carbon atoms) in these several series at which the upper limit of water solubility is reached is determined wholly by the altogether arbitrary proportions of solvent and solute chosen for use in this scheme of separation. It would have been equally easy and perhaps as satisfactory to employ a ratio of solute to solvent which would place the limit elsewhere.

The tendency of certain oxygen-containing compounds to form hydrates also contributes to water solubility. The stability of these hydrates is, therefore, a factor in determining water and ether solubility. Such compounds as chloral probably owe their great solubility in water to hydrate formation.

Low-molecular-weight esters of formic and pyruvic acids are hydrolyzed by water at room temperature, as indicated by the fact that the aqueous solution becomes distinctly acid to litmus.

*Solubility in Dilute Hydrochloric Acid.* Aliphatic amines, primary, secondary, and tertiary, form salts (polar compounds) with hydrochloric acid. Hence aliphatic amines are readily soluble in dilute hydrochloric acid.

Aryl groups diminish the basicity of the nitrogen atom; primary aromatic amines, although more weakly basic than primary aliphatic amines, are soluble in dilute hydrochloric acid; diarylamines and triarylamines are not soluble. Diphenylamine, triphenylamine, and carbazole, for example, are insoluble. Arylalkylamines containing not more than one aryl group are soluble.

Disubstituted amides ($RCONR_2$) which are of sufficiently high molecular weight to be water insoluble are soluble in dilute hydrochloric acid. This behavior contrasts with that of the simple amides ($RCONH_2$), which are neutral compounds. Most monosubstituted amides (RCONHR) also are neutral. *N*-Benzylacetamide, however, is basic.

It should be noted that amines may react with 5% hydrochloric acid to form insoluble hydrochlorides. Compounds of this type may appear to be insoluble. For example, certain arylamines, such as α-naphthylamine, form hydrochlorides which are sparingly soluble in dilute hydrochloric acid. By warming the mixture slightly and diluting it with water, solution sometimes may be effected. The appearance of the solid usually will show whether the amine has undergone a change. In order to decide doubtful cases, the solid should be separated and its melting point compared with that of the original compound. A halogen test with alcoholic silver nitrate would indicate formation of a hydrochloride.

A few types of oxygen-containing compounds that form oxonium salts upon treatment with hydrochloric acid also are basic.

*Solubility in Dilute Sodium Hydroxide Solutions and in Dilute Sodium Bicarbonate Solutions.* Carboxylic acids, sulfonic acids, sulfinic acids, phenols, some enols, imides, primary and secondary nitro compounds, arylsulfonyl derivatives of primary amines, unsubstituted arylsulfonamides,

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oximes, thiophenols, and many less familiar types of compounds are soluble in dilute sodium hydroxide solutions. Of these only the three first-mentioned groups are soluble in dilute solutions of sodium bicarbonate.

Aldehydes and ketones are sufficiently acidic to react with aqueous alkali to yield anions which serve as reaction intermediates in such processes as the aldol condensation; they are far too weakly acidic, however, to dissolve to any measurable extent in sodium hydroxide solution. When two carbonyl groups are attached to the same carbon atom, as they are in acetoacetic and malonic esters and in 1,3-diketones, the acidity increases sharply because of the added stabilization of the anion in which the negative charge can be distributed on two oxygen atoms as well as the carbon atom.

$$\ddot{O}{=}C{-}\bar{C}{-}C{=}\ddot{O} \leftrightarrow {}^{-}\ddot{O}{-}C{=}C{-}C{=}\ddot{O} \leftrightarrow \ddot{O}{=}C{-}C{=}C{-}\ddot{O}^{-}$$

Dissociation constants that illustrate this point are the following.[14]

| | |
|---|---|
| $CH_3COCH_2COOC_2H_5$ | $2 \times 10^{-11}$ |
| $CH_3COCH(C_2H_5)COOC_2H_5$ | $2 \times 10^{-13}$ |
| $CH_2(COOC_2H_5)_2$ | $5 \times 10^{-14}$ |
| $CH_3COCH_2COCH_3$ | $1 \times 10^{-9}$ |

It should be noted that, although these substances are approximately as acidic as the phenols, the rate of proton removal from carbon may be a relatively slow reaction[14] and the rate of solution of such substances may be so slow that they appear to be insoluble in base.

Even one nitro group confers sufficient acidity on a substance to make it soluble in dilute sodium hydroxide. Thus nitroethane[15] has a $K_a$ of about $3.5 \times 10^{-9}$.

It is of interest that nitro compounds have a tautomeric form, the *aci* form, which is approximately as strong an acid as the carboxylic acids. The *aci* form of nitroethane has a $K_a$ of $3.6 \times 10^{-5}$.

Just as the grouping

$$-\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{\underset{|}{C}}}-\overset{O}{\overset{\|}{C}}-$$

is acidic, so is the imide grouping

$$-\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{N}}-\overset{O}{\overset{\|}{C}}-$$

and imides are soluble in dilute sodium hydroxide solution but not in sodium bicarbonate. Even a *p*-nitrophenyl group or an $RSO_2$ grouping makes the —CONH— function weakly acidic in aqueous solution. Thus *p*-nitroacetanilide and sulfonamides ($RSO_2NH_2$) dissolve in sodium hydroxide solution but not sodium bicarbonate. Oximes, which have a hydroxyl group attached to a nitrogen atom, show similar solubility behavior.

Esters containing five or six carbon atoms that are almost completely soluble in water may be hydrolyzed by continued shaking with dilute sodium hydroxide solution. The alkali should not be heated, and the solubility or insolubility should be recorded after 1 to 2 minutes.

Fatty acids containing twelve or more carbon atoms react with the alkali slowly, forming salts which are soaps. The mixture is not clear but consists of an opalescent colloidal dispersion that foams when shaken. Once this behavior has been observed it is easily recognized.

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Certain of the sodium salts of highly substituted phenols are insoluble in sodium hydroxide. This property may be detected by trying the solubility of any residue in water. Certain phenols which are very insoluble in water may precipitate owing to hydrolysis and hence appear to be insoluble in alkali.

*Solubility of Amphoteric Compounds.* Compounds containing both an acidic and a basic group are amphoteric. Low-molecular-weight amino acids exist largely as dipolar salts.

$$\underset{\textstyle RCHCOOH}{\overset{NH_2}{|}} \qquad \underset{\textstyle RCHCOO^-}{\overset{NH_3^+}{|}}$$

They are soluble in water and may give solutions neutral to litmus.

The water-insoluble amphoteric compounds act both as bases and as strong or weak acids, depending on the relative basicity of the amino group, since the basicity determines the extent to which the acidic group will be neutralized by inner salt formation. If the amino group carries only aliphatic substituents, the compounds will dissolve in hydrochloric acid and sodium hydroxide but not in sodium bicarbonate. Compounds of the following types illustrate this group.

$$\underset{\textstyle RCHCO_2H}{\overset{NH_2}{|}} \qquad \underset{\textstyle RCHCO_2H}{\overset{NHR}{|}} \qquad \underset{\textstyle RCHCO_2H}{\overset{NR_2}{|}}$$

The presence of an aryl group on the nitrogen atom, however, diminishes its basicity so that such compounds are soluble even in aqueous bicarbonate solution. This is illustrated by the following compounds.

$$C_6H_5NHCH_2CO_2H \qquad H_2N\text{–}C_6H_4\text{–}CO_2H \qquad \underset{\textstyle RCHCO_2H}{\overset{C_6H_5NCH_3}{|}}$$

If two aryl groups are attached to the nitrogen atom the compound is not basic but behaves simply as a strong acid.

$$(C_6H_5)_2NCH_2CO_2H$$

*Solubility in Cold Concentrated Sulfuric Acid.* This solvent is used with neutral, water-insoluble compounds containing no elements other than carbon, hydrogen, and oxygen. If the compound is unsaturated, is readily sulfonated, or possesses a functional group containing oxygen, it will dissolve in cold concentrated sulfuric acid. Solution in sulfuric acid frequently is accompanied by a reaction such as sulfonation, polymerization, dehydration, or addition of the sulfuric acid to olefinic or acetylenic linkages; but in many cases ions[15] are produced from which the solute may be recovered by dilution with ice water. The following illustrate some of the more common reactions.

$$RCH{=}CHR + H_2SO_4 \longrightarrow [RCH_2{-}\overset{+}{C}HR] \xrightarrow{HSO_4^-} RCH_2\overset{OSO_3H}{\overset{|}{C}}HR$$

$$RCH_2OH + 2H_2SO_4 \longrightarrow RCH_2OSO_3H + H_3O^+ + HSO_4^-$$

(The water arising from sulfate ester formation is converted to hydronium ion by concentrated sulfuric acid.)

$$(RCH_2)_2CHOH + 2H_2SO_4 \longrightarrow (RCH_2)_2CHOSO_3H + H_3O^+ + HSO_4^-$$

$$(RCH_2)_3COH + H_2SO_4 \longrightarrow (RCH_2)_2C{=}CHR + H_3O^+ + HSO_4^-$$

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$$CH_3OC_6H_5 + 2H_2SO_4 \rightarrow CH_3OC_6H_4SO_3H + H_3O^+ + HSO_4^-$$

$$(CH_3)_3C_6H_3 + 2H_2SO_4 \rightarrow (CH_3)_3C_6H_2SO_3H + H_3O^+ + HSO_4^-$$

$$(C_6H_5)_3COH + 2H_2SO_4 \rightarrow (C_6H_5)_3C^+ + H_3O^+ + 2HSO_4^-$$

$$(C_6H_5)_2C{=}O + H_2SO_4 \rightarrow (C_6H_5)_2\overset{+}{C}OH + HSO_4^-$$

$$C_6H_5COOH + H_2SO_4 \rightarrow C_6H_5\overset{+}{C}(OH)_2 + HSO_4^-$$

$$(CH_3)_3C_6H_2CO_2H + 2H_2SO_4 \rightarrow (CH_3)_3C_6H_2\overset{+}{C}{=}O + H_3O^+ + 2HSO_4^-$$

$$CH_3COCOCH_3 + 3H_2SO_4 \rightarrow 2CH_3\overset{+}{C}(OH)_2 + HSO_4^- + HS_2O_7^-$$

Paraffins, cycloparaffins, and their halogen derivatives are insoluble in sulfuric acid. Simple aromatic hydrocarbons and their halogen derivatives do not undergo sulfonation under these conditions and are insoluble. However, the insertion of two or more alkyl groups in the benzene nucleus permits the compound to be sulfonated easily, and hence polyalkylbenzenes dissolve rather readily in sulfuric acid. For this reason isodurene and mesitylene are soluble. Occasionally the solute may react in such a manner as to yield an insoluble product.

A few high-molecular-weight ethers such as phenyl ether undergo sulfonation so slowly at room temperature that they may not dissolve.

Many secondary and tertiary alcohols are dehydrated readily by concentrated sulfuric acid to give olefins which then undergo polymerization. The resulting polymers are insoluble in cold concentrated sulfuric acid and hence form a distinct layer on top of the acid. Benzyl alcohol and its substitution products dissolve in concentrated sulfuric acid, which causes condensation to form orange-colored precipitates.

Gas-chromatographic procedures for studying the solubility behavior of organic compounds have greatly improved the sensitivity of the observations. For example, the slight solubility that is not detected by standard solubility techniques may indicate functional groups that are masked by the larger portion of the insoluble molecules. *n*-Butanol is considered soluble in water, whereas *n*-pentanol is considered insoluble.[46] For example 1 part of *n*-butanol is soluble in 15 parts of water; less than 3 percent *n*-pentanol is soluble, and *n*-hexanol is still less soluble. After thorough shaking and standing, these solubilities can be determined by injecting the water portion of the alcohol-water mixture into the instrument. The ratios of the peak areas (water/alcohol) will give a good estimate of the solubilities of the alcohols in the water at the measured temperatures. Similarly, the solubility of water in the alcohols can be measured by injection of the alcohol layers

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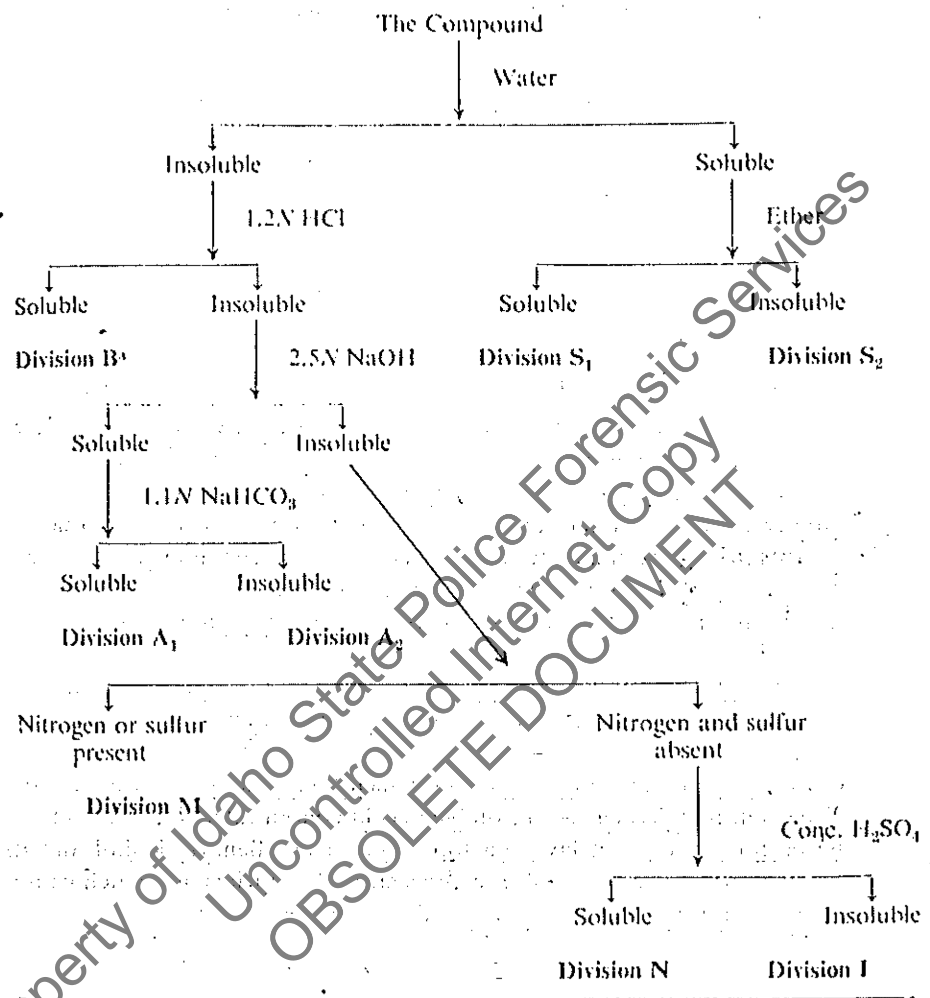
## AN OUTLINE FOR SOLUBILITY CLASSIFICATION

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[a] If the water-insoluble compound is soluble in HCl, determine its solubility in NaOH to detect amphoteric compounds.

### Designation for the Solubility Divisions

**Division $S_1$.** This division includes the compounds that are soluble in both water and ether.

**Division $S_2$.** This division includes the compounds that are soluble in water but insoluble in ether.

**Division B.** Compounds that are insoluble in water but soluble in 1.2*N* hydrochloric acid belong in this division. They all contain nitrogen. Not all the amines will dissolve in dilute acid, however, and many of them fall in Division M.

**Division $A_1$.** The compounds in this division are insoluble in water but soluble in both 1.1*N* sodium bicarbonate solution and 2.5*N* sodium hydroxide solution.

**Division $A_2$.** This division includes the compounds that are insoluble in water and insoluble in a $1.1N$ sodium bicarbonate solution, but which are soluble in a $2.5N$ sodium hydroxide solution.

**Division M.** Compounds that contain nitrogen or sulfur, and which have been found insoluble in all of the solvents used thus far, are placed in this miscellaneous division. The list of compounds that fall in this division is very long. Only the most common chemical classes are included in the solubility tables of this chapter. Halogens may, of course, be present in the compounds of this division.

**Division N.** Compounds that are soluble in concentrated sulfuric acid but are insoluble in the other solvents used belong in this division. Nitrogen and sulfur are both absent, since their presence would classify the compound in Division M.

**Division I.** Compounds that are insoluble in all the classification solvents and do not contain nitrogen or sulfur belong in this division.

An outline of the solubility classification procedures is provided.

## DIVISIONAL SOLUBILITY CLASSIFICATIONS[a,d]

**Division $S_2$[b]**

1. Only C, H, and O present:
   - DIBASIC AND POLYBASIC ACIDS[d]
   - HYDROXY ACIDS
   - POLYHYDROXY ALCOHOLS
   - POLYHYDROXY PHENOLS
   - Simple carbohydrates
2. Metals present:
   - SALTS OF ACIDS AND PHENOLS
   - Miscellaneous metallic compounds
3. Nitrogen present:
   - AMINE SALTS OF ORGANIC ACIDS
   - AMINO ACIDS
   - AMMONIUM SALTS
   - Amides
   - Amines
   - Amino alcohols
   - Semicarbazides
   - Semicarbazones
   - Ureas
4. Halogen present:
   - HALO ACIDS
   - Acyl halides (by hydrolysis)
   - Halo alcohols, aldehydes, etc.
5. Sulfur present:
   - SULFONIC ACIDS
   - Alkyl sulfuric acids
   - Sulfinic acids
6. Nitrogen and halogen present:
   - Amine salts of halogen acids
7. Nitrogen and sulfur present:
   - AMINO SULFONIC ACIDS
   - BISULFATES OF WEAK BASES
   - Cyano sulfonic acids
   - Nitro sulfonic acids

**Division $S_1$[c]**

1. Only C, H, and O present:
   - ALCOHOLS
   - ALDEHYDES AND KETONES[e]
   - CARBOXYLIC ACIDS
   - Acetals
   - Anhydrides
   - Esters
   - Ethers
   - Some glycols
   - Lactones
   - Polyhydroxy phenols
2. Nitrogen present:
   - AMIDES
   - AMINES
   - Amino heterocyclics
   - Nitriles
   - Nitro paraffins
   - Oximes
3. Halogen present:
   - Halogen-substituted compounds of 1 above
4. Sulfur present:
   - Hydroxy heterocyclic sulfur compounds
   - Mercapto acids
   - Thio acids
5. Nitrogen and halogen present:
   - Halogenated amines, amides, and nitriles
6. Nitrogen and sulfur present:
   - Amino heterocyclic sulfur compounds

**Division B**

- AMINES[e]
- Amino acids
- Amphoteric compounds (e.g., amino phenols, amino thiophenols, amino sulfonamides)
- Aryl substituted hydrazines
- N, N-Dialkyl amides
- Some salts

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### Division $A_1$

1. Only C, H, and O present:
   ACIDS[f] and ANHYDRIDES
2. Nitrogen present:
   AMINO ACIDS
   NITRO ACIDS
   Cyano acids
   Heterocyclic nitrogen carboxylic acids
   Imides
   Polynitro phenols
3. Halogens present:
   HALO ACIDS
   ACID HALIDES
   Polyhalo phenols
4. Sulfur present:
   SULFONIC ACIDS
   Sulfinic acids
5. Nitrogen and sulfur present:
   Amino sulfonic acids
   Nitro thiophenols
   Sulfates of weak bases
   Sulfonamides
6. Sulfur and halogens present:
   SULFONYL HALIDES

### Division $A_2$

1. Only C, H, and O present:
   ACIDS[g]
   ANHYDRIDES
   PHENOLS, including esters of phenolic acids
   Enols
2. Nitrogen present:
   AMINO ACIDS
   NITRO PHENOLS
   Amides[h]
   Amino phenols
   Amphoteric compounds
   Cyano phenols
   Imides
   N-monoalkyl aromatic amides
   N-substituted hydroxylamines
   Oximes
   *p*- and *s*-Nitroparaffins
   Trinitro aromatic hydrocarbons
   Ureides
3. Halogens present:
   HALO PHENOLS
4. Sulfur present:
   Mercaptans (thiols)
   Thiophenols
5. Nitrogen and halogen present:
   Polynitro halogenated aromatic hydrocarbons
   Substituted phenols
6. Nitrogen and sulfur present:
   Amino sulfonamides
   Amino sulfonic acids
   Amino thiophenols
   Sulfonamides
   Thioamides

### Division M[i]

1. Nitrogen present:
   ANILIDES AND TOLUIDIDES
   AMIDES AND IMIDES
   NITRO ARYLAMINES
   NITRO HYDROCARBONS
   Amino phenols
   Azo, hydrazo, and azoxy compounds
   Di- and triarylamines
   Dinitro phenylhydrazines
   Nitrates
   Nitriles
2. Sulfur present:
   N-dialkyl sulfonamides
   Sulfates; sulfonates
   Sulfides; disulfides
   Sulfones
   Thio esters
   Thiourea derivatives
3. Nitrogen and sulfur present:
   Sulfonamides
   Thiocyanates
4. Nitrogen and halogen present:
   Halogenated amines, amides, nitriles, and nitro compounds

### Division N[j]

ALCOHOLS
ALDEHYDES AND KETONES
ESTERS
ETHERS
UNSATURATED HYDROCARBONS[k]
Acetals
Anhydrides
Lactones
Polysaccharides[l]

### Division I

HYDROCARBONS[m]
Halogen derivatives of hydrocarbons
Diaryl ethers
All perfluoro esters, ethers, aldehydes, and ketones

---

[a] Nitrogen, halogens, and sulfur are absent unless specified.
[b] Moderate-weight compounds with two or more polar groups, except for the sulfonic and sulfinic acids when only one polar group is necessary.
[c] Generally, mono functional compounds with 5 carbons or less.
[d] In this table, the more common classes are printed in SMALL CAPITAL letters.
[e] Amines with sufficiently strong negative substituents as well as diaryl and triarylamines fall in Division M.
[f] Generally with 10 carbons or less; many form colloidal soap solutions.
[g] High-molecular-weight acids form colloidal soaps.
[h] Including N-monoalkyl amides.
[i] Only the most common classes are listed.
[j] Halogens may be present as substituents.
[k] Noncyclic unsaturated hydrocarbons, and those unsaturated cyclics that are easily sulfonated, such as di- or pol lkyl-substituted benzenes.
[l] Char in the acid.
[m] Including most of th lic hydrocarbons, and all of the saturated, noncyclic hydrocarbons.

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# *Functional-Group and Classification Tests*

In the examination of an organic compound, the use of classification tests follows the determination of the boiling point (or melting point), solubility, and behavior on ignition. From these data and the appearance of the compound (color, physical state, odor), it is generally possible to uncover clues as to the type of functional groups that may be present. The next step in the identification is to seek specific information concerning the presence or absence of common functional groups.

At this point one must stop and concider all the information that has been amassed concerning the unknown. The solubility tests now give us a list of possible types of compounds. However, an unknown compound in a crime lab may, at this point, have had a number of other tests preformed. It may have initially been screened as a possible drug sample. The negative (or positive) screening tests can give clues. For instance: neg Marquis - excludes a number of types of aromatics and alkaloids, neg Cobalt thiocyanate - excludes most tertiery amines, neg Van Urks - excludes certain indoles, etc,etc.

Now is the time to concider running some spectra (assuming of course that you haven't already done so, used a 'spec finder', and struck out and that's the reason you're using this approach in the first place). With the spectra and some interpretation aids in hand, a great deal of information about the unknown can be gleaned.

The identification of organic compounds has been revolutionized by the introduction of instruments which make it possible to measure quickly and conveniently the infrared, nuclear magnetic resonance, and ultraviolet spectra of substances even with small amounts of material. Mass spectroscopy has become increasingly important because of the perfection of reliable spectrometers and advances in the interpretation of the spectra. This chapter will be limited, however, to a brief discussion of the use of infrared, nuclear magnetic resonance, and ultraviolet spectroscopy for functional group determination.

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## INFRARED SPECTROSCOPY

A detailed discussion of infrared spectroscopy is necessarily beyond the scope of this book, and the reader should consult the following general references.

(A) Bellamy, *The Infra-red Spectra of Complex Molecules*, 2nd ed., Methuen & Co., London; John Wiley and Sons, New York, 1958. A very good introduction to the subject of infrared spectroscopy as it is used by the organic chemist, this book is more comprehensive than the other references listed below.

(B) Jones and Sandorfy, Chapter IV in *Chemical Applications of Spectroscopy*, edited by W. West, Interscience Publishers, New York, 1956. This chapter contains a valuable discussion of infrared spectroscopy with emphasis on techniques.

(C) Nakanishi, *Infrared Absorption Spectroscopy—Practical*, Holden-Day, San Francisco, Calif., 1962. This book is of particular value because of the large number of problems, including spectra, and, in many cases, specific questions about various aspects. The spectra are then discussed in a separate section where answers are given.

(D) Hershenson, *Infrared Absorption Spectra, Index for 1945-1957*, Academic Press, New York, 1959. This work provides the most convenient means of locating infrared spectra in the literature of the years covered.

(E) Silverstein and Bassler, *Spectrometric Identification of Organic Compounds*, John Wiley and Sons, New York, 1963. This reference book provides a good introduction to the use of infrared, nuclear magnetic resonance, mass spectroscopy, and ultraviolet spectroscopy for identification work.

The tremendous power of the infrared method lies in the fact that many stretching and bending motions are essentially independent of changes of structure in other parts of the molecule. Thus, the absorption maximum caused by the carbon-oxygen stretching of the C=O group in cyclohexanone (I) is at almost exactly the same frequency as that in pregnane-3-one (II).

$CH_2CH_3$ $H_3C$ $H_3C$ O O

I II

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It is of great help to bear in mind the effects on absorption to be expected from certain changes in structure. A double bond between two atoms in general has a larger force constant (i.e., acts as a stiffer spring) than a single bond between the same two atoms. Most single bonds fall in the region of frequencies less than 1600 cm.$^{-1}$, most double bonds in the region 1600 to 2000 cm.$^{-1}$, and most triple bonds between 2000 and 2500 cm.$^{-1}$ Between 2500 and 3700 cm.$^{-1}$ are the stretching frequencies of the bonds between hydrogen and oxygen, sulfur, and nitrogen.

A knowledge of organic structural theory is essential in any use of infrared spectroscopy. Thus, knowledge that conjugation of a carbon-carbon double bond with a carbonyl group decreases the amount of double-bond character in both the carbonyl and carbon-carbon double bond leads to the expectation that each of these frequencies should be shifted toward the single-bond region, i.e., lowered. This is illustrated below:

$CH_3C(=O)CH_2CH_3$ (C=O 1720 cm.$^{-1}$) $CH_3CH_2CH=CH_2$ (C=C 1647 cm.$^{-1}$)

$[CH_3C(=O)—CH=CH_2 \leftrightarrow CH_3C(—O^-)=CH—CH_2^+]$

C=O 1685 cm.$^{-1}$
C=C 1623 cm.$^{-1}$

### Generalizations

Certain generalizations about the effect of structure on the infrared spectra may be helpful. These are illustrated by Fig. 22.

*Conjugation*, as mentioned above, lowers the frequency of both double bonds concerned.

*Cumulation* of double bonds, on the other hand, has the opposite effect.

Thus allenes show carbon-carbon double-bond absorption at about 1950 cm.$^{-1}$ rather than 1650 cm.$^{-1}$, the position of an isolated double bond. The carbon-oxygen double bond in ketenes and isocyanates is raised from its normal position of about 1710 cm.$^{-1}$ to 2100–2300 cm.$^{-1}$

*Hydrogen bonding* lowers both the hydrogen stretching frequency and the carbonyl frequency when a carbonyl group is involved. Thus the carbonyl frequency of *N,N*-diethylacetamide in dioxane solution is 1647 cm.$^{-1}$ (no hydrogen bonding), but in methanol, where there is hydrogen bonding with the solvent, it is 1615 cm.$^{-1}$

*Negative substituents* may raise the carbonyl frequency in aldehydes, ketones, or acids and their derivatives. Thus ethyl acetate has its carbonyl absorption at 1745 cm.$^{-1}$, but ethyl trichloroacetate absorbs at 1768 cm.$^{-1}$. Such shifts depend upon the angle between the carbon-oxygen and the carbon-X bonds, as was first shown with complex α-bromocyclohexanones. Thus α-chloroacetone in the liquid phase shows two carbonyl bands: one at 1722 and the second at 1745 cm.$^{-1}$ Each of these corresponds to a different rotational conformation.

*Ring strain* raises the stretching frequency of an exocyclic double bond. This is illustrated by the following series.

| Cyclohexane ring $=CH_2$ | Cyclopentane ring $=CH_2$ | Cyclobutane ring $=CH_2$ | Cyclopropane ring $=CH_2$ |
|---|---|---|---|
| 1651 cm.$^{-1}$ | 1657 cm.$^{-1}$ | 1690 cm.$^{-1}$ | 1750 cm.$^{-1}$ |

Carbon-hydrogen stretching frequencies are also raised by ring strain. Thus, the carbon-hydrogen stretching frequency occurs at 3030, 3007, and 3015 cm.$^{-1}$ in cyclopropane, ethylene oxide, and ethyleneimine[3] as compared with 2960 cm.$^{-1}$ in cyclohexane.[4] In effect, contraction of the angle between carbon atoms attached to the double bond, $>C{=}CH_2$, from its normal angle of nearly 120° to values of [illegible], 90, and 60° (which it must assume in rings of five, four, and three members, respectively), leads to successive increases in stretching frequency.

*Steric strain*, which acts in the opposite direction, may occur in molecules with the general formula $R_2C{=}CH_2$, if the R groups are sufficiently large. Spreading the $>C{=}CH_2$ angle in this way lowers the carbon-carbon double-bond stretching frequency, as the following examples illustrate.[5]

$(CH_3CH_2)_2C{=}CH_2$ — 1652 cm.$^{-1}$

$(CH_3)_2CH{-}C(CH_3)_2$ and $(CH_3)_2CH$ attached to $C{=}CH_2$ — 1639 cm.$^{-1}$

$(CH_3)_3C{-}C(CH_3)_2$ and $(CH_3)_3C$ attached to $C{=}CH_2$ — 1621 cm.$^{-1}$

In Fig. 22 are summarized the positions of the absorption maxima of the principal functional groups. (See pp. 182–183.)

Ideally, spectra should be measured in the vapor phase, where the interactions between molecules are minimized. For practical reasons, however, the compromise of measuring them in solution in a non-polar solvent with as little absorption as possible, such as carbon tetrachloride or carbon disulfide, is most satisfactory. Intermolecular interactions in solution or in the pure liquid regularly give shifts of bands from their positions when measured in the vapor phase. Spectra of liquids and solutions are therefore not comparable with data obtained from the vapor.

Solids insoluble in solvents suitable for infrared measurements are generally run as suspensions in paraffin oil. Spectra of the "mulls" are

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often very much less satisfactory than those obtained in solution, since the peaks may show unexpected shifts when the spectra are measured in the mull because of interactions between the molecules which are packed close together in the crystal.

An example of the utility of infrared spectroscopy in structure elucidation is given by the spectrum shown in Fig. 23 (p. 186) of a substance with the molecular formula $C_{14}H_{12}O$. From the ratio of hydrogen to carbon atoms it is seen that the compound is nine pairs of hydrogen atoms short of saturation (see Chapter 12 for interpretation of molecular formulas). The absorption above 3000 cm.$^{-1}$ (aromatic or olefinic carbon-hydrogen stretching) together with the bands at 1500 and 1600 (substituted phenyl ring) and the strong band at 690 cm.$^{-1}$ (monosubstituted phenyl) all indicate that at least one aromatic ring is present. The absence of absorption between 2900 and 3000 cm.$^{-1}$ shows that there are few, if any, aliphatic hydrogen atoms (as can also be seen from the molecular formula). The absence of strong absorption above 3100 cm.$^{-1}$ indicates that there is no hydroxyl group, and the strong absorption at 1725 cm.$^{-1}$ shows that the single oxygen atom is almost certainly part of a carbonyl group. The two bands at 2720 and 2800 cm.$^{-1}$ (aldehyde hydrogen) indicate that the carbonyl functional group is an aldehyde function. The position of the aldehyde carbonyl absorption argues that it is not attached directly to a phenyl ring (where it would be conjugated). This leaves the part formula

$$\begin{array}{l} C_6H_5 \\ \quad \searrow CHCH{=}O \\ C_6H_5 \nearrow \end{array}$$

where one of the $C_6H_5$ units is strongly indicated by the spectrum to be a benzene ring and the other may be presumed to be, although this last point does not follow from the spectral interpretation made thus far. It should be noted that here, as in infrared spectra in general, there is evidence of traces of an impurity. The absorption at 1660 and 1275 cm.$^{-1}$ is suggestive of the presence of a few per cent of benzophenone, which is an almost universal contaminant of diphenylacetaldehyde.

Listed on pp. 187–193 are some of the common functional groups with their more characteristic infrared bands. For further details the references mentioned should be consulted.

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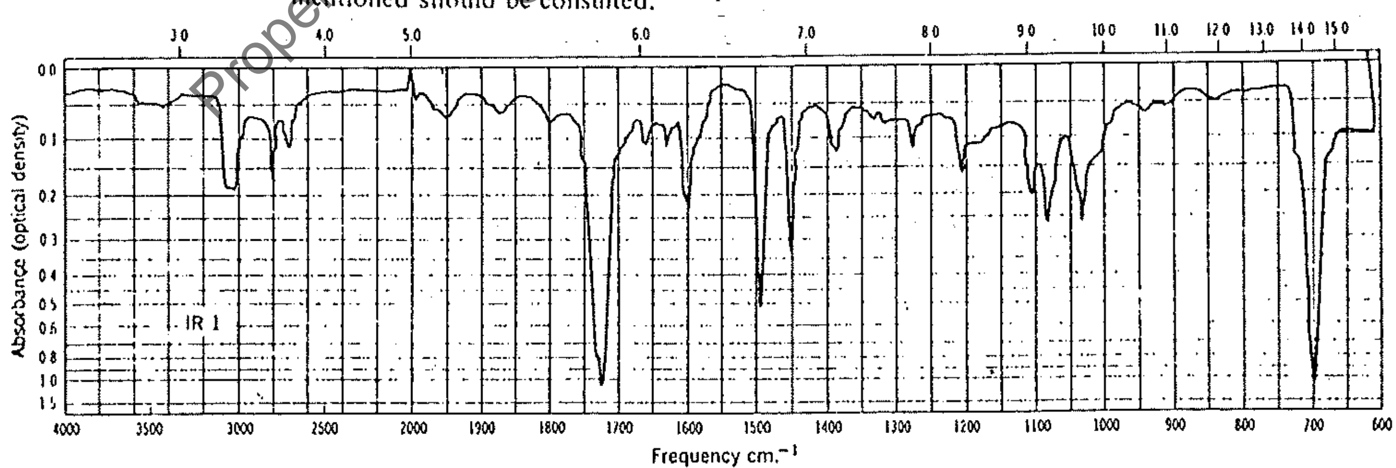


Figure 23. Infrared spectrum of diphenylacetaldehyde in carbon tetrachloride solution.

*Acetylenes.*[6] Simple monosubstituted acetylenes of the type RC≡CH have a moderately strong band due to triply bound carbon-carbon stretching at 2105-2150 cm.$^{-1}$ There is also a characteristic carbon-hydrogen stretching band at about 3300 cm.$^{-1}$ (Olefins and saturated hydrocarbons have their carbon-hydrogen stretching frequencies below 3200 cm.$^{-1}$) Since, in order to be infrared-active, a stretching vibration must lead to a change of dipole moment, disubstituted acetylenes, RC≡CR, where the R's are similar alkyl groups, show no absorption in the triple-bond region.

*Acid Anhydrides.* The anhydride grouping is one of the relatively few simple carbonyl functions which show two absorption frequencies corresponding to carbon-oxygen stretching. Thus, saturated aliphatic acid anhydrides have one band at about 1800–1825 cm.$^{-1}$ and a second at 1740–1770 cm.$^{-1}$ The effects of conjugation and ring strain are qualitatively the same as the effects on the frequencies of other carbonyl functions. Bands of crotonic and benzoic anhydride, for example, are lowered some 20 to 40 cm.$^{-1}$ Succinic anhydride, with its carbonyl groups attached to a five-membered ring, has bands at 1865 and 1782 cm.$^{-1}$ A correlation of the effect of structure on the distance between the two carbonyl maxima has also been made.[7]

*Acid Halides.* Saturated acid chlorides have their carbonyl absorption maxima at 1780–1810 cm.$^{-1}$ Conjugation again presumably lowers the position of the maximum, but relatively few examples of conjugated halides have been studied.

*Acids, Carboxylic.*[8] Although the carboxylic acid function is often detected readily by the solubility of the unknown substance in base, infrared analysis may also be useful. Strong hydrogen bonding affects the positions of both the oxygen-hydrogen single-bond and carbon-oxygen double-bond stretching frequencies, making them somewhat uncertain. The OH absorption of acids is at 2500–3000 cm.$^{-1}$ The carbonyl frequency occurs in simple saturated acids at 1700 cm.$^{-1}$ In general the effect of conjugation and negative substituents is the same as for esters (see p. 181).

*Alcohols.* The rather severe limitations of the functional group tests for alcohols make infrared spectroscopy particularly valuable for their detection. The most readily recognized absorption characteristic of the —OH function is the oxygen-hydrogen stretching frequency which occurs in the region 3100–3600 cm.$^{-1}$ Non-hydrogen-bonded hydroxyl groups absorb at about 3600 cm.$^{-1}$, and highly hindered tertiary alcohols may show a single sharp absorption at that frequency. However, most alcohols in solution exist as an equilibrium mixture of a small fraction of non-hydrogen-bonded molecules (small peak at 3600 cm.$^{-1}$) together with a large fraction of molecules which are hydrogen-bonded to varying degrees and give rise to a broad band at about 3400 cm.$^{-1}$ Since hydrogen-bonded hydrogen-oxygen stretching absorption might be mistaken for hydrogen-nitrogen stretching, the spectra of more dilute solutions should be measured in order to avoid confusion. Dilution decreases the amount of intermolecular hydrogen bonding and tends to shift the hydrogen-oxygen absorption, if present, toward the free-hydroxyl region at 3600 cm.$^{-1}$ In certain cases an overtone of the carbonyl absorption (approximately $2 \times 1700$ cm.$^{-1}$) has been found at 3400 cm.$^{-1}$, and care must be taken when carbonyl groups are present not to mistake this band for hydroxyl absorption.

The effect of differences in internal hydrogen bonding between *cis*- and *trans*-glycols has been used to assign configurations of such compounds.[9] The carbon-oxygen stretching frequency near 1100 cm.$^{-1}$ is also found in spectra of alcohols, and a considerable amount of work has been done to



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correlate the position of this band with the environment of the hydroxyl group (see References on p. 178).

*Aldehydes.* Simple saturated aldehydes show absorption ascribed to the carbon-oxygen double bond at about 1725 $cm.^{-1}$ Conjugation with a carbon-carbon double bond or aromatic ring lowers this value to about 1700 $cm.^{-1}$ Additional help in identifying an aldehyde function may be gained from the carbon-hydrogen stretching frequency associated with the C—H of the formyl group. This absorption appears in the region 2695–2720 $cm.^{-1}$ for aliphatic aldehydes, at about 2730 $cm.^{-1}$ for many aromatic aldehydes, and at 2760 $cm.^{-1}$ for certain *ortho*-substituted ones.[10] Often two bands near 2720 and 2820 $cm.^{-1}$ are observed.[11]

*Amides.* Saturated amides (in carbon tetrachloride or chloroform solution) have the carbonyl absorption between 1645 and 1680 $cm.^{-1}$ As with other carbonyl compounds, conjugation of the carbonyl group lowers this frequency somewhat, and inclusion of the amide linkage in a strained ring raises it. Thus five-membered lactams have the carbonyl absorption at about 1700 and four-membered lactams at about 1730–1760 $cm.^{-1}$ Amides with hydrogen attached to the nitrogen atom show also the nitrogen-hydrogen stretching frequency at about 3200 $cm.^{-1}$, and it is often possible to distinguish primary, secondary, and tertiary amides from one another. Detailed discussions of the spectra of amides in the solid state and in solution are available[12] and should be consulted.

*Amines.* The nitrogen-hydrogen stretching absorption appears in the region 3150–3400 $cm.^{-1}$ Although hydrogen-bonded hydrogen-oxygen stretching absorption may also appear in this region, it can often be distinguished from nitrogen-hydrogen absorption by measuring the spectrum of the unknown in more dilute solutions. Dilution tends to destroy intermolecular hydrogen bonds, and so the hydrogen-oxygen band shifts back toward the non-hydrogen-bonded region (3600 $cm.^{-1}$). Primary amines also show a band associated with the functional group H—N—H at 1600–1625 $cm.^{-1}$

*Esters.* Simple saturated aliphatic esters absorb at about 1740 $cm.^{-1}$ Conjugation of the carbonyl group with a carbon-carbon double bond or phenyl ring lowers the carbonyl frequency to about 1720–1725 $cm.^{-1}$ Substitution of electron-withdrawing groups on the $\alpha$-carbon may give large shifts to higher frequencies. Thus, ethyl cyanoacetate absorbs at 1751 and ethyl trichloroacetate at 1768 $cm.^{-1}$ Enols of $\beta$-keto esters show an unusual shift in the carbonyl absorption, which appears as a very strong band at about 1650 $cm.^{-1}$ This shift is probably due to contribution of the resonance structure shown.[13] Contribution from such a structure is

```
            H
           /
        (+)O        O(−)
           ‖        |
    CH3—C          C—OC2H5
           \      /
             C
             H
```

reinforced by hydrogen bonding. Similarly, butyl *o*-hydroxybenzoate has its carbonyl absorption at 1675 $cm.^{-1}$

Although six-membered lactones show normal ester carbonyl absorption, five-membered lactones show a shift to about 1770 $cm.^{-1}$ and four-membered lactones to 1820 $cm.^{-1}$ or above.

The ester carbonyl frequency is strongly influenced by substitution in the alcohol part of the molecule. Thus phenyl and vinyl esters have their carbonyl stretching absorption at 1750–1770 $cm.^{-1}$ Substituents in the phenyl ring may shift the absorption even farther. Thus *o*-nitrophenyl acetate absorbs at 1786 $cm.^{-1}$

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*Ethers.* The presence of oxygen in an unknown, together with evidence for the absence of other oxygen-containing functional groups, provides evidence for an ether. However, carbon-oxygen single-bond stretching leads to absorption in the infrared at 1050–1150 cm.$^{-1}$ in simple saturated ethers and at 1200–1260 cm.$^{-1}$ in aryl and vinyl ethers.

*Hydrocarbons (Saturated).* Aliphatic compounds with carbon-hydrogen bonds show absorption maxima because of the carbon-hydrogen stretching vibration at 2850–2970 cm.$^{-1}$, and also deformations of the $—CH_2—$ group give rise to absorption at 1465 cm.$^{-1}$ and the $CH_3—$ group to absorption at 1450 cm.$^{-1}$ In addition a maximum at 1375 cm.$^{-1}$ is due to the $CH_3—$, which is quite characteristic. More detailed discussions may be consulted.[14,15] The precise locations of these bands may be of considerable value in structure determination since they are influenced by adjacent carbonyl groups or carbon-carbon double bonds.[16] Hydrogen attached to a multiply bound carbon gives rise to absorption at somewhat higher carbon-hydrogen stretching frequencies. Thus, $R_2C{=}CH_2$ shows absorption at about 3080 cm.$^{-1}$, $RC{\equiv}C—H$ at 3300 cm.$^{-1}$, and aromatic hydrogen at about 3050 cm.$^{-1}$ In strained rings the carbon-hydrogen stretching frequency is increased.[17] Thus, cyclopropanes show absorption at 3024–3058, cyclobutanes at about 2970–2995, cyclopentanes at 2940–2950, and cyclohexanes at about 2915–2924 cm.$^{-1}$ It has been found further that the position of deuterium (and, therefore, presumably of hydrogen also), when it is in a cyclohexane ring, depends upon whether it is axial or equatorial.[18]

*Hydrocarbons (Aromatic).* A phenyl ring is often indicated, not only by the carbon-hydrogen stretching band at 3000–3090 cm.$^{-1}$, but also by bands near 1500 and 1600 cm.$^{-1}$, although one or both of the last two may be missing. Monosubstituted benzenes have a strong band near 700 cm.$^{-1}$

*Ketones.* Saturated aliphatic ketones have the carbonyl band at about 1710 cm.$^{-1}$ As in the case of other carbonyl compounds, this band is shifted to 1670–1690 cm.$^{-1}$ when the carbonyl group is conjugated with a carbon-carbon double bond or aromatic ring. The enols of $\beta$-diketones show carbonyl absorption as a strong broad band at 1540–1640 cm.$^{-1}$, for the reason indicated in the discussion of the enols of $\beta$-keto esters (p. 189). The effect of ring strain on carbonyl absorption parallels that on the olefinic double bond. Although cyclic six-membered ketones exhibit normal aliphatic carbonyl absorption, five-membered ketones have their carbonyl absorption displaced to 1740 cm.$^{-1}$ and four-membered ketones to 1780 cm.$^{-1}$ As with olefinic double bonds, spreading of the bond angle by steric strain between bulky substituents lowers the carbonyl absorption frequency.[19] Thus, di-*t*-butyl ketone has its carbonyl absorption at 1686 cm.$^{-1}$, and *t*-butyl triptyl ketone (hexamethyl-3-hexanone) at 1675 cm.$^{-1}$

Substituents may have a considerable effect on the position of the carbonyl bond. The effect of a chlorine atom on an $\alpha$-carbon atom has been correlated with its orientation in space with respect to the carbon-oxygen bond.[20]

Similarly, substituents in the aryl ring may give rise to shifts in the carbonyl frequency of aromatic ketones (conjugated).[21]

Another structural effect which may lead to significant shifts of the carbonyl frequency of a ketone is illustrated by the transannular interaction of a nitrogen atom of an amine group. Thus the amino ketone shown below has its carbonyl stretching frequency at 1681 cm.$^{-1}$ In acid solution

O N CH3 | O⊖ N + CH3 | H O N+ CH3 $ClO_4^-$

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or in the solid perchlorate salt there is no absorption in the carbonyl region, but hydroxyl absorption is observed which leads to assignment of the oxygen-protonated structure above.[22]

It was pointed out in the discussion of anhydride spectra that interaction of two equivalent or nearly equivalent carbonyl groups connected by an oxygen atom leads to a splitting of the carbonyl stretching absorption into two bands. Such splitting is said to be due to Fermi resonance, and its occurrence is well known in simple molecules.[23] Interactions may also occur between a carbonyl stretching motion and an overtone of some other vibration which accidently happens to be of the correct frequency. For example, 3,4-diphenyl-2-cyclopenten-1-one shows two bands of comparable intensity in the carbonyl region at 1695 and 1718 cm.$^{-1}$ This splitting has been shown to be due to a band at 860 cm.$^{-1}$, which has its first overtone close to the natural carbonyl frequency. That interaction of these two leads to splitting of the carbonyl frequency was suggested by the fact that in a series of related compounds only those with the lower absorption showed the carbonyl splitting. A guess that the 860 cm.$^{-1}$ band (and its overtone) in the compound shown was due to an out-of-plane bending of the vinyl hydrogen on the carbon atom *alpha* to the carbonyl group led to the synthesis and determination of the spectrum of the analogous compound with the ring protons replaced by deuterium in order to change the bending frequency. The splitting of the carbonyl absorption was found to disappear in the deuterated compound.[24]

The danger of faulty interpretation of spectra because of failure to consider such interactions need hardly be pointed out. Cyclopentanone[2] shows similar behavior;[25] a second peak is observed which disappears with deuteration of the α-carbon atoms. It is probable that such splitting due to Fermi resonance is quite common among carbonyl compounds,[25] and since the two interacting frequencies are likely to have different dependence on solvent the curve shapes may be quite solvent dependent.[26]

*Nitriles.*[27] Simple saturated nitriles show the C≡N absorption as a medium band at 2250 cm.$^{-1}$ Conjugation lowers the frequency to 2225 cm.$^{-1}$

*Nitro Compounds.* Both aliphatic and aromatic nitro compounds[28] have two bands characteristic of the nitro group at about 1350 and 1520 cm.$^{-1}$

*Olefins.* Simple unconjugated olefins, of the type $R_2C{=}CH_2$, show absorption at about 1650 cm.$^{-1}$ However, more highly substituted olefins may be so constituted that the carbon-carbon stretching vibration gives rise to little change in dipole moment. In this case there is no absorption in the infrared in the double-bond region. Sheppard and Simpson have discussed spectra of olefins in more detail.[29]

Conjugation with a second carbon-carbon double bond, an aromatic ring, or a carbonyl group may shift the carbon-carbon double-bond absorption to 1600 cm.$^{-1}$ or slightly lower. As might be expected, polarization of a carbon-carbon double bond by conjugation with a carbonyl group or unsymmetrical substitution with other polar groups increases the intensity of absorption. The effects of changing the bond angles by ring strain or steric strain were discussed on p. 182.

The degree of substitution of an olefin and even the configuration may often be determined. For example, the carbon-hydrogen bending band

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in olefins of the type $R_2C{=}CH_2$, where R is an alkyl group, occurs at about 910 cm.$^{-1}$, while the corresponding absorption of olefins of the type RCH=CHR depends upon whether the olefin has *cis* or *trans* configuration; the *trans* band is at about 960 cm.$^{-1}$, and the *cis* (a rather less certain one) at 680–715 cm.$^{-1}$ The positions of these bands may be appreciably altered by the substitution of functional groups for R in the structures above.[30]

*Miscellaneous Nitrogen Functions.* Studies of certain nitrogen-containing functional groups have yielded data that do not appear in the general references previously cited. Some of these data are mentioned here. A few nitrite esters (strong bands at 1725, 1640, and 1610 cm.$^{-1}$) and nitrosamines (bands at 1350–1410 cm.$^{-1}$) have been examined.[31] Oximes show the carbon-nitrogen double-bond frequency at about 1640 cm.$^{-1}$, and certain $\alpha$- and $\beta$-oximes can be distinguished by the position of the oxygen-hydrogen stretching frequency.[32] Ketimines absorb in the same region.[33] Dialkylketimines show the nitrogen-carbon double-bond absorption at 1640–1645 cm.$^{-1}$ and alkyl aryl at about 1620 cm.$^{-1}$ Diphenylketimine gives a band at 1602 cm.$^{-1}$

Isocyanates, both aromatic and aliphatic, show the carbon-oxygen stretching absorption[34] at 2269 $\pm$ 6 cm.$^{-1}$ Diazo compounds show intense absorption in the region 2000–2200 cm.$^{-1}$ Thus diazomethane[35] shows strong nitrogen-nitrogen absorption at 2101 cm.$^{-1}$ Diphenyldiazomethane has its corresponding absorption at 2045 cm.$^{-1}$ Certain diazocyclohexadienones[36] also show absorption at 2015–2150 cm.$^{-1}$ Benzenediazonium fluorborate, apparently the only diazonium salt studied,[36] has a medium strong absorption at 2296 cm.$^{-1}$

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## ULTRAVIOLET SPECTROSCOPY

Although a comprehensive discussion of ultraviolet spectroscopy is beyond the scope of this book, an attempt will be made to indicate briefly the types of structural problems that it might be expected to solve. The following general references are recommended and should be consulted by anyone interested in using the method for structure determination.

(A) Jaffé and Orchin, *Theory and Applications of Ultraviolet Spectroscopy*, John Wiley and Sons, New York, 1962.

Gillam and Stern, *An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry*, 2nd ed., Edward Arnold, Ltd., London, 1957.

These books provide the most comprehensive and generally useful sources of information for the organic chemist.

(B) Braude, *Determination of Organic Structures by Physical Methods*, edited by Braude and Nachod, Academic Press, New York, 1955, Chapter 4.

(C) Mason, *Quart. Revs.*, 15, 287 (1961).

Streitwieser, *Molecular Orbital Theory for Organic Chemists*, John Wiley and Sons, New York, 1961.

These two reviews provide a good introduction to the theory of ultraviolet and visible light absorption.

(D) Hershenson, *Ultraviolet and Visible Absorption*, Academic Press, New York, 1956. This work provides a relatively simple route to spectra published between 1930 and 1954.

(E) Friedel and Orchin, *Ultraviolet Spectra of Aromatic Compounds*, John Wiley and Sons, New York, 1951. This book is largely a collection of spectra, as its title indicates, although there is also some discussion of techniques and general principles in the beginning pages.

*Identification of Functional Groups from Knowledge of Positions of Absorption Maxima.* This use of spectroscopy, which is a common infrared technique, is rare in the ultraviolet for two reasons. First, most simple functional groups absorb weakly or not at all, and second, the spectra of most molecules are relatively simple; that is, they have only one or two maxima instead of the ten or twenty common in an infrared curve. Necessarily, therefore, many types of functional groups have absorption in the same region. Nevertheless, inspection of the ultraviolet spectrum by an experienced observer has occasionally suggested the presence of a previously unsuspected functional group. Probably such inspections are most commonly made to reveal the class of aromatic or heterocyclic ring present in a natural product of unknown structure. As another example, the presence of a nitrophenyl group in chloromycetin was suggested by Doub and Vandenbelt on the basis of the ultraviolet spectrum.[48b] In general, however, some information about possible functional groups is required before the ultraviolet spectrum is useful. Of course, the absence of functional groups which are known to absorb in the ultraviolet region may often be unambiguously inferred.

## PROTON MAGNETIC RESONANCE SPECTROSCOPY

the following references are recommended.

(A) Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon Press, New York, 1959.

Roberts, *Nuclear Magnetic Resonance, Applications to Organic Chemistry*, McGraw-Hill Book Company, New York, 1959.

Roberts, *An Introduction to Spin-spin Splitting in High Resolution Nuclear Magnetic Resonance Spectra*, W. A. Benjamin, New York, 1961.

These three books provide discussion intended primarily for the practicing organic chemist and constitute a good introduction to the subject.

(B) Pople, Schneider, and Bernstein, *High-resolution Nuclear Magnetic Resonance*, McGraw-Hill Book Company, New York, 1959. This is the standard reference work on nuclear magnetic resonance and is a valuable source of information both for the expert and for the occasional user of NMR.

(C) Martin, "NMR Spectroscopy as an Analytical Tool in Organic Chemistry," *J. Chem. Educ.*, 38, 286 (1961).

Conroy, "Nuclear Magnetic Resonance in Organic Structural Elucidation," in *Advances in Organic Chemistry*, Vol. II, edited by Raphael, Taylor, and Wynberg, Interscience Publishers, New York, 1960.

Each of these is a shorter review and provides a good introduction to the subject of NMR.

(D) Bhacca, Johnson, and Shoolery, *NMR Spectra Catalog*, Varian Associates, Palo Alto, Calif., 1962. A catalog of 339 high-resolution spectra, several of which are used in the subsequent discussion of this chapter.

Wiberg and Nist, *Interpretation of NMR Spectra*, W. A. Benjamin, New York, 1962. This work is a collection of theoretical splitting patterns calculated by an IBM-709 computor, with the results printed by the output equipment so as to resemble actual spectra.

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At this point a very brief discussion of the classical method of classification tests will undertaken. This is the 'screening test' type of testing. Since there are hundreds of such tests, most of which will never be used in a criminalistics lab, I will only provide a list of tests for the most common functional groups. With the name of the test in hand, it is easy to look up the actual proceedure in any Qual O book. Feigl's book Spot Tests in Organic Analysis and the 5TH edition of The Merck Index are both excellent sources of information on spot tests. The Merck Index alone lists 4,510 tests.

A List of the Tests

*Class Detected and Reagents*

Tests for Aromatic Structures
- A. Chloroform and Aluminum Chloride
- B. Formaldehyde and Sulfuric Acid
- C. 2,4,7-Trinitrofluorenone

Tests for Active Unsaturation
- A. Bromine in Carbon tetrachloride
- B. Permanganate

Tests for Oxidizable Compounds
- A. Chloranil
- B. Ceric Nitrate
- C. Ferricyanide
- D. Iodic Acid
- E. Nitrochromic Acid
- F. Tollens' Reagent

Tests for Acidic Substances
- A. Iodate-Iodide Reagent
- B. Rhodamine B-Uranyl Acetate
- C. Liberation of Nitrous Acid

Acids
- A. Carboxylic Acids
- B. Amino Acids
- C. Sulfonic Acids

Acid Anhydrides

Acid Halides
- A. Amide Formation
- B. Ferric Hydroxamate Test

Alcohols
- A. Vanadium-Oxine
- B. Ferric Hydroxamate
- C. Xanthate
- D. N-Bromosuccinimide
- E. Lucas
- F. Chromic Acid

Alkyl and Aryl Halides
- A. Alcoholic Silver Nitrate
- B. Hydrolysis, followed by Silver Nitrate
- C. Formaldehyde-Sulfuric Acid

Amides, Unsubstituted
- A. Ammonia Liberation
- B. Ferric Hydroxamate
- C. Distinguishing Aliphatic from Aromatic
- D. Sulfonamides
- E. Ureas

Amides, N-Substituted
- A. N-Alkyl Substituted
- B. Anilides
- C. Other Amides

Amines
- A. Tetraphenyl Borate
- B. Copper Ions
- C. Fluorescein Chloride
- D. Basicity Test
- E. Quinhydrone
- F. N-Halosuccinimide
- G. 3,3',5,5'-Tetrabromophenolphthalein
- H. Hinsberg's Test
- I. Diazotization
- J. Lignin
- K. Chloranil
- L. 2,4-Dinitrofluorobenzene
- M. Tests for Primary Amines
- N. Tests for Secondary Amines
- O. Tests for Tertiary Amines

Carbohydrates
- A. Anthrone
- B. *p*-Toluidine
- C. Molisch's Test
- D. Resorcinol
- E. Oxidation by Copper Ions
- F. Test for Ketoses
- G. Test for Pentoses

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Carbonyl Compounds
- A. 2,4-Dinitrophenylhydrazine
- B. 3,5-Dinitrobenzoic Acid
- C. N-Hydroxybenzenesulfonamide
- D. Schiff's Test
- E. Methone
- F. Benedict's and Tollens' Tests
- G. 2-Hydrazinobenzothiazole
- H. Tests for Ketones

Thiols
- A. Iodine-Azide
- B. Nitroprusside
- C. Isatin
- D. Lead Test

Nitro Compounds
- A. Ferrous Hydroxide
- B. Diphenylamine
- C. Di- and Trinitro- Hydrocarbons
- D. Nitroparaffins
- E. Nitrophenols

Phenols
- A. Ferric Chloride
- B. Coupling with a Diazonium Salt
- C. Indicator Formation
- D. Indophenol Formation
- E. Millon's Test
- F. 4-Aminoantipyrine

The following suggestions offer a means of initiating the attack on the problem of establishing or excluding the presence of functional groups. *At the same time that a test is applied to an unknown compound, a control test should be carried out on the known compounds suggested in each experiment.* Thus, it is possible to make direct and immediate comparisons between the results of the experiments on the known and unknown compounds. It is very important to observe just what positive and negative tests look like. Wherever possible the classification tests carried out should have been suggested by information previously obtained about the unknown.

The compound classes refered to here are the same designation as the s olubility chart on page  refers to. The numbers after the name of each test refer to the number of th t test in reference #3 and can be disregarded.

**Class $A_1$ Compounds. Acidic Compounds in Class $S_1$ or $S_2$.** Carboxylic acids, sulfonic acids, and certain substituted phenols are most likely to be in these classes. Often no other functional group tests are necessary, and it is most advantageous to prepare a list of possibilities and proceed with the preparation of derivatives and the determination of further physical constants as described in the next chapter.

It should be noted that low-molecular-weight acid chlorides and anhydrides, which may be in Class $A_1$ by virtue of their hydrolysis to acids during the solubility test, can be recognized by their ready conversion to anilides (1*b*). The phenols in this class can often be distinguished by the phenol tests described below.

**Class $A_2$ Compounds.** Phenols are the commonest type of compounds in this class. They are indicated by the acetyl chloride test (1*d*), bromine water (6), ceric nitrate (7), and nitrous acid (17) as well as reduction of permanganate (20).

**Class $S_1$ and $S_2$ Compounds Containing Nitrogen and Basic to Litmus; Class B Compounds.** Basic primary, secondary, and tertiary amines are found in these solubility classes and are detected by means of the Hinsberg test (4) or nitrous acid (17). Arylamines also undergo bromination readily (6).

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Class $S_2$ Compounds

(*a*) *Salts of Carboxylic Acids.* A solution of the salt is acidified and the free carboxylic acid liberated. If the acid is water insoluble, it is removed by filtration and treated as a Class $A_1$ compound. If the acid is soluble in the aqueous solution, it may be isolated by extraction with ether or chloroform and subsequent removal of the solvent by distillation.

(*b*) *Salts of Sulfonic Acids.* A salt of a sulfonic acid is best converted to the corresponding chloride by treatment with phosphorus pentachloride. The sulfonyl chloride yields a sulfonamide when treated with ammonium hydroxide. When the amide has been purified and its melting point has been determined, a list of possible compounds may be made (see p. 6).

(*c*) *Salts of Amines.* Addition of alkali will liberate the free bases, which, depending on their solubilities, can be assigned to Class $S_1$, Class $S_2$, or Class B and treated accordingly. Usually it is time-saving and more convenient to apply the various tests for amines (1*b*, 4, 17, 16, 15, 6) directly on the amine salt.

(*d*) *Polyfunctional Compounds.* Polybasic acids and hydroxy acids are best characterized by reference to their neutralization equivalents (Chapter 9). The sugars, which give a characteristic charring and a caramel odor in the ignition test, should be treated with Benedict's solution (3) and Tollens' reagent (28). If a reducing sugar is present, the osazone test (19) is applied. If no reduction occurs, the unknown should be boiled for a few minutes with dilute hydrochloric acid and the tests repeated. If a positive test for a sugar is obtained, the optical rotation should be determined (p. 51). Polyatomic alcohols and keto alcohols in Class $S_2$ are detected by reaction with acid halides (1), hydroxylamine (14), phenylhydrazine (19), 2,4-dinitrophenylhydrazine, or periodic acid (18). Some amino acids may fall in Class $S_2$ as well as in Class $A_1$–B, or Class $A_2$–B. These may be tested with nitrous acid (17). Neutralization equivalents (Chapter 9) of the benzoyl derivatives (1*c*) may be determined also.

The carbonyl group in aldehydes and ketones is the functional group in this class for which the most general and reliable tests are available. Either aldehydes or ketones respond to tests with 2,4-dinitrophenylhydrazine (8), hydroxylamine (14), and phenylhydrazine (19). Further differentiation results from the use of fuchsin (11), Benedict's solution (3), Tollens' reagent (28), or sodium hypoiodite (25). Alcohols may be detected by means of acetyl chloride (1*d*), benzoyl chloride (1*c*), or sodium (22). If these tests are positive, further information may be obtained by applying the Lucas test (13), periodic acid (18), the iodoform test, or ceric nitrate (7). Esters may be discovered with the hydroxamic acid test (14*c*) or hydrolysis with sodium hydroxide solution (24*a*) and give useful saponification equivalents (Chapter 9). Anhydrides and acyl halides react with aniline to give anilides (1*b*, 1*c*) and are hydrolyzed by alkali (19*b*). The acid which is produced may be characterized by reference to the neutralization equivalent, the partition coefficient, or the Duclaux constants (see Chapter 9). In the absence of positive tests for other functional groups ethers are generally considered; they may be cleaved with hydriodic acid (12) and, if aromatic, will undergo bromination slowly (5).

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**Class M Compounds.** Hydrolysis with hot sodium hydroxide solution (24) serves to convert amides, substituted amides, nitriles, and certain negatively substituted aromatic amines to the corresponding acids or phenols with liberation of ammonia or amines. Nitro groups may be detected by the use of ferrous hydroxide (10) or zinc and ammonium chloride (29).

**Class I Compounds.** Reactive halogen is indicated by the silver nitrate (21) or the sodium iodide test (26). Fuming sulfuric acid (27) can be used to detect aromatic nuclei whether halogen is present or absent. Condensation with azoxybenzene (2*a*) or with chloroform (2*b*) in the presence of aluminum chloride also serves to detect the presence of aromatic rings.

**Unsaturation.** The tests for double or triple bonds (5, 20) may be applied to compounds in all solubility classes except Class I.

**Halogen Compounds.** Both the alcoholic silver nitrate (21) and the sodium iodide (26) tests may be tried in order to obtain information concerning the reactivity of the halogen.

No routine "scheme of analysis" has been devised which can be guaranteed to lead rapidly to the identification of any unknown organic compound. On the contrary, the most efficient path to the structure of one substance may be entirely different from that to another. There are some general suggestions to be made, however, and these are given below.

Carry out tests with sufficient care so that you are sure a repetition of the test will lead to the same result. It must be realized that the tests have different degrees of reliability and that two different tests for the same functional group may give discordant results. In such cases judgment must be exercised and, when possible, certain tests given more weight than others. For this reason, it is often desirable to place the tests for a given functional group together when they are interpreted on the report sheets. One good positive test can be given more weight than many negative ones. Suppose, for example, that an unknown gives a precipitate with 2,4-dinitrophenylhydrazine but a negative test with phenylhydrazine. Instead of repeating each test a number of times (which generally gives the same results as before) or carrying out other functional group tests, which often just add to the confusion, it is more profitable to examine further the test with 2,4-dinitrophenylhydrazine. The possibility that the product obtained is actually recovered 2,4-dinitrophenylhydrazine or original unknown can easily be excluded by a comparison of melting points and by mixture melting points. The possibility that the 2,4-dinitrophenylhydrazine reagent was too sensitive and gave a positive test because of a minor impurity in the unknown can generally be excluded by a material balance. Once these possibilities are eliminated, the negative phenylhydrazine test and the positive 2,4-dinitrophenylhydrazine test together constitute a positive test for an aldehyde or ketone.

Unknowns are often contaminated with impurities which are difficult to remove and which may interfere seriously with the classification tests. Thus, benzyl alcohols may be contaminated with the corresponding aldehydes (formed by air oxidation) and give a positive carbonyl test. Recrystallization of a solid or distillation of a liquid may help obviate such difficulties or, on the other hand, may create new ones. For instance, dibenzyl ether undergoes partial decomposition to benzaldehyde and toluene

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when distilled at atmospheric pressure, and certain allyl alcohols rearrange to the isomeric saturated aldehydes when heated.

The detailed behavior of even relatively simple compounds is sufficiently complex so that a textbook knowledge may be misleading. The student is advised to refer constantly to the literature for the chemistry of the compounds under consideration. More than once the identification of nitrobenzene has been delayed because the investigator did not realize that it can give ammonia when heated with strong sodium hydroxide. Similarly, the failure of an unknown, believed to be *p*-hydroxybenzaldehyde, to give a solid bisulfite addition product or the expected fuchsin color is a disturbing flaw in the identification until reference to the literature is made; thereafter these observations are valuable confirmation of the postulated structure.

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# The Separation of Mixtures

The separation of mixtures of organic compounds is a problem with which the chemist is constantly confronted and for which he has developed a wide variety of techniques. The methods of separation considered here will be limited to those that *take cognizance of the chemical nature of the compounds to be separated* and, more specifically, of differences in polarity and in acidity and basicity. Extraction and steam distillation are such methods.

Although other methods of separation such as distillation, chromatography, paper chromatography, gas-phase chromatography, and electrophoresis are of tremendous importance in any modern chemical research laboratory, they are too specialized to be included in this book. Good discussions of them are available in the references cited.

An excellent general reference to all the separation methods mentioned here is the following.

K. B. Wiberg, *Laboratory Technique in Organic Chemistry*, McGraw-Hill Book Co., New York, 1960.

### Distillation

Carney, *Laboratory Fractional Distillation*, The Macmillan Co., New York, 1949.
*Distillation*, Vol. IV of *Technique of Organic Chemistry*, edited by Weissberger, Interscience Publishers, New York, 1951.

### Chromatography

Lederer and Lederer, *Chromatography*, Elsevier Publishing Co., New York, 1953.
Cassidy, *Adsorption and Chromatography*, Vol. V of *Technique of Organic Chemistry*, edited by Weissberger, Interscience Publishers, New York, 1951.
Zechmeister, *Progress in Chromatography*, Chapman & Hall, London, 1950.
Strain, *Chromatographic Adsorption Analysis*, Interscience Publishers, New York.
Block, Durrum, and Zweig, *Paper Chromatography and Paper Electrophoresis*, Academic Press, New York, 1955.

### Gas-phase Chromatography

Dal Nogare and Juvet, *Gas-Liquid Chromatography*, Interscience Publishers, New York, 1962.
Keulemans, *Gas Chromatography*, 2nd ed., Reinhold Publishing Corp., New York, 1959.
Pecsok, *Principles and Practice of Gas Chromatography*, John Wiley and Sons, New York, 1959.
Purnell, *Gas Chromatography*, John Wiley and Sons, New York, 1962.

### General Survey

Berg, *Physical and Chemical Methods of Separation*, McGraw-Hill Book Co., New York, 1962.

In addition to the techniques just mentioned, which are a basic part of nearly all research in organic chemistry, the following are of great value in certain areas.

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## Countercurrent Distribution

Craig and Craig, Chapter IV in *Technique of Organic Chemistry*, Vol. III, edited by Weissberger, Interscience Publishers, New York, 1950.

## Electrophoresis

Alberty, *J. Chem. Educ.*, 25, 426, 619 (1948).

## Centrifuging

Nichols and Bailey, Chapter III in *Technique of Organic Chemistry*, Vol. I, 2nd ed., edited by Weissberger, Interscience Publishers, New York, 1949.

Gold, Chapter III in *Technique of Organic Chemistry*, Vol. III, edited by Weissberger, Interscience Publishers, New York, 1950.

# LABORATORY PROCEDURES FOR THE SEPARATION OF MIXTURES

The identification of the components of a mixture involves first a separation into individual compounds and second the characterization of each of the latter according to the procedures in the following chapters. It is very rarely possible to identify the constituents of a mixture without previous separation. The separation of the compounds in a mixture should be as nearly quantitative as possible in order to give some idea of the actual percentage of each component. It is far more important, however, to carry out the separation in such a manner that each compound is obtained in the pure state, since this renders the individual identification much easier.

The method of separation chosen should be such that the compounds are obtained as they existed in the original mixture. Derivatives of the original compounds are not very useful unless they may be reconverted readily into the original compounds. This criterion of separation is necessary because the identification of a compound rests ultimately on agreement between physical constants of the original and of a derivative with similar data obtained from the literature.

The history of a mixture will usually furnish sufficient information to indicate the group to which the mixture belongs and hence the general mode of separation to be used.

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## Preliminary Examination of Mixtures

1. The physical state is noted. If a solid is suspended in a liquid, the solid is removed by filtration and is examined separately. If two immiscible liquids are present. they also are separated and examined individually.
2. The solubility or insolubility of the mixture in water is determined.
3. With liquid mixtures, 2 ml. of the solution is evaporated to dryness on a watch crystal or porcelain crucible cover and the presence or absence of a residue noted. The ignition test is applied to the residue. For a solid mixture the ignition test is applied directly.
4. In liquid mixtures the presence or absence of water is detected by (*a*) determining the miscibility of the solution with ether; (*b*) anhydrous copper sulfate; (*c*) distillation test for water. The distillation test is the most reliable and is carried out in the following manner. Five milliliters of the liquid mixture and 5 ml. of anhydrous toluene are placed in a small distilling flask. The mixture is heated gently until distillation occurs, and 2 ml. of distillate is collected. About 5 to 10 ml. of anhydrous toluene is

added to the distillate. The presence of two layers or distinct drops suspended in the toluene indicates water. If the solution is only cloudy, traces of water are indicated.

5. If water has been found to be absent, the presence or absence of a volatile solvent in a liquid mixture is determined by placing 10 ml. of the mixture in a 25-ml. distilling flask. The flask is placed in a beaker of cold water which is heated to boiling. Any liquid that distils under these conditions is classified as a volatile solvent. The distillate, which may be a mixture of readily volatile compounds, and the residue in the flask are examined separately.

It frequently happens that distillation of a mixture originally water soluble yields a volatile solvent and a water-insoluble residue. The separation of such a mixture is, therefore, carried out by removing all the volatile solvent. The residue is then treated as a water-insoluble mixture.

If the residue after distillation is a water-soluble liquid it is best not to remove the solvent at this stage since the separation is usually not quantitative.

If, however, the residue after distillation is a water-soluble solid and the removal of the solvent seems quantitative, then it is desirable to remove all the volatile solvent and to examine distillate and residue separately.

It is to be noted that if water is present no such separation should be attempted.

6. The reaction of an aqueous solution or suspension of the mixture to litmus and phenolphthalein is determined. If the mixture is distinctly acid, 5 ml. should be titrated with 0.1*N* sodium hydroxide solution in order to determine whether considerable amounts of free acid are present or whether the acidity is due to traces of acids formed by hydrolysis of esters. The titration must be performed in an ice-cold solution and the first pink color of phenolphthalein taken as the end point.

7. Two milliliters of the mixture is acidified with hydrochloric acid, and the solution is cooled. The evolution of a gas or the formation of a precipitate is noted. Dilute sodium hydroxide solution is now added, and the result is noted.

8. Two milliliters of the mixture is made distinctly alkaline with sodium hydroxide solution. The separation of an oil or solid, the liberation of ammonia, and any color changes are noted. The solution should be heated just to boiling and then cooled. The odor is now compared with that of the original mixture. The presence of esters is often indicated by a change in odor. Dilute hydrochloric acid is now added, and the result is noted.

9. In the case of water-insoluble mixtures an elementary analysis should be made. If water or a large amount of a volatile solvent is present in a water-soluble mixture the elementary analysis of the mixture is omitted. If the water-soluble mixture is composed of solids an elementary analysis is made.

10. If water is absent the effect of the following classification reagents is cautiously determined: (*a*) metallic sodium; (*b*) acetyl chloride.

11. The action of the following classification reagents should be determined on an aqueous solution or suspension of the original mixture: (*a*) bromine water; (*b*) potassium permanganate solution; (*c*) ferric chloride solution; (*d*) alcoholic silver nitrate solution; (*e*) fuchsin-aldehyde reagent; (*f*) phenylhydrazine.

At this stage of the examination the results of the foregoing tests are summarized and as much information as possible is deduced from the behavior of the mixture. The preliminary study will show the group in which the mixture should be classified and will, therefore, indicate which of the following procedures should be used in its separation.

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## Mixtures of Water-soluble Compounds

The schematic diagram on p. 99 represents one method for the separation of this type of mixture. It may and should be modified for certain mixtures when the preliminary examination indicates that such modification is expedient.

*Procedure*

1. About 50 ml. of the mixture is placed in a 500-ml. round-bottomed flask arranged for steam distillation. A steam trap and safety tube should be used. By means of steam distillation from 50 to 60 ml. of distillate 1 is collected. Residue 1 in the flask is placed in an evaporating dish, and the water is evaporated by means of a steam cone. Occasionally the last traces of water may best be removed under diminished pressure. The residue, liquid or solid, is examined.

2. Distillate 1 is acidified with phosphoric acid and steam-distilled to yield 40 to 50 ml. of distillate 2. Residue 2, consisting of the phosphate of the amine, is made alkaline, and the amine is distilled. If the amine is very volatile it should be collected in hydrochloric acid and isolated as the hydrochloride. Less volatile amines may be separated from the aqueous solution by means of potassium carbonate.

3. Distillate 2 is made just slightly alkaline with dilute sodium hydroxide solution and is again steam-distilled, 30 to 40 ml. of distillate 3 being collected. Residue 3 (in the flask) is composed of the sodium salt of the acidic compound. Half of it is acidified with phosphoric acid and steam-distilled. Duclaux numbers are determined on the distillate. The other half of the solution of the sodium salt is used for the preparation of a derivative.

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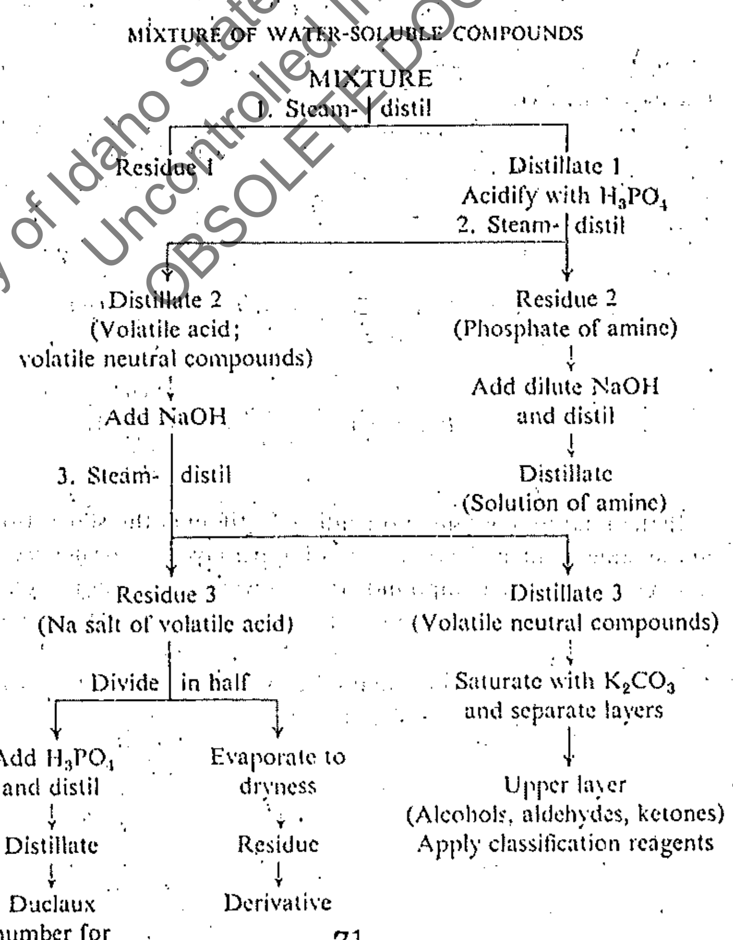
MIXTURE OF WATER-SOLUBLE COMPOUNDS

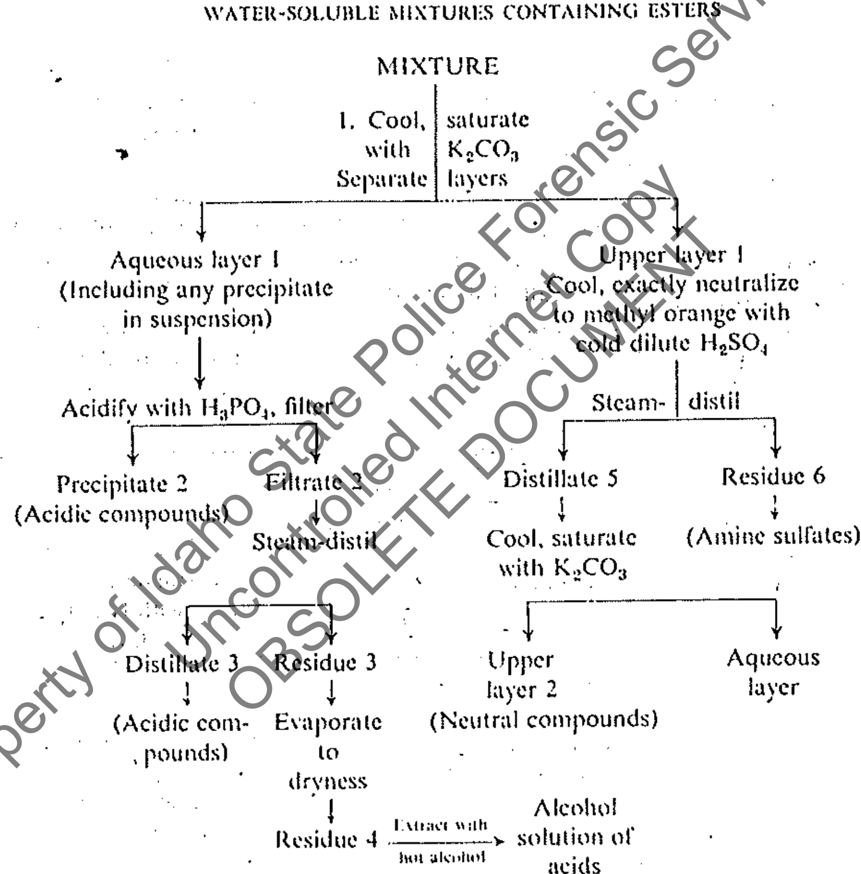


4. Distillate 3 contains volatile neutral compounds—alcohols, aldehydes, and ketones. These may be separated from the water by saturation with potassium carbonate. Classification reagents are applied to determine whether one or all of these are present. If only one is present, the liquid is distilled, the boiling point is noted, and a derivative is prepared.

*Discussion.* In the first steam distillation it should be remembered that amine salts of weak acids (e.g., aniline acetate) may undergo hydrolysis to such an extent that both acid and base will be found in the distillate. Salts of strong acids (e.g., aniline hydrochloride) are unaffected by steam distillation; they remain behind in the distilling flask.

Note that the procedure outlined on pp. 98–99 would cause hydrolysis of any esters that might be present. If the presence of an ester is indicated by odor or by change of odor in preliminary test 8, the procedure may be modified as shown by the following scheme.

WATER-SOLUBLE MIXTURES CONTAINING ESTERS

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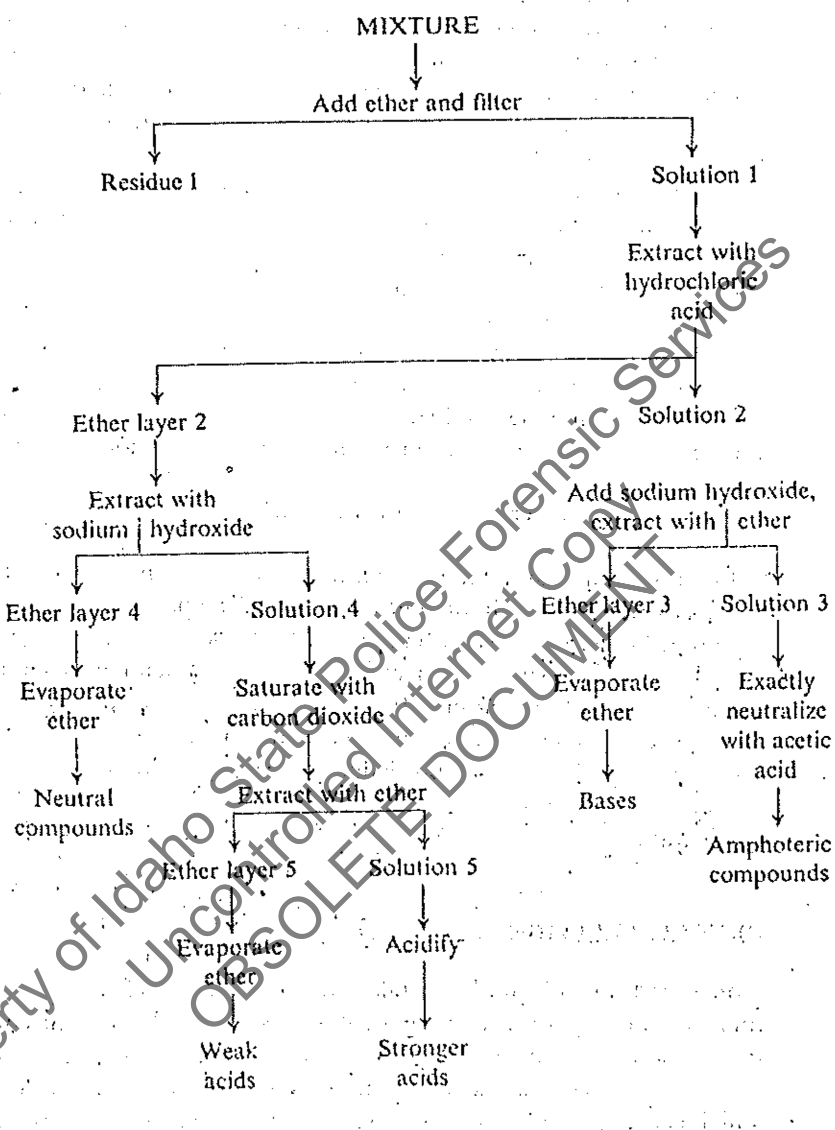
If the mixture contains no acidic constituents the separation may start at the same point as the treatment of upper layer 1. In such a case residue 6 may contain acidic compounds (which were originally present as salts) in addition to the amine sulfates. It may be separated by the first method given.

This modified procedure may also be used to advantage with mixtures containing acids and alcohols; it provides no opportunity for esterification to take place.

## Mixtures of Water-insoluble Compounds

After the removal of any volatile solvent the following procedure may be used for the separation of a water-insoluble mixture.

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*Procedure*

From 25 to 50 g. of the mixture is mixed with 75 ml. of ether, and any insoluble compounds (residue 1) are separated on a filter and washed with ether. The ether washings are added to the original ether solution, and the ether solution (1) is then extracted with three 30-ml. portions of 5% hydrochloric acid solution. If a solid amine hydrochloride separates during this extraction, the hydrochloric acid should be diluted with water until a solution results.

The hydrochloric acid extracts are combined (solution 2) and rendered alkaline with sodium hydroxide solution, and the resulting mixture is extracted with several 25-ml. portions of ether. The ether layer (3) is dried with sodium sulfate, and the ether is distilled. The residue is composed of bases.

The solution (3) after ether extraction is carefully neutralized with acetic

acid. If a solid separates it may be removed by filtration, but it is better to extract the solution four or five times with 25-ml. portions of ether in order to recover any amphoteric compounds.

The ether layer (2) from the hydrochloric acid extraction is now extracted with three 30-ml. portions of 5% sodium hydroxide solution. If a soapy emulsion is formed, more water and a little alcohol may be added in order to cause the separation of two layers.

The ether layer (4) is dried with sodium sulfate, and the ether is distilled. The residue contains the neutral compounds. Classification reagents should be applied as well as tests designed to determine whether or not the residue is a mixture. This mixture may often be further separated by a steam distillation. If an aldehyde is present it should be extracted by means of sodium bisulfite solution, with the addition of ether to facilitate separation. If the residue is a solid it may often be fractionally crystallized from alcohol. If the residue is a liquid and no chemical separation appears possible it may be fractionally distilled.

The sodium hydroxide solution (4) is cooled and saturated with carbon dioxide. Any weak acids are extracted with several portions of ether (ether layer 5). To remove any dissolved ether, solution 5 is warmed on a steam cone for a few minutes and stirred. It is then acidified and cooled; and the stronger acids are removed by filtration or by extraction with ether.

### Mixtures Encountered in Synthetic Work

Every student has already separated many mixtures during his first year's laboratory work in organic chemistry. The principles used in devising procedures for the isolation and purification of an organic compound from a reaction mixture are identical with those which have been pointed out in the preceding sections. In fact, the very great improvement in the yields of organic compounds obtainable from a given reaction has been due to the application of knowledge concerning solubility and behavior toward classification reagents to the outlining of the most efficient method of separation.

## CRYSTALLIZATION

The separation of solid crystals from a solution of a substance or a mixture of compounds usually involves dissolving the organic compound in a suitable solvent, preferably one in which it is more soluble hot than cold. The solid can also be dissolved in a water-miscible solvent, which causes the substance to separate into crystals when water is added.

It is sometimes necessary to wash the crystals with water or other immiscible solvents that will extract impurities. For example benzoate esters are washed with dilute sodium carbonate solutions to remove unreacted benzoic acids or to destroy excess benzoyl chlorides.

### Solvent Choice

The proper choice of solvent is important in purifying derivatives by crystallization. Unfortunately the ideal solvent cannot be chosen strictly by theoretical considerations or rules of thumb. The literature on derivatives gives some information on the solvent to be used but seldom gives solubility data. However, the solubility can quickly be determined by using the gas-chromatographic technique (see p 44). The purity can be determined on the first crop of crystals. If it is substantially purer than the origin. , the proper solvent has been used; if not, another must be found.



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A few general rules will make selection of a solvent easier:

1. Since "like usually dissolves like," a solid will be most soluble in that liquid which it most resembles in structure. For example, solid esters are most soluble in ethyl acetate or other solvent esters (as well as in methanol or ethanol).

2. If possible, it is better to select that solvent in which the impure product is most soluble when hot but only slightly soluble when cold, thus retaining the impurities in the solvent.

3. Mixed solvents are useful when the impure compound is quite soluble in one of the cold solvents but only slightly soluble in the other cold solvent. For example, if the compound is very soluble in acetone, it is dissolved in a small amount of this solvent; then a hydrocarbon is added slowly to force the crystallization.

4. In purification by crystallization, it is preferable for the compound being purified to be as soluble as possible and the impurities insoluble or much less soluble. For example, a phenylurethan derivative of an alcohol dissolves in hot petroleum ether, but the impurity, diphenylurea, is insoluble. Gas-chromatographic examination of the solvents before and after crystallization will establish whether the impurities are being removed.

5. Naturally the analyst avoids using a solvent that reacts with the compound being purified unless the impurities are removed in the reaction and the original compound thus purified is recovered. For example, a variety of acidic substances can be purified by solution in aqueous sodium hydroxide and subsequent filtration to remove impurities; then treatment with mineral acids precipitates the original acidic substance.

6. Some compounds are so new that the literature does not give a suitable solvent. Depending upon the nature of the compound, try:

*a.* Methanol, ethanol, or a mixture of the lower alcohols

*b.* Water, acetone, or a mixture of acetone and alcohol

*c.* Benzene, a mixture of benzene and toluene, petroleum ether, or a mixture of benzene and petroleum ether

*d.* Glacial acetic acid or aqueous acetic acid

Table 2-1 lists solvents and solvent pairs useful in the crystallization of various derivatives. The ideal technique is to choose a solvent in which the compound to be purified is very soluble and then add to the solution a solvent in which the compound is insoluble or only slightly soluble. Both solvents must be mutually miscible. Heating the solutions may assist in the purification. The solution should be examined before and after crystallization in the gas chromatograph to determine whether a degree of purification has been attained.

For substances that dissolve only with difficulty the more powerful solvents, such as dimethyl sulfoxide (**caution**: hazardous on skin!), tetrahydrofuran, nitro paraffins, etc., may be used alone or in combination.

Induction of crystallization can be either *chemical* or *physical.* Chemical induction involves addition of a *seed crystal,* another solvent, or a solid substance that will induce crystallization. Physical induction involves warming or cooling, scratching, or local drying to produce induction sites for crystallization.

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**TABLE 2-1 Solvents and Solvent Pairs for Derivative Crystallization**[a]

Listed in Order of Decreasing Solvent Polarity

| Solvent or solvent pair | Derivative to be crystallized |
|---|---|
| Water | Carboxylic acids, amides, and substituted amides |
| Methanol | Acetates, benzoates, 3,5-dinitrobenzoates, other esters, amides, *p*-toluidides, nitro and bromo derivatives, etc. |
| Methanol-water | Benzyl and *p*-nitrobenzyl esters, anilides, sulfonamides, picrates, semicarbazones, hydrazones, and substituted hydrazones |
| Ethanol | Compounds similar to those listed with methanol and methanol-water mixtures; molecular complexes |
| Dioxane-water | Xanthylamides |
| Ethyl acetate | Quaternary ammonium salts, esters |
| Isopropyl ether | Quaternary ammonium salts |
| Acetone-alcohol | Osazones, bromo compounds, nitro compounds |
| Petroleum ether | Phenylurethans, α-naphthylurethans, etc. |
| Petroleum ether–benzene | *p*-Nitrophenylurethans, 3,5-dinitrophenylurethans |
| Benzene | Picrates, molecular complexes |
| Chloroform and carbon tetrachloride | Sulfonyl chlorides, acid chlorides, anhydrides |

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By reference to the literature prepare a list of compounds which boil or melt within 5° of the value observed for the unknown and which have the same elementary composition, the same solubility, and the same behavior toward the classification reagents as the unknown. Many of these are to be found in the tables in Chapter 11. However, there are a number of other reference works that contain lists of compounds in order of increasing melting point or boiling point. The following are recommended.

1. Frankel, Patai, Farkas-Kadmon, and Zilkha, *Tables for Identification of Organic Compounds, Supplement to Handbook of Chemistry and Physics*, Hodgman, Editor-in-Chief, Chemical Rubber Publishing Company, Cleveland, Ohio, 1960. This work contains the most complete set of tables of compounds available, arranged by melting point and boiling point, with more than 4380 parent compounds.
2. Huntress and Mulliken, *Identification of Pure Organic Compounds, Order I.* (Compounds of carbon, hydrogen, and oxygen), 1941.
3. Huntress, *Organic Chlorine Compounds*, 1948.
4. Utermark, *Schmelzpunkt Tabellen organischer Verbindungen*, 1951.

Older works which may be useful also are the following:

5. Clarke, *Handbook of Organic Analysis*, 1931.
6. Mulliken, *Identification of Pure Organic Compounds*, 1904.

Once a compound has been suggested as a possibility, a literature search can be made for derivatives of the substance. The most direct way to make a thorough search for a particular compound is to look for the molecular formula in the formula indices of each of the following works in order.

Beilstein's *Handbuch der organischen Chemie*, 4th ed., Index to the 2nd Supplement. This covers the literature to 1929. The use of Beilstein will be discussed further below.

*Chemisches Zentralblatt*, Collective Indices: 1922–1924, 1925–1929, 1930–1934.

*Chemisches Zentralblatt*, Annual Formula Indices, 1935–1939.

After 1939 the most reliable index is the *Chemical Abstracts* Subject Index. Decennial Indices cover the years 1937–1946 and 1947–1956; the annual indices must be consulted for later years. Although there is a Collective Formula Index to *Chemical Abstracts* for the years 1920–1946 as well as annual Formula Indices for later years, these are not complete and should not be depended on when a thorough search is desired.

The importance of Beilstein's *Handbuch* is such that further discussion of its use is warranted.[1] The main work or *Hauptwerk* covers the literature through 1909 in 27 volumes. The organization is based on structure in such a way that it is possible to find a desired compound rather easily without using the index once one is familiar with the work. Thus, acyclic hydrocarbons, alcohols, aldehydes, and ketones are in Vol. 1; acyclic acids in Vol. 2; acyclic hydroxy, aldehydo, and keto acids in Vol. 3; sulfonic acids, amines, and phosphines in Vol. 4. In Vol. 5 cyclic (including aromatic) hydrocarbons are treated, and the presentation of cyclic compounds continues along these lines until Vol. 27, where the discussion of heterocyclic compounds begins.

Once a particular compound is located in the main work it is easily found in the supplements. The First Supplement (*Erstes Ergänzungswerk*),



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which covers the literature from 1910 to 1919, has, as do the later supplements, the same arrangement as the main work. Thus a compound found in Vol. I of the main work is in Vol. I of the First Supplement. Furthermore, an auxiliary set of page numbers in the middle of each page of each of the supplements relates the material on that page to the corresponding page in the main work. The Second Supplement covers the material from 1920 to 1929 in the same way. The Third Supplement, which covers a longer period (1930–1949), is still in progress.

Elsevier's *Encyclopedia of Organic Chemistry* was initially intended to cover the literature of organic chemistry with the same thoroughness as Beilstein's *Handbuch.* Unfortunately, publication was discontinued after the appearance of a few volumes. The published volumes, however, provide a valuable supplement for Beilstein's *Handbuch.*

Compendia on a smaller scale are the following.

Heilbron, *Dictionary of Organic Compounds*, 1953.
Rodd, *Chemistry of Carbon Compounds;* completed, 1962.

In deciding whether a compound actually possesses the physical constants observed, considerable latitude must be allowed for experimental error. Thus, if the boiling point is very high or the melting point very low, the range must be extended somewhat beyond 5°. Other constants such as specific gravity , refractive index , and neutralization equivalents , but not Duclaux constants , may be used, with proper allowance for experimental error, to exclude compounds from the list of possibilities. A complete list of possible compounds with derivatives for each should always be made even though a product obtained in the classification tests appears to be a suitable derivative.

At the same time that a list of possible compounds is being prepared, notes should be made concerning derivatives for the compounds. In this way much time may be saved. It is best to consult literature on derivatives and note the types of compounds suitable for final identification. The same reference works cited above are used to obtain this information, and in addition the original literature references must be consulted.

Examination of the list of possibilities often suggests further functional group tests to be attempted. For example, if a list of possible nitro compounds contains a nitro ketone, carbonyl tests may be valuable.

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## THE PREPARATION OF DERIVATIVES

The list of possible compounds that results from the preceding steps in the examination of an unknown usually contains compounds belonging to one or two types. The next step in the identification is the confirmation of the identity of one of these possibilities with the unknown and the simultaneous demonstration that each of the remaining possibilities differs in some way from the unknown. This final proof is accomplished by the preparation of derivatives.

In eliminating compounds from the list of possibilities, one is not restricted to the use of derivatives. Any sufficiently characteristic property, such as specific gravity, refractive index, optical rotation, or neutralization equivalent, may be employed.

*The Properties of a Satisfactory Derivative.* (*a*) A satisfactory derivative should be easily and quickly made and readily purified. This generally means that the derivative must be a solid, because, in the isolation and purification of small amounts of material, solids afford greater ease of manipulation, and melting points are more accurately and more easily determined than boiling points. The most suitable derivatives melt above 50° but below 250°. Most compounds that melt below 50° are difficult to crystallize, and a melting point above 250° is undesirable on account of possible decomposition and because the stem correction of the thermometer often amounts to several degrees.

(*b*) The derivative must be prepared by a reaction that produces a compound in good yield. Processes accompanied by rearrangements and side reactions are to be avoided.

(*c*) The derivative should possess properties distinctly different from those of the original compound. Generally, this means that there should be a marked difference between its melting point and that of the parent substance.

(*d*) The derivative chosen should be one that will single out uniquely one compound from among all the possibilities. Hence the melting points of the derivatives to be compared should differ from each other by at least 5°.

Consult the literature and select a suitable derivative from those suggested. It will be noted that derivatives that are satisfactory for purposes of identification are numerous but often of limited scope. A few of the most useful are listed below; these should receive first attention when a derivative is sought.

*Acids*

(*a*) Amides and anilides.
(*b*) *p*-Nitrobenzyl and *p*-bromophenacyl esters.

Duclaux constants, partition coefficients, and the neutralization equivalents are very useful in this type of work and may take the place of a derivative. However, it seldom is advisable to depend upon them alone.

*Alcohols*

(*a*) Phenyl- and α-naphthylurethans.
(*b*) 3,5-Dinitrobenzoates.

*Aldehydes and Ketones*

(*a*) Phenylhydrazones, *p*-nitrophenylhydrazones.
(*b*) 2,4-Dinitrophenylhydrazones.
(*c*) Semicarbazones.
(*d*) Oximes.

*Acid Anhydrides*

(*a*) Acids.
(*b*) Amides.
(*c*) Anilides.

*Acid Chlorides*

(*a*) Acids.
(*b*) Amides.
(*c*) Anilides.

*Alkyl and Aryl Halides*

(*a*) Anilides.
(*b*) Alkylmercuric halides.

*Amines (Primary and Secondary)*

(*a*) Benzenesulfonamides.
(*b*) *p*-Toluenesulfonamides.
(*c*) Acetamides.
(*d*) Benzamides.
(*e*) Phenylthioureas.

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*Amines (Tertiary)*
Addition compounds with
(*a*) Chloroplatinic acid.
(*b*) Methyl *p*-toluenesulfonate.
(*c*) Methyl iodide.
(*d*) Picric acid.
*Aromatic Hydrocarbons*
(*a*) Nitro derivatives.
(*b*) Aroylbenzoic acids.
*Ethers (Aromatic)*
(*a*) Nitro derivatives.
(*b*) Bromo derivatives.
*Phenols*
(*a*) α-Naphthylurethans.
(*b*) Bromo derivatives.
(*c*) Acetates.
(*d*) Benzoates.

Many types of compounds can be hydrolyzed to acids, amines, alcohols, etc., and often are most easily identified by reference to such products. In this group are acetals, acid anhydrides, acid chlorides, amides, esters, certain ethers, and nitriles.

Nitro, nitroso, azo, and hydrazo compounds can be reduced to the corresponding amines, and many compounds may be identified readily by reference to such reduction products.

The next step in the identification is the preparation of the derivative. Procedures for many of these are to be found in literature.

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## REPORTS OF UNKNOWNS

After the identification of an unknown has been completed the results are reported on special forms supplied by the instructor. The following specimen reports illustrate the correct procedure.

Compound *n-Butyl Alcohol* Name *John Smith*
Unknown No. *1* Date *June 1, 1963*

1. Physical Examination:
   (*a*) Physical state *Liquid.* (*b*) Color *None.* (*c*) Odor *Choking.*
   (*d*) Ignition test *Burns with bluish flame, no residue.*
2. Physical Constants:
   (*a*) M.P.: observed ; corr. (*b*) Sp. gr. $0.812^{20°}_{4}$.
   B.P.: observed *114–117* ; corr. *115–118* . (*c*) $n^{20}_{D}$ *1.3988.*
3. Elementary Analysis:
   F –, Cl –, Br –, I –, N –, S –, Metals *None.*
4. Solubility Tests:

| $H_2O$ | NaOH | $NaHCO_3$ | HCl | $H_2SO_4$ |
|---|---|---|---|---|
| + | | | | |

Reaction to litmus *None:* to phenolphthalein *None.*

7. Preliminary Examination of the Literature:

| Possible Compounds | M.P. or B.P. | Suggestions for Further Tests |
|---|---|---|
| *Isobutyl alcohol* | *108°* | |
| *Methylisopropylcarbinol* | *113* | *A sec-methyl carbinol—should give iodoform test* |
| *3-Pentanol* | *116* | |
| *n-Butyl alcohol* | *117* | |
| *2-Pentanol* | *119* | *A sec-methyl carbinol—should give iodoform test* |
| *Methyl-t-butylcarbinol* | *120* | |
| | | |
| | | |
| | | |

8. Further Classification and Special Tests:

| Reagent | Results | Inferences |
|---|---|---|
| *Iodoform test* | *No ppt.* | *Not a sec-methyl carbinol* |
| | | |
| | | |
| | | |
| | | |



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9. Probable Compounds:

| Name | Useful Derivatives and Their M.P.'s, N.E., etc. | | | |
|---|---|---|---|---|
| | *3,5-Dinitro-benzoate* | *α-Naphthyl-urethan* | *Phenylurethan* | *Sp. Gr.* |
| *n-Butyl alcohol* | 64° | 71° | 61° | 0.810 |
| *Isobutyl alcohol* | 86 | 104 | 86 | 0.805 |
| *3-Pentanol* | 97 | 71 | 49 | 0.820 |

10. Preparation of Derivatives:

| Name of Derivative | Observed M.P. | Reported M.P. |
|---|---|---|
| *3,5-Dinitrobenzoate* | 62–63° | 64° |
| *α-Naphthylurethan* | 68–69 | 71 |
| *Phenylurethan* | 57–59 | 61 |
| | | |

11. Special Comments:

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## REFERENCES

(E) Editorial comment

1. Sadtler IR Interpretation Course, 4th edition, Sadtler Research Laboratories, Inc.

2. Shriner, Fuson and Curtin, The Systematic Identification of Organic Compounds, John Wiley & Sons, New York, 5th ed.

3. Crippen, Identification of Organic Compounds with the Aid of Gas Chromatography, McGraw-Hill Book Co., 1973.

4. Cheronis, Entriken, Hodnett, Semi-micro Qualitative Organic Analysis, Interscience Publishers, 3rd ed., 1965.

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# THE ANOR (ALTERNATE NON-AQUEOUS ORGANIC RATIO) EXTRACTION PROCEDURE

Allen R. Adair, B. S., M.S., Montgomery Division
F. Taylor Noggle, Jr., B.S., M.S., Auburn Division
Martha S. Odom, B.S., Huntsville Division
Mary A. Rhodes, B.S., M.S., Birmingham Division

ALABAMA DEPARTMENT OF FORENSIC SCIENCES

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## INTRODUCTION

Increasing case loads of the forensic drug chemist have necessitated the discovery of "shortcuts" to the traditional extraction procedures. The ANOR Extraction Procedure has proven to be such a "shortcut" method by this laboratory system. This procedure produces drugs of purity equal to or higher than drugs extracted by traditional methods, e.g. morphine sulfate and pentobarbital. The ANOR Extraction Procedure is a dry extraction technique for rendering certain solvents (chloroform, petroleum ether, hexane) either acidic or basic to obtain the free acid, ion-pair, or free base of a drug. The amounts of chemicals, glassware, and time required are generally less than required with traditional extraction methods.

## MATERIALS

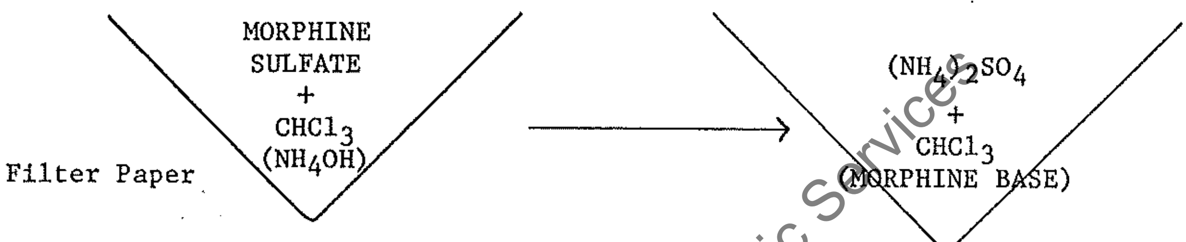
1. APPARATUS: 250 ml. separatory funnels
   Whatman #1 PS filter paper
   50 ml. beakers
   200 ml. reagent bottles
   Disposable pipettes
2. REAGENTS: Chloroform
   Petroleum Ether
   Hexane
   Concentrated Hydrochloric Acid
   Ammonium Hydroxide (30%)
   (All reagents obtained from Fisher)
3. INSTRUMENTATION: Infrared Spectrophotometer
   (Illustrated spectra recorded on Beckman Acculab 9)

## METHOD

The ANOR Extraction Procedure utilizes an organic solvent saturated with ammonium hydroxide ($NH_4OH$) or hydrochloric acid (HCl). The $NH_4OH$ or HCl is mixed with the desired solvent at a ratio of approximately one part acid or base to ten parts solvent. If the $NH_4OH$ or HCl is lighter than the solvent, such as the case with chloroform, a separatory funnel is useful for storing the solvent until ready for use. If the $NH_4OH$ or HCl is heavier than the desired solvent, such as the case with

petroleum ether and hexane, a reagent bottle can be utilized for storing the solvent and the upper layer is pipetted with a disposable pipette.

Morphine (an amphoteric drug) sulfate tablets may be dry extracted by the ANOR procedure with chloroform saturated with $NH_4OH$ to obtain morphine as the base. The following reaction takes place in the filter paper when chloroform saturated with $NH_4OH$ is added to morphine sulfate powder:




The morphine base flows through the filter paper along with the chloroform. The chloroform is evaporated to dryness on a steam bath and the morphine residue mixed with a suitable quantity of potassium bromide (KBr), a disk prepared, and the infrared spectrum run. Figure 1 is the infrared spectrum of morphine base after extraction by the ANOR procedure. Many other bases can be extracted from their salts utilizing the same method (Table I).

By proper selection of the organic solvent saturated with $NH_4OH$ certain drug combinations may be successfully separated. d-Propoxyphene base (Figure 2) can be extracted from Darvon-N, a commercial preparation containing d-propoxyphene napsylate and acetaminophen, by utilizing hexane saturated with $NH_4OH$. Other drug combinations as well as single drug entities may be extracted using hexane saturated with $NH_4OH$ (Table II).

The base derived from the ANOR Extraction Procedure may be converted to the hydrochloride salt after mild heating on a steam bath to dissipate excess ammonium hydroxide. This step is necessary to prevent formation of ammonium chloride which will interfere with the infrared spectrum. A piece of moistened pH paper may be extended over the beaker to assure that all the ammonium hydroxide has been removed. The salt may be formed by bubbling HCl fumes into the solution of the organic base and the organic solvent. The organic solvent is evaporated to dryness and the residue which remains is suitable for identification by infrared spectrophotometry.

The barbiturates or other organic acids may be obtained from their salts in a similar manner. Pentobarbital is obtained as the free acid from sodium pentobarbital by using chloroform saturated with HCl. The following reaction takes place in the filter paper when chloroform saturated with HCl is added to the sodium pentobarbital powder:

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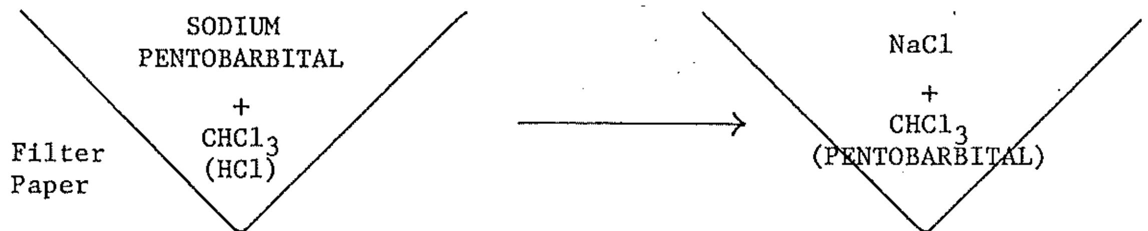


The free acid of pentobarbital flows through the filter paper with the chloroform. The infrared spectrum of pentobarbital extracted by the ANOR procedure is shown in Figure 3. This procedure can be used to obtain organic acids not only from other barbiturate salts but other organic acid salts (Table III).

Another group of drugs which may be extracted by the ANOR procedure are those drugs whose ion pairs are soluble in organic solvents, especially chloroform. Among those drugs are heroin, cocaine, phencyclidine, and pentazocine as the hydrochloride salts. Cocaine base may be placed in a piece of filter paper and dry extracted with chloroform saturated with HCl. The spectrum of cocaine HCl so obtained is shown in Figure 4. A list of drugs which have been extracted as the ion pair are shown in Table III.

By varying the organic solvents (Table IV), using combinations of solvents at various ratios, and adjusting the pH with $NH_4OH$ or HCl not only may single drug items be separated by the ANOR procedure, but mixtures containing several drugs may be selectively separated (Tables V and VI).

A general procedure for utilizing the ANOR Extraction Procedure consists of:

1. Determining proper solvent system.
2. Placing drug sample as a powder in a piece of Whatman #1 PS paper.
3. Eluting powder with proper solvent system and catching eluate in a beaker placed under the filter paper.
4. Evaporating of solvent to obtain the free acid, free base, ion pair or salt (if HCl gas is bubbled through eluate).

## DISCUSSION

The ANOR Extraction Procedure has proven to be extremely valuable in many drug cases analyzed by this laboratory system. Many of the infrared spectra obtained by this procedure appear superior to the spectra obtained by traditional extraction techniques. The ease of application, economy of time and solvents, and the results obtained have made this the procedure of choice in a majority of cases.

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TABLE I

Drugs extracted utilizing $NH_4OH$ saturated $CHCl_3$

Hydromorphone

Morphine

Benzphetamine (Didrex) inner portion

Diazepam (Valium)

Lorazepam (Ativan)

Flurazepam (Dalmane)

All Benzodiazepines should be applicable (Except Valrelease capsules)

Phentermine (Ionamin)

Chlordiazepoxide ( Librium)

Cocaine

Pentazocine (Talwin)

Methaqualone

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TABLE II

Drugs extracted utilizing $NH_4OH$ saturated hexane

Phentermine (Fastin)

Propoxyphene ( Darvon, Darvon-N)

Codeine (Empirin #3, Phenaphen with Codeine capsules, Phenaphen with Codeine tablets)

Diethylpropion (Tenuate)

Diazepam (Valium)

Chlorphentermine (Pre-Sate)

Amphetamine ( From mixtures of Caffeine, followed by conversion to d-Mandelic acid derivative)

Ephedrine (from caffeine mixture)

Phenylpropanolamine (from caffeine mixture)

Methamphetamine (Syndrox)

3,4,-Methylenedioxy amphetamine

Clortermine

Meperidine (Demerol, Demerol APAP)

TABLE III

Drugs extracted utilizing HCl saturated $CHCl_3$

Phenobarbital
Secobarbital
Pentobarbital
All barbiturates should be applicable
Diphenylhydantoin Na (Dilantin)
Heroin
Phencyclidine
Diazepam (Valium)
Pentazocine (Talwin)
Cocaine
Meperidine

TABLE IV

Drugs extracted utilizing $NH_4OH$ saturated petroleum ether

Cocaine
Cocaine-lidocaine mixtures may be extracted by first washing with 1,4-dioxane to obtain the lidocaine, then with $NH_4OH$ saturated petroleum ether to obtain the cocaine.

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TABLE V

Drugs extracted utilizing HCl saturated diethyl ether/hexane(4/1)

From a mixture of caffeine, acetaminophen and butalbital, the butalbital may be extracted using this solvent.



TABLE VI

Drugs extracted utilizing $NH_4OH$ saturated $CHCl_3$/ hexane (4/1)

From a mixture of chlordiazepoxide-amitriptyline, chlordiazepoxide may be extracted using this solvent

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# EVALUATION OF A SCREENING PROCEDURE FOR BASIC AND NEUTRAL DRUGS: N-BUTYL CHLORIDE EXTRACTION AND MEGABORE CAPILLARY GAS CHROMATOGRAPHY

M.E. SHARP[1]

## ABSTRACT

**A procedure for the comprehensive screening of blood for basic and neutral drugs is described and evaluated. A rapid n-butyl chloride extraction method is used. Extraction recoveries are determined using megabore capillary and packed column gas chromatography with nitrogen-specific detection. Recoveries and retention times are recorded in tabular form. Detection limits are below 200 nanograms of drug per millilitre of blood.**

## RÉSUMÉ

**Une procédure de dépistage des drogues basiques et neutres est décrite et évaluée. Une méthode d'extraction rapide avec le chlorure de N-butyl est utilisée. Les recouvrements sont évalués par chromatographie gazeuse sur colonne capillaire mégabore et colonne garnie avec détection spécifique à l'azote. Les recouvrements et temps de rétention sont présents sous forme de tableaux. Les limites de détection sont inférieures à 200 nanogrammes par millilitre de sang.**

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## INTRODUCTION

Basic and neutral drugs are of great importance to the forensic toxicologist. Biological fluids must be screened for these drugs and frequently quantitation is required. It would be desirable to use a single extraction procedure for screening and quantitating basic and neutral drugs. A number of solvents (chloroform, methylene chloride, ethyl acetate, ether, hexane, n-heptane — 1.5% isopropyl alcohol) and a range of pH levels have been used for isolating drugs from biological fluids (1-6). Wolen and Gruber (6) introduced n-butyl chloride as a solvent for direct extraction (direct solvent contact without the use of support material such as kieselguhr) of propoxyphene from plasma. Subsequently n-butyl chloride, alone or in combination with isopropyl alcohol (1%), was used as an extraction solvent in screening for basic and neutral drugs in plasma and blood (7-9). However, previous investigators reported detection limits above those required for detecting therapeutic levels of many basic and neutral drugs (7,8). To determine whether n-butyl chloride extraction would provide a comprehensive screen of suitable sensitivity for forensic investigations, nearly 200 drugs of forensic significance were selected for the present study. This list includes many of the drugs most frequently prescribed in the province of Saskatchewan[2]. For several of the selected drugs,

[2]Saskatchewan Prescription Drug Plan, Unpublished Data.

[1] Toxicology Section, Forensic Laboratory Regina, R.C.M. Police, Box 6500, Regina, Saskatchewan. S4P 3J7

extractability in a toxicologic screen has not been previously reported.

Gas chromatography is the most widely used analytical tool in toxicologic screening for the presence of drugs. When coupled with nitrogen-specific detection, high sensitivity and reasonable specificity have been achieved with conventional packed glass columns. However, technological advances have provided capillary columns which are more inert and offer increased sensitivity and separation power (10-12). Capillary GC has been used to analyze a wide range of drugs and chemicals and has allowed separations which were not previously possible. Recently, there has been increasing interest in Megabore capillary columns ("Megabore" is a registered trademark of J & W Scientific, Inc.). These wide bore columns (I.D. 0.30-0.70mm) have a greatly enhanced sample-handling capacity. Megabore columns accommodate non-split injections and therefore may be directly substituted for a packed column following installation of an injection port adapter. Since an injection port inlet splitter is not required, conversion to Megabore is far less expensive than conversion to narrow bore capillary columns (I.D. 0.20-0.30mm). An evaluation of Megabore columns in screening for drugs has not previously been reported in the literature.

The present study is divided into two parts: firstly, an evaluation of a screening procedure, comparing Megabore and packed column GC; and secondly, a thorough investigation of extraction recovery for a selected group of drugs.

## EXPERIMENTAL

### Materials

Absolute ethanol (Consolidated Alcohols Ltd.), n-butyl chloride (BDH Chemicals) and ammonium hydroxide (Fisher Scientific Ltd.) were reagent grade. Outdated infusion blood (which contained caffeine) or caffeine-free blood from volunteers was used in this study. Glass tubes with teflon-lined screw caps (13 x 100mm, Canlab Ltd.) were used for extractions. The tubes were cleaned with 2% Decon 75 Concentrate® (BDH Chemicals) then rinsed with distilled water. Disposable glass tubes (12 x 75mm, Canlab Ltd.) were used in collection of the extracting solvent and subsequent storage of extraction residue.

### Standard Solutions

Drug standards were obtained and reference standards were prepared at a concentration of 1 milligram per millilitre of ethanol (calculated as the free base). In cases where the salts of standards were not readily soluble in ethanol, the free base was prepared (chloroform extraction of basic aqueous solution). Working standards were prepared by diluting reference standards (above) to 10 micrograms per millilitre of ethanol. Drug standards were stored at 4° C.

For extraction, drugs were divided into groups according to GC retention times. Extraction groups were composed of three to ten drugs. Each drug standard was injected separately to check for interfering peaks. Standards, and consequently GC retention times, were verified by GC-mass spectrometry (Finnigan 9500 GC coupled with a Finnigan 3200 MS and an Incos 2300 data system). Retention times on Megabore GC were corrected to 10.95 min for amitriptyline. Retention indices (RI) were not calculated because the hydrocarbon standards are not detected by NPD (Nitrogen Phosphorus Detector) under normal conditions.

### Extraction Procedure

The general procedure for extracting basic and neutral drugs from blood is

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outlined in Figure 1. Prazepam was used as an internal standard to check reproducibility and extraction recoveries. 200 nanograms of internal standard was added to screw-capped tubes and evaporated under a gentle flow of $N_2$. One millilitre of blood was added to each tube. The contents were mixed (Vortex®) for 5 seconds. 200 microlitres of ammonium hydroxide was added to the blood and mixed for 5 seconds. The mixture was extracted with 5 millilitres of n-butyl chloride by shaking for 10 minutes on a horizontal shaker and centrifuging for 3 minutes (to clarify the solvent layer). The organic layer was transferred to disposable glass tubes and evaporated at room temperature (under $N_2$). The residue was redissolved in 40 microlitres of ethanol immediately prior to injecting a 1 to 2 microlitre aliquot into the GC.

Drugs were extracted in duplicate for the screening procedure and in triplicate for the extraction recovery, according to the general procedure (Figure 1). 200 nanograms of each standard was added to the screw-capped tubes and the solvent was evaporated ($N_2$) prior to adding 1 millilitre of blood. For three drugs with high therapeutic blood levels, working standards and the internal standard were adjusted such that 2 micrograms of drug was extracted. To determine extraction recovery, appropriate drug standards were added to glass tubes (in duplicate) and pooled with an extract of 1 millilitre of drug-free blood. This mixture was evaporated and subsequently redissolved in 40 microlitres of ethanol, as described above. Percentage recovery was determined by comparison of the extracted standards with the unextracted standards. In the screening procedure, packed column or Megabore capillary GC was used for analysis; in the extraction recovery, exclusively Megabore GC was used. To determine extraction recovery for prazepam, the internal standard chosen was diazepam. To ensure the absence of drugs and interfering peaks in the infusion blood,

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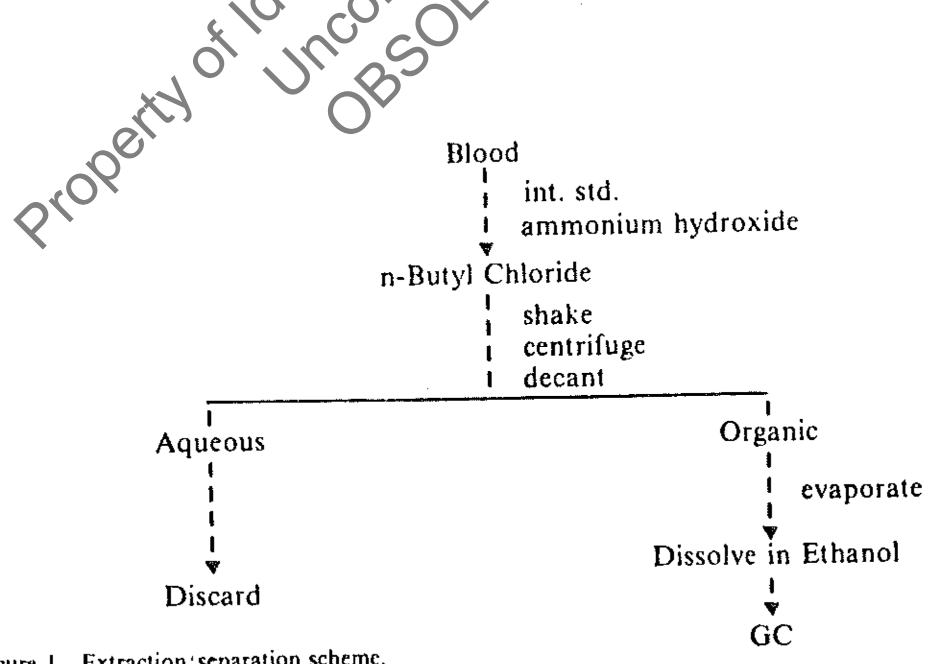


Figure 1 Extraction/separation scheme.

a 1 millilitre sample was extracted and analyzed on both GC columns.

### Instrumentation

Two Hewlett-Packard Model 5710A gas chromatographs equipped with nitrogen-phosphorus specific detectors were used in this study. The first was fitted with a 1.8m × 2mm I.D. glass column containing 3% OV-101 on 80/100 Gas Chrom Q (Chromatographic Specialties Ltd.). The oven temperature was programmed from 150°C to 290°C at 8°/min and held at the final temperature for 16 min. The second GC was fitted with a 10m Megabore column (fused silica, 0.53mm I.D., Hewlett-Packard Canada Ltd.) coated with a 1.5 μm film of methyl silicone (crosslinked). The oven temperature was programmed from 115° to 290°C at 8°/min and held at the final temperature for 8 min. For both instruments, helium carrier gas flow was 20 mL/min. The flow rates for detector gases were 3 mL/min for hydrogen and 70 mL/min for air. Injector and detector temperatures were maintained at 250° and 300°C, respectively. Total analysis time was approximately 30 minutes. Peak areas were determined using Hewlett-Packard Model 3385A and 3380A integrators.

## RESULTS AND DISCUSSION

The present study demonstrated the efficiency of n-butyl chloride as an extraction solvent for a wider range of basic and neutral drugs and metabolites than has been reported previously (2,4,6-9). Tables 1 and 2 list drugs with extraction recoveries exceeding 25% (see Tables for exceptions). Retention data for both packed and Megabore columns are presented to accommodate laboratories that use either column. The temperature program for the Megabore column was designed to provide drug retention times that paralleled those for the packed column. Megabore GC retention times were reproducible over the six month study period. However, over a period of one year, drugs with retention times less than 4 minutes or greater than 16 minutes were found to shift slightly (less than 10%).

Extraction recoveries for a selected group of drugs are presented in Table 3. For all drugs, recoveries were reproducible (variation within triplicate samples was generally less than 10%). In addition, recovery of the internal standard, prazepam, was consistent over the course of the study.

Extraction recovery of many drugs was dependent on the temperature used to evaporate the extraction solvent. The following drugs were susceptible to volatilization in the presence of heat and were not recovered unless the extraction solvent was evaporated at room temperature: diphenhydramine, ephedrine, fenfluramine, mephentermine, methoxyphenamine, N-methylphenethylamine, phentermine, phenylpropanolamine and 3, 4-MDA.

As indicated in the literature, the Megabore capillary column offered superior inertness in comparison with the packed column. For example, on the packed column, broad, tailing peaks and poor sensitivity for certain drugs prevented detection of the extracts by packed column GC. However, these drug standards and extracts chromatographed well on the Megabore column and were readily detected. This phenomenon was observed for the following drugs: antazoline, bisacodyl, chloroquine, cyproheptadine, diethazine, pericyazine, phenylpropanolamine, nortriptyline, tetrahydrozoline, thioproperazine and xylometazoline. Chromatography was improved on the Megabore column and provided lower detection limits in comparison with packed column GC. The degree of improvement varied from drug to drug.

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Certain drugs exhibited two peaks when chromatographed on the Megabore column (Tables 1-3). However, with the exception of ergotamine, only one of the components chromatographed on the packed column. The multiple peaks were investigated by GC-MS. For chlordiazepoxide and physostigmine, the second GC peaks gave spectra consistent with the intact drugs whereas the first GC peaks appeared to be decomposition products. The reverse was apparent for ethopropazine; the early GC peak was consistent with the intact drug. For ergotamine and mazindol, both GC peaks appeared to be decomposition products. Chlorprothixine and thiothixine both yielded minor, late-eluting peaks which exhibited spectral features similar to the first peaks. For phenelzine, both GC peaks appeared to be the result of consolidation reactions (spectral ions had greater molecular weights than the intact drug).

The present extraction method, coupled with Megabore GC and nitrogen-specific detection, provided detection limits well below 200 nanograms per millilitre of blood for most drugs (e.g. approximately 20 ng/mL for diazepam). This is a significant improvement over detection limits previously reported for packed columns (2,7,8). Figure 2 shows a chromatogram for a group of drugs extracted from

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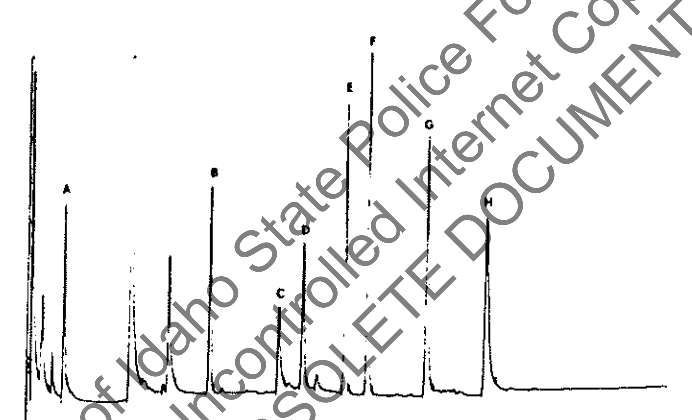


Figure 2 Megabore capillary gas chromatogram of a blood sample spiked with eight drugs (200 ng/ml). Drugs and retention times (min.) are: A, phenylpropanolamine, 1.68; B, orphenadrine, 8.19; C, pindolol, 11.22; D, maprotiline, 12.30; E, methotrimeprazine, 14.19; F, prazepam (internal standard), 15.18; G, haloperidol, 17.68; H, pericyazine, 20.21.

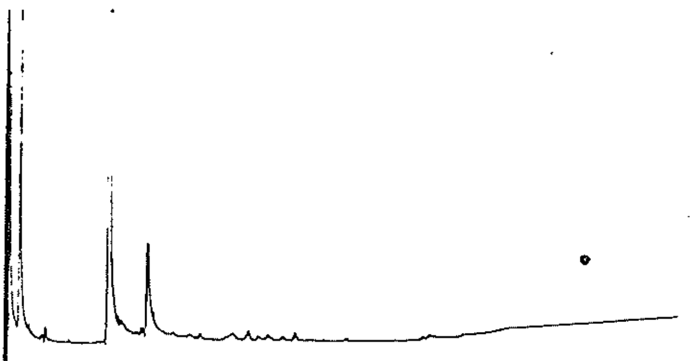

Figure 3 Megabore capillary gas chromatogram of a drug-free sample.

blood and analyzed by Megabore GC. For the drugs investigated, chromatograms were free from interfering peaks. Figure 3 shows a chromatogram for an extract of drug-free blood. Samples also were checked for blood artifact interference by comparing chromatograms of the unextracted standards (pooled with an extract of drug-free blood) with chromatograms of the pure drug standards.

Table 4 presents a list of drugs which were not detected at a concentration of 200 nanograms per millilitre of blood. Many of these drugs may be extracted but were not detected because of a poor chromatographic response (chromatography on the Megabore column was improved in comparison with the packed column). Certain drugs may be detected at blood concentrations above 200 nanograms per millilitre because chromatography improved with an increased quantity of drug. For example, a 50 nanogram injection of piperacetazine produced a peak at 22.62 min. Furthermore, piperacetazine is readily detected at blood concentrations exceeding 500 nanograms per millilitre.

The procedure described in this paper enables the analyst to screen a sample of blood for the presence of more than 170 basic and neutral drugs in approximately one hour. This method is applicable to toxicologic screening at therapeutic levels for many of the drugs. The addition of an internal standard such as nimetazepam may provide an indication of drug concentration and facilitates comparison among samples. The present method has compared favorably with other screening procedures. For example, there is a substantial reduction in time required for sample preparation in comparison with methods for screening liver tissue.

The n-butyl chloride extraction procedure is extremely versatile. Extracts may be analyzed using electron-capture detection, flame-ionization or GC-mass spectrometry. For example, using electron-capture detection (Megabore GC) triazolam is readily detected at 5 nanograms per millilitre of blood. The present method has been employed successfully for quantitation of therapeutic levels of several drugs. Examples are: amitriptyline, chlorpheniramine, chlorpromazine, cocaine, dextromethorphan, diazepam, diphenhydramine, propoxyphene, and verapamil. Lorazepam, oxazepam, and triazolam have been quantitated using ECD.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Stoner, R.E. and Parker, C. (1974). Single-pH Extraction Procedure for Detecting Drugs of Abuse. *Clin. Chem.* 20: 309-311.

2. Foerster, E.H. and Mason, M.F. (1974). Preliminary Studies on the Use of n-Butyl Chloride as an Extractant in a Drug Screening Procedure. *J. Forensic Sci.* 19:155-161.

3. Horning, M.G., Gregory, P., Nowlin, J., Stafford, M., Letratanangkoon, K., Butler, C., Stillwell, W.G., and Hill, R.M. (1974). Isolation of Drugs and Drug Metabolites from Biological Fluids by Use of Salt-Solvent Pairs. *Clin. Chem.* 20:282-287.

4. Verebely, K. and Inturrisi, C.E. (1973). The Simultaneous Determination of Propoxyphene and Norpropoxyphene in Human Biofluids Using Gas-Liquid Chromatography. *J. Chromatogr.* 75: 195-205.

5. Curry, S.H. (1976). Gas Chromatographic Methods for the Study of Chlorpromazine and Some of its Metabolites in Human Plasma. *Psychopharmacol. Commun.* 2:1-15.

6. Wolen, B.L. and Gruber, C.M. (1968). Determination of Propoxyphene in Human Plasma by Gas Chromatography. *Anal. Chem.* 40:1243-1246.

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7. Pierce, W.O., Lamoreaux, T.C., Urry, F.M., Kopjak, L. and Finkle, B.S. (1978). A New, Rapid Gas Chromatography Method for the Detection of Basic Drugs in Postmortem Blood, Using a Nitrogen Phosphorus Detector. Part I. Qualitative Analysis. *J. Anal. Toxicol.* 2:26-31.

8. Kopjak, L., Finkle, B.S., Lamoreaux, T.C., Pierce, W.O. and Urry, F.M. (1979). A New, Rapid Gas Chromatography Method for the Detection of Basic Drugs in Postmortem Blood Using a Nitrogen Phosphorus Detector. Part II. Quantitative Analysis. *J. Anal. Toxicol.* 3:155-157.

9. Sharp, M.E., Wallace, S.M., Hindmarsh, K.W. and Peel, H.W. (1983). Monitoring Saliva Concentrations of Methaqualone, Codeine, Secobarbital, Diphenhydramine and Diazepam After Single Oral Doses. *J. Anal. Toxicol.* 7:11-14.

10. Freeman, R.R. (Ed.) (1981). High Resolution Gas Chromatography. 2nd Ed. *Hewlett-Packard Company.*

11. Novak, J. and Roth, M. (1984). Sensitivity of Gas Chromatographic Analysis on Packed and Capillary Columns With Different Types of Detector Under Resolution-Normalized Conditions. *J. Chromatogr.* 292:149-157.

12. Plotczyk, L.L. (1982). Application of Fused-Silica Capillary Gas Chromatography to the Analysis of Underivatized Drugs. *J. Chromatogr.* 240: 349-360.

13. Caddy, B., Kidd, C.B.M. and Leung, S.C. (1982). Identification of Drugs Using Capillary Gas Liquid Chromatography. *J. Forens. Sci. Soc.* 22:3-31.

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**Table 1**

**BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY***

**Listed in Alphabetical Order**

| Drug | Retention Time** (min) | |
|---|---|---|
| | Megabore Column | Packed Column |
| Acepromazine | 15.75 | 15.7 |
| Alphaprodine | 06.60 | 06.7 |
| Aminopromazine | 13.35 | 13.4 |
| Amitriptyline | 10.95 | 11.0 |
| Amoxapine | 14.94 | 15.0 |
| Anileridine | 17.46 | 17.1 |
| Antazoline | 11.97 | 12.0 |
| Atropine | 10.77 | 10.9 |
| Azapetine | 08.07 | 08.2 |
| Benactyzine | 11.68 | 11.7 |
| Benztropine | 12.20 | 12.2 |
| Biperiden | 11.74 | 11.8 |
| Bisacodyl | 16.88 | 16.8 |
| Bromazine | 10.53 | 10.5 |
| Brompheniramine | 09.93 | 09.9 |
| Bupivacaine | 11.85 | 11.9 |
| Butacaine | 13.54 | 13.5 |
| Butylaminobenzoate | 05.99 | 06.1 |
| Caramiphen | 08.68 | 08.6 |
| Chlophedianol | 09.81 | 09.9 |
| Chlorcyclizine | 11.25 | 11.3 |
| Chlordiazepoxide | 13.85 | 13.8 |
| Chlormezanone*** | 10.80 | 10.7 |
| Chloroquine | 14.95 | 15.1 |
| Chlorpheniramine | 08.90 | 08.9 |
| Chlorphenoxamine | 09.94 | 09.9 |
| Chlorpromazine | 13.81 | 13.9 |
| Chlorprothixene | 13.84, 14.11 | 13.9 |
| Clemastine | 13.28 | 13.3 |
| Clomipramine | 13.11 | 13.1 |
| Clorprenaline | 04.04 | 04.3 |
| Cocaine | 10.91 | 11.0 |
| Codeine | 12.51 | 12.7 |
| Cotinine | 04.85 | 05.2 |
| Cyclobenzaprine | 11.36 | 11.4 |
| Cycrimine | 10.19 | 10.4 |
| Cyproheptadine | 12.49 | 12.5 |

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## BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY*

| Drug | Retention Time** (min) Megabore Column | Retention Time** (min) Packed Column |
|---|---|---|
| Desipramine | 11.36 | 11.4 |
| Desmethyldiazepam | 13.68 | 13.8 |
| Dextroamphetamine | 00.68 | 01.3 |
| Dextromethorphan | 10.26 | 10.3 |
| Diazepam | 13.14 | 13.3 |
| Dibucaine | 15.86 | 15.9 |
| Dicyclomine | 10.04 | 10.1 |
| Diethazine | 12.75 | 12.8 |
| Diethylpropion† | 03.24 | 03.5 |
| Dihexyverine | 12.15 | 12.2 |
| Dimethindene | 11.82 | 11.9 |
| Dimethothiazine | 18.70 | 18.9 |
| Diphenhydramine | 07.37 | 07.4 |
| Diphenidol | 12.86 | 12.9 |
| Diphenoxylate† | 21.65 | 24.3 |
| Diphenylpyraline | 09.81 | 09.9 |
| Disopyramide*** | 13.99 | 14.1 |
| Doxapram | 17.33 | 17.3 |
| Doxepin | 11.26 | 11.3 |
| Ephedrine | 02.05 | 02.6 |
| Ergotamine | 12.27, 11.76 | 12.3, 11.5 |
| Ethoheptazine | 07.20 | 07.3 |
| Ethopropazine† | 16.01, 12.69 | 12.7 |
| Ethylmorphine | 12.99 | 13.0 |
| Fenfluramine | 01.27 | 01.8 |
| Fluopromazine | 11.29 | 11.3 |
| Flupentixol | 18.75 | — |
| Fluphenazine | 23.76 | — |
| Flurazepam | 16.42 | 16.4 |
| Haloperidol | 17.68 | 17.7 |
| Hydrocodone | 13.53 | 13.3 |
| Hydroxyzine | 17.26 | 17.2 |
| Imipramine | 11.22 | 11.2 |
| Ketamine | 06.93 | 07.2 |
| Levallorphan | 12.42 | 12.5 |
| Levorphan | 11.04 | 11.1 |
| Lidocaine | 07.42 | 07.4 |
| Loperamide | 24.88 | — |
| Lorazepam | 12.90 | 12.9 |

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## BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY*

| Drug | Retention Time** (min) Megabore Column | Packed Column |
|---|---|---|
| Maprotiline | 12.30 | 12.3 |
| Mazindol | 12.67, 12.25 | 12.3 |
| Meclizine† | 18.58 | 18.7 |
| Meperidine | 05.97 | 06.1 |
| Mephentermine | 01.35 | 01.9 |
| Mepivacaine | 09.45 | 09.5 |
| Mesoridazine | 21.03 | 23.2 |
| Methadone | 10.48 | 10.5 |
| Methamphetamine | 00.99 | 01.5 |
| Methapyrilene | 08.61 | 08.8 |
| Methaqualone | 10.24 | 10.3 |
| Methotrimeprazine | 14.19 | 14.3 |
| Methoxamine | 05.70 | 05.9 |
| Methoxyphenamine | 02.17 | 02.7 |
| Methylnicotinate | 01.07 | 01.5 |
| Methylphenidate | 05.70 | 05.9 |
| Metoclopramide | 15.00 | 15.0 |
| Metoprolol | 09.21 | 09.2 |
| Metyrapone | 07.23 | 07.4 |
| Nitrazepam | 16.83, 16.13 | 16.3 |
| Normethadone | 10.01 | 10.0 |
| Norpropoxyphene | 13.05 | 13.1 |
| Nortriptyline | 11.05 | 11.1 |
| N-methylphenethylamine | 00.84 | 01.3 |
| Nylidrin | 12.31 | 12.3 |
| Orphenadrine | 08.19 | — |
| Oxazepam | 12.10 | 12.1 |
| Oxycodone | 13.82 | 14.0 |
| Oxyprenolol | 07.79 | 07.8 |
| Pargyline | 01.21 | 01.6 |
| Pentazocine | 11.78 | 11.8 |
| Pericyazine | 20.21 | 21.3 |
| Perphenazine | 21.80 | 23.0 |
| Phenacaine | 14.62 | 14.6 |
| Phenazopyridine | 11.54 | 11.6 |
| Phencyclidine | 07.60 | 07.7 |
| Phendimetrazine | 02.91 | 03.3 |
| Phenelzine | 10.76, 03.97 | — |
| Pheniramine | 06.64 | 06.8 |
| Phenmetrazine | 02.65 | 03.2 |

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## BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY*

| Drug | Retention Time** (min) Megabore Column | Packed Column |
|---|---|---|
| Phentermine | 00.87 | 01.6 |
| Phenoxybenzamine | 11.30 | 11.4 |
| Phenylpropanolamine | 01.68 | 02.3 |
| Phenyltoloxamine | 08.23 | 08.3 |
| Physostigmine | 11.13 | 06.8 |
| Pindolol | 11.22 | 11.3 |
| Pizotyline | 12.60 | 12.7 |
| Pramoxine | 11.81 | 11.8 |
| Prazepam | 15.18 | 15.2 |
| Prenylamine | 14.51 | 14.5 |
| Prilocaine | 06.93 | 07.0 |
| Procainamide***† | 11.06 | 11.2 |
| Procaine | 08.99 | 09.0 |
| Prochlorperazine | 17.93 | 18.0 |
| Procyclidine | 10.67 | 10.8 |
| Promazine | 12.13 | 12.2 |
| Promethazine | 11.67 | 11.8 |
| Proparacaine | 12.44 | 12.4 |
| Propoxyphene | 10.94 | 10.9 |
| Propranolol | 10.42 | 10.5 |
| Propylhexedrine | 00.99 | 01.5 |
| Protriptyline | 11.42 | 11.5 |
| Pseudoephedrine | 02.08 | 02.6 |
| Pyrilamine | 11.35 | 11.4 |
| Pyrrobutamine | 13.24 | 13.3 |
| Quinidine | 16.49 | 16.5 |
| Quinine | 16.46 | 16.5 |
| Scopolamine | 11.93 | 12.1 |
| Strychnine | 18.97 | 19.7 |
| Tetrahydrozoline | 06.58 | 06.7 |
| Thioproperazine | 22.83 | 33.1 |
| Thioridazine | 19.16 | 19.6 |
| Thiothixene | 23.15, 22.69 | — |
| Thonzylamine | 11.02 | 11.0 |
| Timolol | 11.83 | 11.9 |
| Tranylcypromine | 01.14 | 01.7 |
| Trazodone | 20.64 | — |
| Triazolam | 18.30 | 18.6 |
| Trifluoperazine | 15.56 | 14.8 |

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## BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY*

| Drug | Retention Time** (min) Megabore Column | Packed Column |
|---|---|---|
| Trihexyphenidyl | 11.43 | 11.5 |
| Trimeprazine | 11.96 | 12.0 |
| Trimethobenzamide | 19.75 | 21.0 |
| Trimipramine | 11.23 | 11.2 |
| Tripelennamine | 08.63 | 08.7 |
| Triprolidine | 11.56 | 11.6 |
| Verapamil | 19.74 | 20.3 |
| Xylometazoline | 08.24 | — |
| 3, 4-MDA | 02.94 | 03.2 |

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* Blood spiked at 200ng/mL unless otherwise indicated. Analyzed on packed or Megabore column. Extraction recovery greater than 25% unless otherwise indicated (see Table III for extraction recovery of selected drugs).

** More than one number indicates multiple peaks.

*** Blood spiked at 2.0ug/mL.

† Extraction recovery less than 25%.

Table 2

BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY*

Listed in Order of Retention Time

| Drug | Retention Time** (min) Megabore Column | Packed Column |
|---|---|---|
| Dextroamphetamine | 00.68 | 01.3 |
| N-methylphenethylamine | 00.84 | 01.3 |
| Phentermine | 00.87 | 01.6 |
| Propylhexedrine | 00.99 | 01.5 |
| Methamphetamine | 00.99 | 01.5 |
| Methylnicotinate | 01.07 | 01.5 |
| Tranylcypromine | 01.14 | 01.7 |
| Pargyline | 01.21 | 01.6 |
| Fenfluramine | 01.27 | 01.8 |
| Mephentermine | 01.35 | 01.9 |
| Phenylpropanolamine | 01.68 | 02.3 |
| Ephedrine | 02.05 | 02.6 |
| Pseudoephedrine | 02.08 | 02.6 |
| Methoxyphenamine | 02.17 | 02.7 |
| Phenmetrazine | 02.65 | 03.2 |
| Phendimetrazine | 02.91 | 03.3 |
| 3,4-MDA | 02.94 | 03.2 |
| Diethylpropion† | 03.24 | 03.5 |
| Clorprenaline | 04.04 | 04.3 |
| Cotinine | 04.85 | 05.2 |
| Methoxamine | 05.70 | 05.9 |
| Methylphenidate | 05.70 | 05.9 |
| Meperidine | 05.97 | 06.1 |
| Butylaminobenzoate | 05.99 | 06.1 |
| Tetrahydrozoline | 06.58 | 06.7 |
| Alphaprodine | 06.60 | 06.7 |
| Pheniramine | 06.64 | 06.8 |
| Prilocaine | 06.93 | 07.0 |
| Ketamine | 06.93 | 07.2 |
| Ethoheptazine | 07.20 | 07.3 |
| Metyrapone | 07.23 | 07.4 |
| Diphenhydramine | 07.37 | 07.4 |
| Lidocaine | 07.42 | 07.4 |
| Phencyclidine | 07.60 | 07.7 |
| Oxyprenolol | 07.79 | 07.8 |
| Azapetine | 08.07 | 08.2 |
| Orphenadrine | 08.19 | — |
| Phenyltoloxamine | 08.23 | 08.3 |

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## BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY*

| Drug | Retention Time** (min) Megabore Column | Packed Column |
|---|---|---|
| Xylometazoline | 08.24 | — |
| Methapyrilene | 08.61 | 08.8 |
| Tripelennamine | 08.63 | 08.7 |
| Caramiphen | 08.68 | 08.6 |
| Chlorpheniramine | 08.90 | 08.9 |
| Procaine | 08.99 | 09.0 |
| Metoprolol | 09.21 | 09.2 |
| Mepivacaine | 09.45 | 09.5 |
| Diphenylpyraline | 09.81 | 09.9 |
| Chlophedianol | 09.81 | 09.9 |
| Brompheniramine | 09.93 | 09.9 |
| Chlorphenoxamine | 09.94 | 09.9 |
| Normethadone | 10.01 | 10.0 |
| Dicyclomine | 10.04 | 10.1 |
| Cycrimine | 10.19 | 10.4 |
| Methaqualone | 10.24 | 10.3 |
| Dextromethorphan | 10.26 | 10.3 |
| Propranolol | 10.42 | 10.5 |
| Methadone | 10.48 | 10.5 |
| Bromazine | 10.53 | 10.5 |
| Procyclidine | 10.67 | 10.8 |
| Phenelzine | 10.76, 03.97 | — |
| Atropine | 10.77 | 10.9 |
| Chlormezanone*** | 10.80 | 10.7 |
| Cocaine | 10.91 | 11.0 |
| Propoxyphene | 10.94 | 10.9 |
| Amitriptyline | 10.95 | 11.0 |
| Thonzylamine | 11.02 | 11.0 |
| Levorphan | 11.04 | 11.1 |
| Nortriptyline | 11.05 | 11.1 |
| Procainamide***† | 11.06 | 11.2 |
| Physostigmine | 11.13 | 06.8 |
| Pindolol | 11.22 | 11.3 |
| Imipramine | 11.22 | 11.2 |
| Trimipramine | 11.23 | 11.2 |
| Chlorcyclizine | 11.25 | 11.3 |
| Doxepin | 11.26 | 11.3 |
| Fluopromazine | 11.29 | 11.3 |
| Phenoxybenzamine | 11.30 | 11.4 |

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## BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY*

| Drug | Retention Time** (min) Megabore Column | Packed Column |
|---|---|---|
| Pyrilamine | 11.35 | 11.4 |
| Cyclobenzaprine | 11.36 | 11.4 |
| Desipramine | 11.36 | 11.4 |
| Protriptyline | 11.42 | 11.5 |
| Trihexyphenidyl | 11.43 | 11.5 |
| Phenazopyridine | 11.54 | 11.6 |
| Triprolidine | 11.56 | 11.6 |
| Promethazine | 11.67 | 11.8 |
| Benactyzine | 11.68 | 11.7 |
| Biperiden | 11.74 | 11.8 |
| Pentazocine | 11.78 | 11.8 |
| Pramoxine | 11.81 | 11.8 |
| Dimethindene | 11.82 | 11.9 |
| Timolol | 11.83 | 11.9 |
| Bupivacaine | 11.85 | 11.9 |
| Scopolamine | 11.93 | 12.1 |
| Trimeprazine | 11.96 | 12.0 |
| Antazoline | 11.97 | 12.0 |
| Oxazepam | 12.10 | 12.1 |
| Promazine | 12.13 | 12.2 |
| Dihexyverine | 12.15 | 12.2 |
| Benztropine | 12.20 | 12.2 |
| Ergotamine | 12.27, 11.76 | 12.3, 11.5 |
| Maprotiline | 12.30 | 12.3 |
| Nylidrin | 12.31 | 12.3 |
| Levallorphan | 12.42 | 12.5 |
| Proparacaine | 12.44 | 12.4 |
| Cyproheptadine | 12.49 | 12.5 |
| Codeine | 12.51 | 12.7 |
| Pizotyline | 12.60 | 12.7 |
| Mazindol | 12.67, 12.25 | 12.3 |
| Diethazine | 12.75 | 12.8 |
| Diphenidol | 12.86 | 12.9 |
| Lorazepam | 12.90 | 12.9 |
| Ethylmorphine | 12.99 | 13.0 |
| Norpropoxyphene | 13.05 | 13.1 |
| Clomipramine | 13.11 | 13.1 |
| Diazepam | 13.14 | 13.3 |
| Pyrrobutamine | 13.24 | 13.3 |

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## BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY*

| Drug | Retention Time** (min) Megabore Column | Packed Column |
|---|---|---|
| Clemastine | 13.28 | 13.3 |
| Aminopromazine | 13.35 | 13.4 |
| Hydrocodone | 13.53 | 13.3 |
| Butacaine | 13.54 | 13.5 |
| Desmethyldiazepam | 13.68 | 13.8 |
| Chlorpromazine | 13.81 | 13.9 |
| Oxycodone | 13.82 | 14.0 |
| Chlorprothixene | 13.84, 14.11 | 13.9 |
| Chlordiazepoxide | 13.85 | 13.8 |
| Disopyramide*** | 13.99 | 14.1 |
| Methotrimeprazine | 14.19 | 14.3 |
| Prenylamine | 14.51 | 14.5 |
| Phenacaine | 14.62 | 14.6 |
| Amoxapine | 14.94 | 15.0 |
| Chloroquine | 14.95 | 15.1 |
| Metoclopramide | 15.00 | 15.0 |
| Prazepam | 15.18 | 15.2 |
| Trifluoperazine | 15.56 | 14.8 |
| Acepromazine | 15.75 | 15.7 |
| Dibucaine | 15.86 | 15.9 |
| Ethopropazine† | 16.01, 12.69 | 12.7 |
| Flurazepam | 16.42 | 16.4 |
| Quinine | 16.46 | 16.5 |
| Quinidine | 16.49 | 16.5 |
| Nitrazepam | 16.83, 16.13 | 16.3 |
| Bisacodyl | 16.88 | 16.8 |
| Hydroxyzine | 17.26 | 17.2 |
| Doxapram | 17.33 | 17.3 |
| Anileridine | 17.46 | 17.1 |
| Haloperidol | 17.68 | 17.7 |
| Prochlorperazine | 17.93 | 18.0 |
| Triazolam | 18.30 | 18.6 |
| Meclizine† | 18.58 | 18.7 |
| Dimethothiazine | 18.70 | 18.9 |
| Flupentixol | 18.75 | — |
| Strychnine | 18.97 | 19.7 |
| Thioridazine | 19.16 | — |
| Verapamil | 19.74 | 20.3 |
| Trimethobenzamide | 19.75 | 21.0 |

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## BASIC AND NEUTRAL DRUGS DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND GAS CHROMATOGRAPHY*

| Drug | Retention Time** (min) Megabore Column | Packed Column |
|---|---|---|
| Pericyazine | 20.21 | 21.3 |
| Trazodone | 20.64 | — |
| Mesoridazine | 21.03 | 23.2 |
| Diphenoxylate† | 21.65 | 24.3 |
| Perphenazine | 21.80 | 23.0 |
| Thioproperazine | 22.83 | 33.1 |
| Thiothixene | 23.15, 22.69 | — |
| Fluphenazine | 23.76 | — |
| Loperamide | 24.88 | — |

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* Blood spiked at 200ng/mL unless otherwise indicated. Analyzed on packed or Megabore column. Extraction recovery greater than 25% unless otherwise indicated (see Table III for extraction recovery of selected drugs).

** More than one number indicates multiple peaks.

*** Blood spiked at 2.0ug/mL.

† Extraction recovery less than 25%.

**Table 3**

**RECOVERY OF BASIC AND NEUTRAL DRUGS EXTRACTED WITH N-BUTYL CHLORIDE AND ANALYZED BY MEGABORE GAS CHROMATOGRAPHY***

| Drug | Recovery (%) | Retention Time (min)** |
|---|---|---|
| Amoxapine | 52 | 14.94 |
| Anileridine | 57 | 17.46 |
| Chlormezanone*** | 66 | 10.80 |
| Chlorprothixene | 27 | 13.84, 14.11 |
| Diazepam | 64 | 13.14 |
| Dicyclomine | 33 | 10.04 |
| Diphenoxylate | 10 | 21.65 |
| Disopyramide*** | 73 | 13.99 |
| Ergotamine | 44 | 12.27, 11.76 |
| Ethopropazine | 8 | 16.01, 12.69 |
| Flupentixol | 72 | 18.75 |
| Haloperidol | 72 | 17.68 |
| Loperamide | 53 | 24.88 |
| Maprotiline | 54 | 12.30 |
| Meclizine | 5 | 18.58 |
| Meperidine | 69 | 05.97 |
| Methotrimeprazine | 30 | 14.19 |
| Metoprolol | 92 | 09.21 |
| Orphenadrine | 48 | 08.19 |
| Oxyprenolol | 92 | 07.79 |
| Pericyazine | 69 | 20.21 |
| Phenelzine | 106 | 10.76, 3.97 |
| Phentermine | 100 | 00.87 |
| Phenylpropanolamine | 100 | 01.68 |
| Pindolol | 66 | 11.22 |
| Pizotyline (Pizotifen) | 44 | 12.60 |
| Prazepam | 60 | 15.18 |
| Procainamide*** | 17 | 11.06 |
| Prochlorperazine | 46 | 17.93 |
| Promethazine | 39 | 11.67 |
| Protriptyline | 60 | 11.42 |
| Scopolamine (Hyoscine) | 81 | 11.93 |
| Thiothixene | 61 | 23.15, 22.69 |
| Timolol | 120 | 11.83 |
| Trazodone | 74 | 20.64 |
| Trifluoperazine | 41 | 15.58 |
| Trihexyphenidyl | 29 | 11.43 |
| Verapamil | 33 | 19.74 |
| Xylometazoline | 79 | 08.24 |

* Blood spiked at 200ng/mL unless otherwise indicated.
** More than one number indicates multiple peaks.
*** Blood spiked at 2.0ug/mL.

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**Table 4**

**DRUGS NOT DETECTED BY N-BUTYL CHLORIDE EXTRACTION AND MEGABORE GAS CHROMATOGRAPHY**

| Drug | Retention Time (min) |
|---|---|
| Drugs not extracted | |
| Clomiphene | 17.74 |
| Hydroxyamphetamine | 02.50 |
| Drugs which have a poor chromatographic response* | |
| Cotarnine | 06.45 |
| Hydralazine | 09.01, 10.25 |
| Hydromorphone | 13.30 |
| Nadolol | 14.37 |
| Nalorphine | 14.58 |
| Naphazoline | 08.90 |
| Nifedipine | 14.45 |
| Oxymetazoline | 10.51 |
| Tolazoline | 03.31 |
| Drugs which do not chromatograph** | |
| Amiloride | |
| Bethanidine | |
| Cimetidine | |
| Dipyridamole | |
| Fenoterol | |
| Phenformin | |
| Pimozide | |
| Piperacetazine | |
| Prazocin | |
| Rescinnamine | |
| Reserpine | |
| Salbutamol | |
| Triamterene | |

* Not detected in blood spiked at 200ng/mL; 5ng injection of standard was detected.
** No peaks before 30 min. 5ng injection of standard.

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## General Guidelines for Identification of a Controlled Substance

One goal of controlled substance analysis is to perform a series of tests or a single test that absolutely verifies the presence of a specific controlled substance.

Here are criteria for identifying a substance with reasonable scientific certainty:

Category I (Infrared spectra generated). The infrared spectra of a substance is dependant upon the arrangement of atoms in the molecule. Minor variations in the molecular configuration or elements present results in substantial variations in the infrared spectra. Since infrared spectra are extremely complex, an identification based only on an infrared spectra meets the criteria of identifying a substance with reasonable scientific certainty. This is true only when the standard scan matches the sample scan. As here defined, matches implies that the absorption bands are located at the same wavelengths and that the absorption bands in the standard and sample have the same relative intensity.

Category II (No infrared spectra or a poor infrared spectra) In some situations, it will not be possible to obtain infrared spectra, or the infrared spectra is obtained is inadequate. In those situations, a combination of identification procedures must be followed. Relative specificity of typically used identification procedures are as follows (usually):

More Specific ↑

- Infrared spectra
- Gas chromatography, microcrystalline test
- Microscopic examination for marihuana
- Thin layer chromatography
- Modified Duquenois
- Ultraviolet spectroscopy
- Presumptive tests, PDR comparison, or look-alike list

↓ Less Specific

Here are various combinations of these techniques that typically would produce identifications with reasonable scientific certainty:

1. Matching GLC, TLC, and microcrystalline test.
2. Matching 2 GLC systems and one microcrystalline test.
3. Matching 1 GLC system and 2 microcrystalline tests.
4. Matching 2 GLC systems and ether and 2 TLC systems, or 1 Toxilab.
5. Matching 1 GLC system and 3 TLC systems.

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6. Matching microscopic examination, Modified Duquenois, and TLC (marihuana).

7. Matching Modified Duquenois and 2 TLC systems (marihuana resin).

8. Matching 2 microcrystalline tests and 2 TLC systems.

9. Matching 3 TLC systems (DEA designed for LSD only). LSD confirmation.

There are other factors to be considered when making a decision about the specificity of a test, which can vary with each situation encountered. These factors will now be briefly discussed.

When performing gas chromatography, specificity will of course increase as retention time increases up to a point where specificity probably does not increase with increased retention time.

TLC spots lose most of their identification value when the spot stays within 10% of the origin or travels to within 90% of the distance traveled by the solvent front. The specificity of the visualizing reagent effects the identification value of TLC. Iodine vapor attaches to most organic compounds. Iodoplatinate spray reacts best with tertiary amines. PDAB sprays reacts to the indole structure to form a purple color. Therefore, of these 3 sprays, PDMAB is by far the most specific.

The identification value of microcrystalline tests varies with the unique shape of the crystals formed, and the experience of the criminalist. For instance, with gold chloride in phosphoric acid, several different compounds related to amphetamine give similar sword-shaped crystals. Obviously, these microcrystallines have low identification value. However, this same reagent produces rare 4-lobed microcrystals with a slight bluish birefringance, that have a great deal of identification value.

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CONTROLLED SUBSTANCE ANALYSIS
**WORKSHEET**

DATE: LAB NO:

SEALS: SUSPECT(S):

EVIDENCE DESCRIPTION:

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CONCLUSION:

RESERVE WEIGHT:

# DRUG WORKSHEET

ITEM NO: ____________________
CRIMINALIST ____________________
GROSS PACKAGE WEIGHT: ____________________

LAB. NO: ____________________

FINDINGS:

| SCREENING TESTS | REACTION | MICRO TESTS | DESCRIPTION |
|---|---|---|---|
| MARQUIS | | GOLD CHLORIDE | |
| MECKE | | GOLD BROMIDE | |
| FROEHDE | | OTHER | |
| P-DMAD | | | |
| DILLE-KOP | | | |
| COTHIO | | | |
| MAYERS | | | |
| NaOH/MeOH | | | |
| SANCHEZ | | | |
| OTHER | | | |
| OTHER | | | |

PDR:

CHROMATOGRAPHY:

TLC:

| | SYSTEM | Rf | UVvis | DEVELOP |
|---|---|---|---|---|
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

GC:

| COLUMN/TEMP | Rt/RRt | KOVAT INDEX | DERIV. REAGENT |
|---|---|---|---|
| | | | |

QUANTITATION:

UV:

QUANTITATION:

IR:

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PLANT MATERIAL ANALYSIS
WORKSHEET

DATE: LAB NO:

SEALS: SUSPECT(S):

EVIDENCE DESCRIPTION:

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CONCLUSION:

RESERVE WEIGHT:

# MARIHUANA WORKSHEET

ITEM NO.__________ LAB. NO.__________

CRIMINALIST__________

GROSS PACKAGE WEIGHT:__________

FINDINGS:

MICROSCOPIC EXAMINATION: CYST. HAIRS LG. UNICELL. HAIRS CHAR SEEDS OF MH

GERMINATION:

STARTED __________

SPROUTED__________

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| MODIFIED DUQUENOIS: | DUQ + HCl | AQUEOUS + $CHCl_3$ |
|---|---|---|
| BLK. | | |
| STD. | | |
| SAMPLE | | |

| THIN LAYER CHROMATOGRAPHY | orange (CBD) | scarlet THC) | purple (CBN) |
|---|---|---|---|
| BLK. | | | |
| STD. | | | |
| SAMPLE | | | |

In conclusion:

[illegible]

[illegible]

[illegible]

Or as stated by Farsi Rawalpindi:

Ama mimi, nami anshukuru kwa upande wangu;kidonda changu kilichonitaabisha dahari kabla ya kujq hospitali, lakini toka kupata malhamu hii ninayoitumia sasa, kidonda knakauka kama kidoa.` Matazame uyle kijana aliuyefungwa matambaa ya shingo. Unamwonal?j Kijana yule alikumwa heshi kuvbimga abingo kwa ndani na kutoka usaha. Kumeza mate, masikini alikuwa hawezi, licha ya kula. Lakini tangu aliponukizwa dawa madaktar, wakayaondosha maribi ayao huko shingoni lmwake, taabu zotye simempungua. Alalazwa hospitali siku chache tu, halafu akapesa ruhusa. Sas mzima.

Amebakia kusukutua dqwa tyu nyumbani, na kutazamwa na daktari kila wiki mara moja. Leo njani nalikutana na mtu mmoja, mwele wa menl Tukafuatana pamoja mpaka hapo, halafu tukapoteana. Sijui kend wapi.

Translated, both mean that it is all magic anyway.

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TABLE OF CONTENTS FOR ULTRAVIOLET SPECTROPHOTOMETRY


I. Introduction

II. Guidelines for Testing

III. References



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# INTRODUCTION

Ultraviolet spectophotometry (UV) is used in the crime laboratory mainly for quantitative analysis. In this capacity it can be used for virtually all of the drugs analyzed. It's use for qualitative analysis is limited, because chemicals of similar structure give spectra that are often indistinguishable.

Spectra are developed by passing UV light through a solution containing the unknown, and plotting the absorption or transmittance of this light as a function of wavelength. The theory of UV spectrophotometry is coverd in the following articles and the references. The analyst should study instrument manuals on use and care of particular instruments and their cuvettes.

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# "AN INTRODUCTION TO ULTRAVIOLET SPECTROPHOTOMETRY"

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By
Robert J. Manning

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Beckman Instruments, Inc.
Fullerton, Ca.

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# PREFACE

This little volume can be used as a textbook for self-study or for a short, formal course in spectrophotometry. It was written to be exactly what its title says, "An Introduction to Ultraviolet-Visible Spectrophotometry". It is hoped that it will convey to the uninitiated reader some basis for understanding the use of an ultraviolet spectrophotometer. Experience has shown that a person who understands how an instrument works and who understands the laws of light absorption will be able to get consistently better results from his use of the instrument. He will also be able more quickly to recognize maladjustments or misuse of an instrument than one whose knowledge is limited to step-by-step operating instructions.

Books on spectroscopy always start off with discussion of the electromagnetic spectrum, and this one is no exception. This is followed by a minimal explanation of the physical and chemical bases of analytical spectrophotometry along with a catalog of many of the ways in which these instruments are used. Quantitative analysis is treated in sufficient detail to enable the reader to understand and perform most single and multi-component analyses. The parts of a spectrophotometer and their functions are described and explained so that the operating controls and conditions can be chosen and set in a logical manner.

A number of laboratory experiments have been designed so that the reader who performs them will gain an understanding of spectrophotometry and some of the finer points of operation of modern spectrophotometers. Essentially no special equipment or accessories are needed, and the solvents and chemicals are very common and readily available in most chemical laboratories.

I want to thank my colleagues in the Applications Research Department of Beckman Instruments for their many helpful suggestions and criticisms. I am grateful to J. D. McCallum for design of two experiments, to L. G. Sims and K. M. Jeong for many of the curves and most of the laboratory work, and to Mrs. Dolly Monroe and Barbara Schiavo who were able to convert my longhand scribbles into final copy. Very special thanks go to Robert O. Brace for his invaluable assistance as editor, artist, and prodder. It was he who provided all of the illustrations and all of the layout work.

Fullerton, California R.J.M.

February 1970

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# 1.0 THE ELECTROMAGNETIC SPECTRUM

Ultraviolet and visible radiation is a manifestation of just one very small portion of the electromagnetic spectrum which includes other forms of radiation such as radio, radar, infrared, X-rays, and cosmic rays.

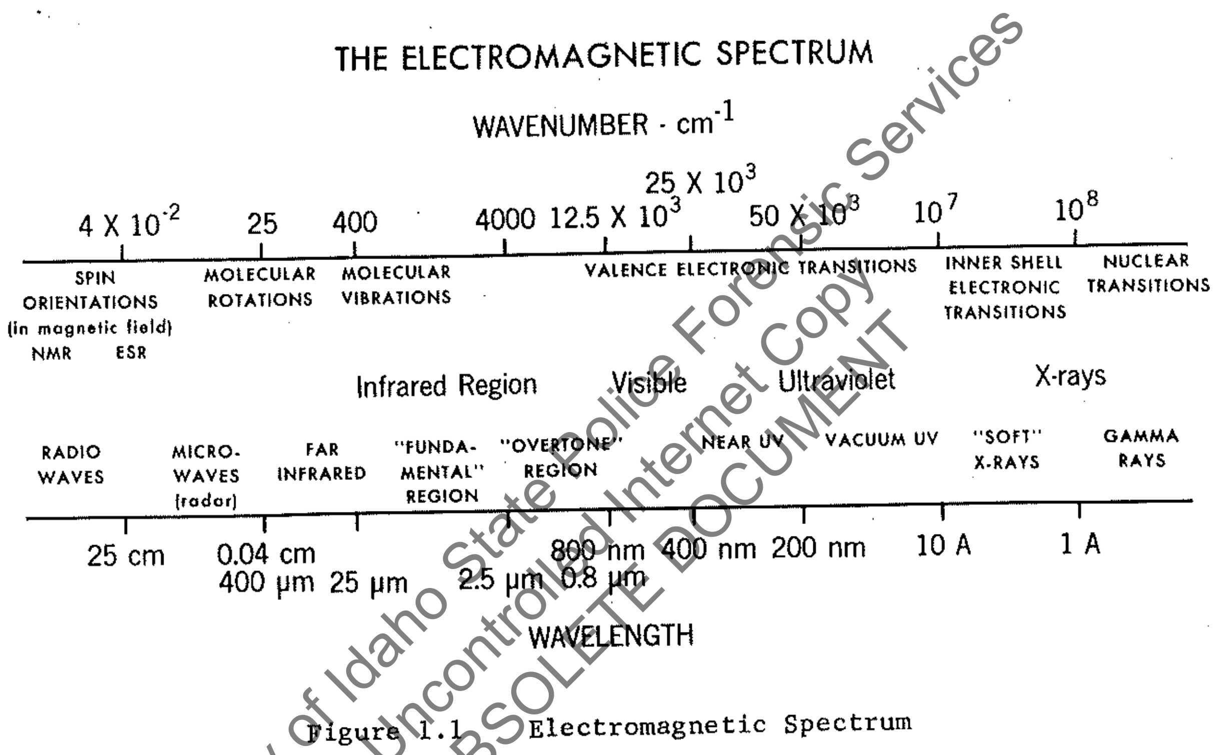


Figure 1.1 Electromagnetic Spectrum

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Electromagnetic radiation can be considered to be an oscillating electric field, with an associated magnetic field, which travels through space with a wave motion. In order to explain the various properties of electromagnetic radiation, a dualistic nature must be assigned to it. It is both an oscillating electromagnetic field and a stream of photons, particles having energy but no mass.

## 1.1 Wavelength and Frequency of Electromagnetic Radiation

Since radiation acts as a wave, it can be classified in terms of either wavelength or frequency. Wavelength is the distance measured along the line of propagation, between the crests of two adjacent waves. Frequency is the number of waves passing a given point per unit time.

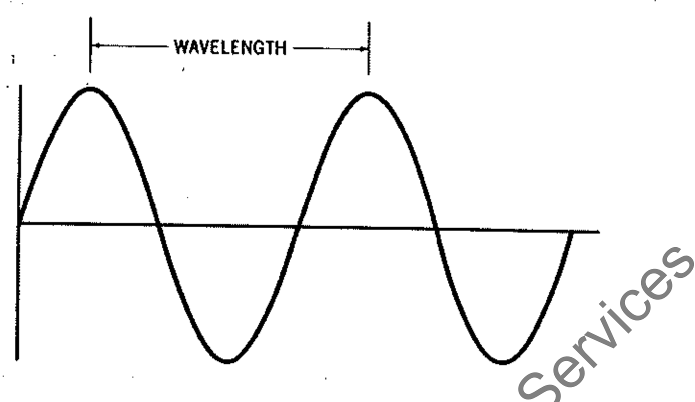


Figure 1.2 Wavelength

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Wavelength, $\lambda$, and frequency, $\nu$, can be related by the equation:

$$\nu = \frac{c}{\lambda}$$

where c is the speed of light (approximately $3 \times 10^{10}$ cm/sec). Common units of wavelength are the nanometer (nm = $10^{-9}$ meter), the micrometer ($\mu$m = $10^{-6}$ meter), and the angstrom (Å = $10^{-10}$ meter). In this book, the term nanometer will be used exclusively in designating wavelength. Sometimes it is more useful to classify radiation in terms of its frequency. For example, for 250 nm radiation:

$$\nu = \frac{c}{\lambda} = \frac{3 \times 10^{10} \text{ cm sec}^{-1}}{250 \times 10^{-9} \text{ m}} = 1.2 \times 10^{15} \text{ Hz}$$

Since frequency is a very large and rather unwieldy number, the number of waves per centimeter or <u>wavenumber</u>, <u>$cm^{-1}$</u>, is used to provide a more convenient term. Wavelength and wavenumber are related by the equation:

$$\bar{\nu} = \frac{10^7}{\lambda \text{ (in nm)}}$$

thus:

$$1000 \text{ nm} = 1 \ \mu\text{m} = 10{,}000 \text{ cm}^{-1}$$
$$500 \text{ nm} = 0.5 \ \mu\text{m} = 20{,}000 \text{ cm}^{-1}$$
$$200 \text{ nm} = 0.2 \ \mu\text{m} = 50{,}000 \text{ cm}^{-1}$$

The ultraviolet-visible range (200-780 nm) corresponds to 50,000 to 12,800 $cm^{-1}$. Note that wavenumber is an inverse function of wavelength, i.e., the longer the wavelength, the lower the frequency.

TABLE 1.1

Regions of the Electromagnetic Spectrum

| Region | Wavelength (nm) | Wavenumber ($cm^{-1}$) |
|---|---|---|
| Far Ultraviolet | 10 - 200 | 1,000,000 - 50,000 |
| Near Ultraviolet | 200 - 380 | 50,000 - 26,300 |
| Visible | 380 - 780 | 26,300 - 12,800 |
| Near Infrared | 780 - 3000 | 12,800 - 3,333 |

## 1.2 Energy

Empirical observations have shown that some forms of radiation are more energetic than others. For example, visible light is harmless, but near ultraviolet rays cause sunburn, and X-rays cause serious burns. Cosmic rays can be detected in deep mines after penetrating hundreds of feet through soil and rock. These observations lead to the conclusion that at the shorter wavelengths the radiation is more energetic. The kinetic energy associated with a photon of electromagnetic radiation is defined by the equation:

$$E = h\nu = \frac{hc}{\lambda}$$

where h is Planck's constant ($6.62 \times 10^{-27}$ erg sec). Thus, energy is directly proportional to frequency and inversely proportional to wavelength. Other energy units can be related to frequency or wavelength. For example:

$$E = h\nu$$

$$E = \frac{Ve}{300}$$

$$V = \frac{300\ E}{e} = \frac{300\ h\nu}{e} = \frac{300\ hc}{e\lambda}$$

where
$E$ = kinetic energy (ergs)

$h$ = Planck's constant = $6.62 \times 10^{-27}$ erg sec

$V$ = electron volts

$e$ = charge on electron = $4.8 \times 10^{-10}$

$c$ = speed of light = $3 \times 10^{10}$ cm $sec^{-1}$

$\lambda$ = wavelength (in cm)

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thus:

$$1000 \text{ nm} = 1 \text{ µm} = 10{,}000 \text{ cm}^{-1} = 1.24 \text{ V}$$

$$500 \text{ nm} = 0.5 \text{ µm} = 20{,}000 \text{ cm}^{-1} = 2.48 \text{ V}$$

$$200 \text{ nm} = 0.2 \text{ µm} = 50{,}000 \text{ cm}^{-1} = 6.20 \text{ V}$$

# 2.0 ORIGIN OF SPECTRA

## 2.1 Absorption of Radiation

When light passes through a transparent material, some of the wavelengths of light may be absorbed. If the intensity of the transmitted light is plotted as a function of wavelength, an absorption spectrum of the material is obtained. It is this selective absorption of radiation that forms the basis for application of absorption spectrophotometry to qualitative and quantitative analysis.

Since light is a form of energy, absorption of a photon of light by a molecule causes an increase in the energy content of the molecule. The amount of this increase is equal to the energy of the photon.

$$\Delta E = h\nu$$

If the molecule is in its normal or ground state before the interaction, the absorption raises its energy content to a higher or "excited" state. Experiments have shown that energy changes produced by light absorption are not smooth, continuous functions but occur only in integral multiples of a unit of energy called a quantum, which is characteristic of each absorbing species. In order for a photon to be absorbed by a molecule, energy of the photon must correspond precisely to the difference between two characteristic energy states of the molecule.

## 2.2 Energy States of Molecules

The total potential energy of a molecule (excluding nuclear energy) can be considered to be the sum of its electronic, vibrational, and rotational energies.

$$E_{total} = E_{electronic} + E_{vibrational} + E_{rotational}$$

Energy differences between rotational states of a molecule are quite small - very much smaller than between electronic states - while vibrational transitions

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are intermediate between the two. Rotational transitions will have absorption bands in the low frequency or long wavelength region of the spectrum (100 to 10 $cm^{-1}$ or 100 - 1000 μm). Vibrational spectra will be between 10,000 and 100 $cm^{-1}$ (1 - 1000 μm). Electronic spectra involve higher energies and occur almost entirely below 1.0 μm wavelength.

The various types of transitions are not independent but are interrelated. Rotational energy levels are superimposed on vibrational levels, and both are superimposed on electronic levels as shown in Figure 2.1.



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Figure 2.1 Molecular Energy Levels

If the atoms of a diatomic molecule such as HCl could be prevented from rotating or vibrating, a pure electronic transition from the normal or ground state of the molecule to the first excited state might be illustrated as in Figure 2-1 (I). If vibration but not rotation is permitted, vibrational transitions are superimposed as shown in Figure 2.1 (II). When rotation is also allowed, further complication is added as shown in Figure 2.1 (III). The lengths of the different arrows correspond to the energies required to effect the transitions. The short arrows represent rotational energies, and the intermediate arrows and long arrows indicate vibrational and electronic energies, respectively. These diagrams also illustrate why the electronic absorption spectra of molecules are not sharp "lines" occurring at a single wavelength, but are rather broad bands spread out over a range of wavelengths.

# 3.0 ABSORPTION SPECTRA OF ORGANIC MOLECULES

## 3.1 Unsaturation

Unsaturation (multiple bonds) has long been recognized as characteristic of ultraviolet absorbing molecules. Saturated compounds are transparent in the ultraviolet region. In molecular orbital theory, electrons forming single bonds are called sigma (σ) electrons and those forming double bonds are called pi (π) electrons. In the near ultraviolet spectral region, transitions of the π electrons give rise to most of the observed absorption bands. Non-bonded or unshared electrons in molecules containing atoms like oxygen or nitrogen are called n electrons, and interactions between π and n electrons are responsible for some important absorptions. Organic groups can be classified according to their effect on the ultraviolet absorption characteristics of the molecules to which they are attached.

ELECTRON TYPES

H, π, n, C=O, H, σ

Figure 3.1 Electron Types

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## 3.2 Chromophores

A chromophore is a group which, when introduced into a saturated hydrocarbon, produces a compound which has a selective absorption somewhere between 185 nm and 1000 nm. For example, n-octane is a saturated hydrocarbon which is quite transparent at all wavelengths between 185 nm and 1000 nm. If a nitrite group is introduced into the octane molecule, a compound, octyl nitrite, is produced which has a strong absorption band centered at 230 nm. Therefore, the nitrite group is classified as a chromophore. Table 3.1 lists typical simple chromophoric groups. Note that each of the groups has at least one multiple bond.

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TABLE 3.1

| Chromophore | System | Example | λ Max(nm) | ε* |
|---|---|---|---|---|
| Carbonyl (ketone) | RR'C=O | Acetone | 271 | 16 |
| Carbonyl (aldehyde) | RHC=O | Acetaldehyde | 293 | 12 |
| Carboxyl | RCOOH | Acetic Acid | 204 | 60 |
| Amide | $RCONH_2$ | Acetamide | 208 | -- |
| Ethylene | RCH=CHR | Ethylene | 193 | 10,000 |
| Acetylene | RC≡CR | Acetylene | 173 | 6,000 |
| Azomethine | >C=N- | Acetoxime | 190 | 5,000 |
| Nitrile | -C≡N | Acetonitrile | < 160 | -- |
| Azo | -N=N- | Azomethane | 347 | 5 |
| Nitroso | -N=O | Nitrosobutane | 300<br>665 | 100<br>20 |
| Nitro | $-NO_2$ | Nitromethane | 271 | 19 |
| Nitrate | $-ONO_2$ | Ethyl Nitrate | 270 | 12 |
| Nitrite | -ON=O | Octyl Nitrite | 370<br>230 | 55<br>2,200 |
| Thiocarbonyl | >C=S | Thiobenzophenone | 620 | 70 |
| Sulfoxide | >S=O | Cyclohexylmethyl Sulfoxide | 210 | 1,500 |
| Sulfone | $>SO_2$ | Dimethyl Sulfone | < 180 | -- |

* ε = Molar absorptivity in liters/mole cm.

Some of the groups, such as the ethylene and nitrile, may sometimes produce absorption bands slightly out of the specified wavelength range, but their other properties conform to those of chromophores. The intensity of absorption of the simple chromophores varies widely from one group to the next. However, all members of a class of compounds containing a single chromophore will normally have absorption bands of approximately equal intensity and within a narrow spectral range. For example, the saturated carboxylic acids in the series from formic to stearic acid ($C_1$ to $C_{18}$) have absorption bands in the region 204 to 210 nm, and their molar absorptivities* range from 40 to 75. Ketones have bands between 270 and 280 nm, with molar absorptivities of 16 to 28. Since each series has its own absorption characteristics, the presence or absence of a particular chromophore may frequently be determined from a study of the absorption spectrum of a compound.

## 3.3 Conjugation

If two or more chromophores occur in a single molecule, their relative positions determine the effect produced. The data presented in Table 3.2 can be used as an example to illustrate this. 1-Hexene has a molar absorptivity of 10,000 at 180 nm and 1,5-hexadiene has an absorptivity of 20,000 at about the same wavelength. 2,4-Hexadiene, on the other hand, has its absorption band at 227 nm with a molar absorptivity of 25,500.

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TABLE 3.2

| Compound | λ Max. | ε |
|---|---|---|
| 1-Hexene | 180 nm | 10,000 |
| 1,5-Hexadiene | 180 nm | 20,000 |
| 2,4-Hexadiene | 227 nm | 25,500 |

The position and intensity of the absorption band of 1,5-hexadiene is approximately what would be expected from two propylene molecules. However, when the two chromophores are on adjacent pairs of carbon atoms as in 2,4-hexadiene, the effect is not what would be expected from a consideration of the two groups separately.

* Molar absorptivity is a measure of the strength of an absorption band. See Section 4.1.2.

There is a definite interaction which shifts the center of the absorption band by 47 nm and increases the intensity. Similar observations on a large number of such compounds have led to the formulation of the following general rules:

a) When two chromophores are in the same molecule and separated by more than one carbon atom, the resulting absorption spectrum is a simple summation of the absorption of each of the two chromophores.

b) When two chromophores in the same molecule are adjacent to each other, the absorption maximum is displaced toward longer wavelengths and the intensity of absorption is increased, as compared with the absorption spectra of compounds containing the two chromophores separated in the molecules.

c) When two chromophores are attached to the same carbon atom, the result is intermediate between the two extremes.

In ultraviolet spectroscopy the most interesting compounds are usually those that contain more than one chromophore, and especially those in which the chromophores are adjacent to each other. Chromophores which are on adjacent carbon atoms (i.e., separated by a single carbon-carbon linkage) are said to be conjugated. Examples of molecules containing conjugated groups are:

1,3-Butadiene, $H_2C=CH-CH=CH_2$

Mesityl Oxide, $(CH_3)_2C=CH-CO-CH_3$

Nitroethylene, $H_2C=CH-NO_2$

Vinyl Acetylene, $H_2C=CH-C\equiv CH$

The presence of conjugated chromophores is almost always accompanied by a shift to longer wavelengths, a bathochromic shift, and by an increase in intensity, a hyperchromic effect. Other types of structural changes in organic compounds can produce effects opposite to those mentioned above. A change to shorter wavelengths is called a hypsochromic shift, and a reduction of intensity is termed a hypochromic effect. Examples of these effects will be illustrated later.

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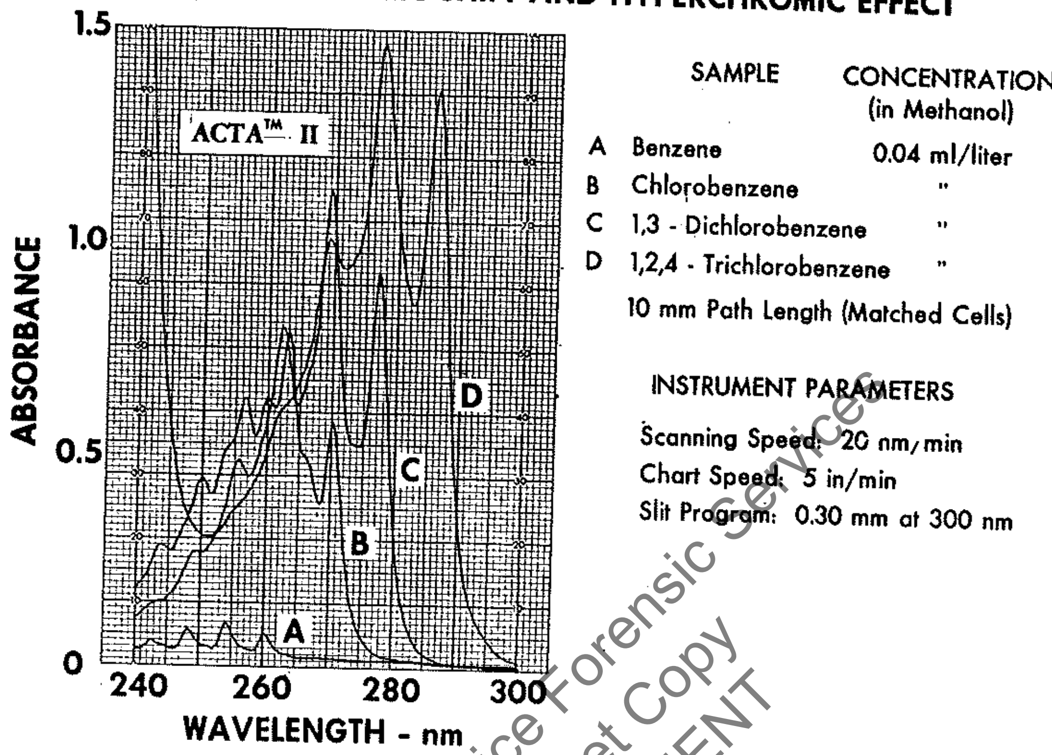


Figure 3.2 Bathochromic Shift and Hyperchromic Effect

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## 3.4 Classification of Chromophores

The conjugated chromophores described above are sometimes referred to as complex chromophores. Among the complex chromophores, two types are most frequently encountered. The first type corresponds to the formula:

$$A - (CH = CH)_n - CH = B$$

where A is H, R, OR, SR, $NR_2$, $O^-$, $S^-$, or $^-NR$, and B is $CH_2$, CHR, $CR_2$, NR, O, S, $^+NR_2$, $^+OR$, or $^+SR$. The active groupings in this case are called K-chromophores and give rise to the so-called K-bands (K from the German "konjugiert"). A second type of complex chromophore is that found in aromatic compounds and is associated with the benzene ring. It gives rise to the so-called B-bands. The simple chromophores listed in Table 3.1 give rise to what are known as R-bands.

The three types of bands listed above can best be differentiated on the basis of their molar absorptivities:

| Bands | Molar Absorptivity (ε) |
|---|---|
| R-bands | 100 or less |
| B-bands | 250 - 3000 |
| K-bands | 10,000 or more |

In conjugated systems, as the number of conjugated chromophores increases, there is a corresponding increase in wavelength and intensity. For example, consider the polyunsaturated acids listed in Table 3.3.

TABLE 3.3

| $CH_3$ - (CH = CH)$_n$ COOH | n | λ Max. | Molar Absorptivity (ε) |
|---|---|---|---|
| Acetic | 0 | 197 | 60 |
| Crotonic | 1 | 208 | 12,500 |
| Sorbic | 2 | 261 | 25,600 |
| 2, 4, 6-Octatrienoic | 3 | 303 | 36,500 |
| 2, 4, 6, 8-Decatetraenoic | 4 | 332 | ~ 50,000 |

As each additional double bond is added to the conjugated system, there are accompanying bathochromic and hyperchromic effects. Similar results can be demonstrated in many other series of compounds.

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## 3.5 Auxochromes

Auxochromes are groups which, when introduced into a chromophoric system, increase the wavelength of the absorption band (i.e., cause a bathochromic shift). Auxochromes do not have absorption bands of their own in the wavelength range under consideration. For example, the hydroxyl group is an auxochrome. Alcohols are so transparent that they are used as solvents even at short wavelengths; however, if the hydroxyl group is introduced into a system containing a chromophore, it will cause a bathochromic shift. In addition to the hydroxyl group, other typical auxochromes are amino groups and their substituted derivatives, halogens, alkyl groups, and many other substituents.

## 3.6 pH and Solvent Effects

Whenever the absorption bands of compounds are discussed, it is necessary to specify the solvent used, because the position and intensity of a band will vary with the solvent. For example, the absorption band of acetone can vary from 259 nm to 279 nm, depending on the solvent. These effects are due to:

a) The nature of the solvent.
b) The nature of the absorption band.
c) The nature of the solute.

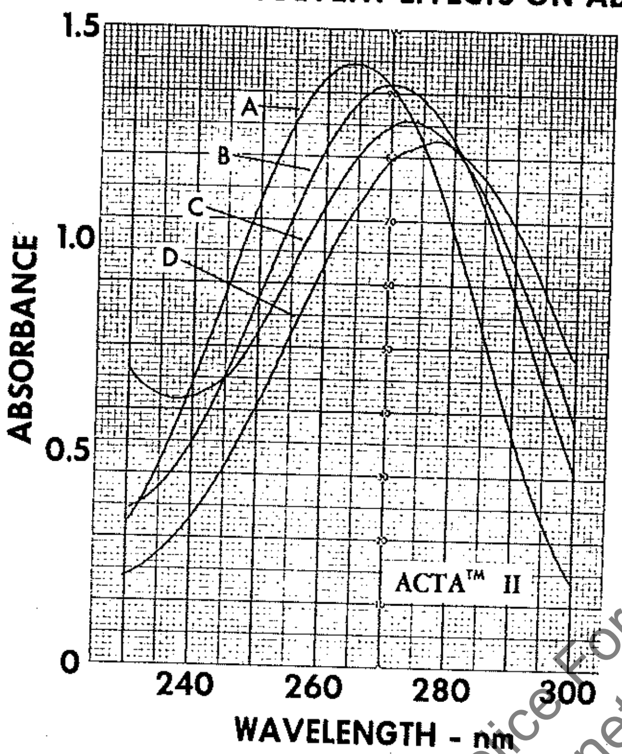


Figure 3.3 Solvent Effects on Absorption Spectra

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In general, magnitude of the effect can be correlated with polarity of the solvent. R-bands are displaced to shorter wavelengths (a hypsochromic shift) with increasing polarity of the solvent, while the opposite is true for K-bands. Polar solutes are affected more by changes of solvent than are non-polar solutes. Typical maximum wavelength displacements are of the order of 5 to 10 nm, although displacements of 20 nm or more have been encountered. Absorptivity varies as much as 10% or more with changes of solvent.

pH effects can be quite striking. Most of the effects are due to shifting equilibria between two different forms such as keto-enol and amino-imino isomers. And, of course, everyone is familiar with the dramatic color changes effected by pH changes on solutions of indicator dyes such as methyl orange and phenolphthalein.

Solvent effects can often be used to great advantage in establishing the nature of a given group by noting the effect on a given band as solvents or pH are changed.

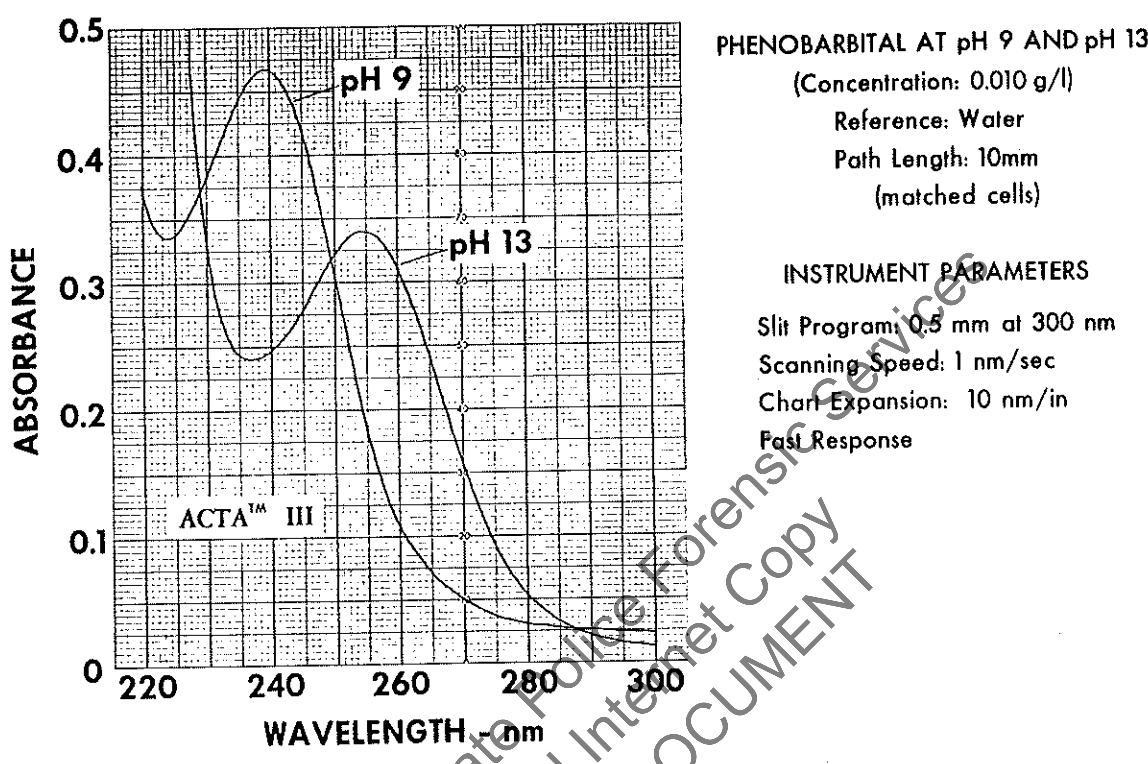


Figure 3.4 Effect of pH on Absorption Spectra

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The most widely used solvents are water, ethanol, cyclohexane, iso-octane, and acetonitrile. For use below 220 nm, it is usually necessary to use the highly purified "spectro grade" solvents which are now widely available. The common 95% ethanol is usually preferable to absolute ethanol, because the latter is frequently prepared by an azeotropic distillation which leaves behind traces of strongly absorbing benzene.

Other solvents which are useful include glycerol, dioxane, ethyl ether, butyl ether, n-hexane, n-heptane, and saturated alcohols from methanol to the pentanols. Chloroform is very useful down to about 250 nm, but it absorbs strongly at shorter wavelengths.

For pH adjustment and control, acetic and sulfuric acids, sodium hydroxide, and the phosphate buffers can be used.

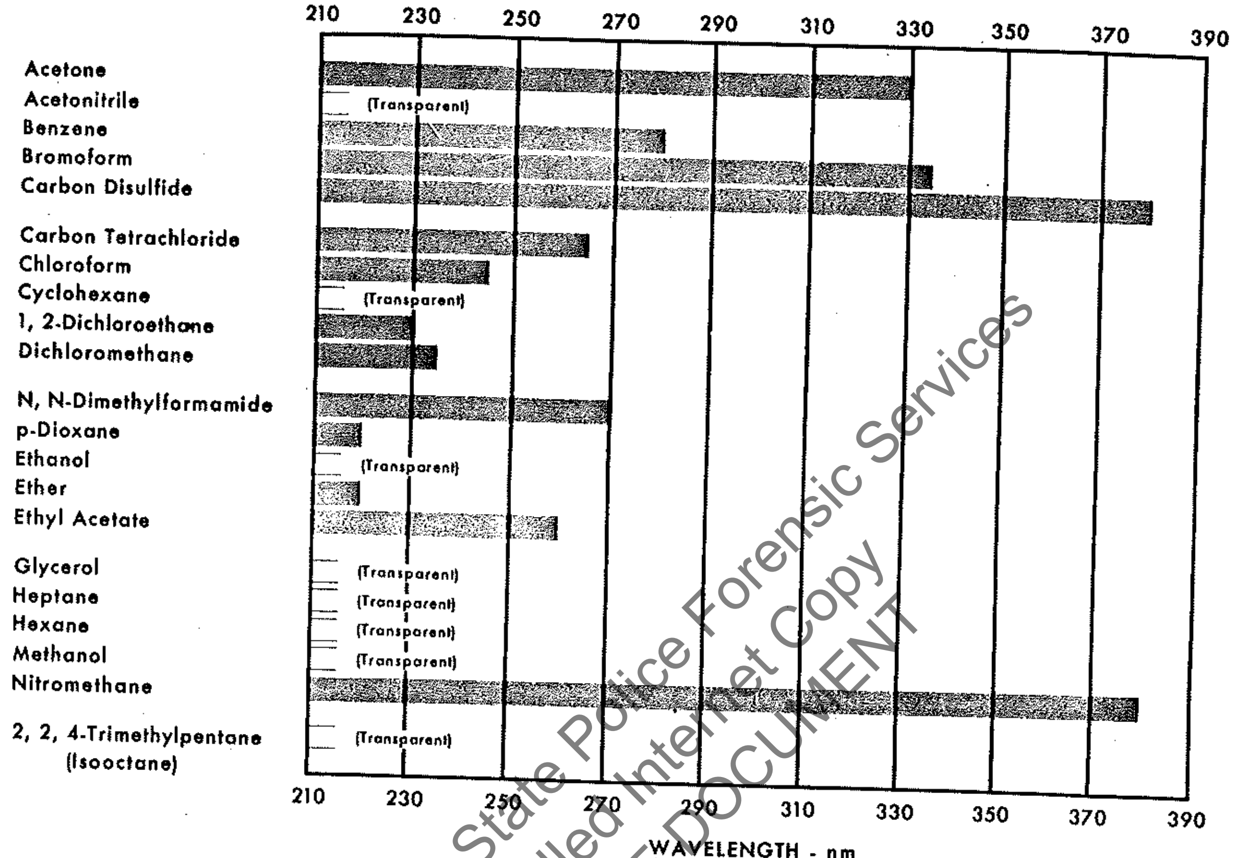


Figure 3.5 Ultraviolet Transmission Characteristics of Common Solvents

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## 3.7 Applications of Ultraviolet Spectrophotometry

### 3.7.1 Detection and Identification of Compounds

Since the various chromophores have absorption bands with distinctive shapes, locations, and intensities, identification and detection of absorbing groups is frequently possible using the ultraviolet region of the spectrum. For example, absorbing groups can usually be identified by use of information such as that in Table 3.1. But identification of individual compounds is more difficult, because absorption bands are characteristic of groups rather than of the molecule as a whole. A frequently used technique for identifying individual compounds is to take the ratio of the absorbances at two different wavelengths. This technique has been particularly useful in the identification of individual barbiturates and amino acids.

Note that the presence of ultraviolet absorbing impurities may lead to gross errors, particularly when the compound of interest is a weak absorber and is in the presence of even small quantities of strongly absorbing impurities. Ultraviolet analyses frequently require use of chromatography, selective extraction, or other separation techniques before obtaining an absorption spectrum of the material.

A technique which has been widely used for increasing accuracy and sensitivity when working with weak absorbers is the preparation of derivatives. For example, the carbonyl group in simple ketones has a molar absorptivity of about 25; by converting the ketone to a thiosemicarbazone, the molar absorptivity is made nearly 1000 times greater.

### 3.7.2 Molecular Structure Determination

The various facts mentioned above will suggest numerous uses in the determination of molecular structure. Some of the more common applications will be pointed out here.

a) Determination of molecular weight is accomplished by preparation of a derivative, a new compound, using a reagent which has a strong absorption band at a wavelength where the original compound does not absorb. The molar absorptivity of the derivative at that wavelength is thus the same as that of the reagent, but the absorptivity will be different, depending on the molecular weight of the compound. The molecular weight (M) can be calculated using the equation:

$$M = \frac{\epsilon w b}{A}$$

where $\epsilon$ is the molar absorptivity, w is the weight of the compound in grams per liter, b is the path length of the cell, and A is the absorbance (see Beer-Bouguer Law, Sec. 4. 1. 1). The 2, 4-dinitrophenylhydrazine derivatives can be used to determine the molecular weights of aldehydes and ketones with an accuracy of about 2%. Other examples include picric acid derivatives of amines, the osazones of sugars, and the 2, 4-dinitrobenzoate esters of alcohols.

b) Dissociation constants of acids and bases can be readily determined spectrophotometrically since, as was mentioned above, absorption spectra of polar compounds will vary significantly with changes in the pH of the solvent. For example, a weak acid such as salicylic acid will dissociate as follows:

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$$HS + H_2O \rightleftarrows H_3O^+ + S^-$$

In strongly basic solution the equilibrium will be shifted so that essentially all of the substance exists as the salicylate ion, $S^-$, while in an acidic solution the undissociated form, HS, will predominate. A gradation from one form to the other will exist at intermediate pH values. Determination of absorptivities of these solutions at various pH levels and different wavelengths thus permits the preparation of a calibration curve from which the dissociation constant can be calculated. Details of the method are given in Rao (p. 75*) and other texts.

c) Keto-enol tautomerism is a dynamic isomerism in which a hydroxyl group and a carbonyl group are readily interconvertible. Since the hydroxyl is non-absorbing while the carbonyl is a chromophore, relative concentrations of the two forms are readily determinable.

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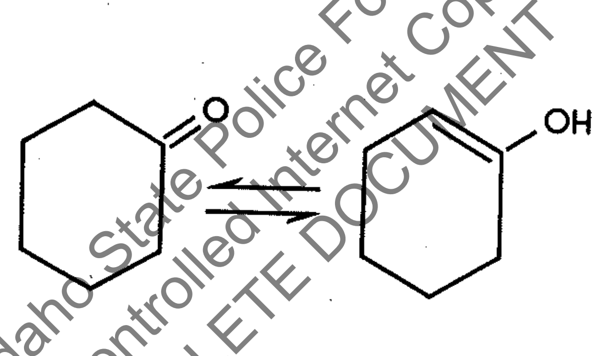


Figure 3.6 Tautomerism

d) Cis-trans isomerism generally involves differing spatial arrangements of groups on either side of a rigid double bond. When one of these groups is a chromophore, differences in wavelength and intensity of the absorption spectra of the two forms will result. While a thorough discussion of this subject would be too lengthy and esoteric for inclusion here, Rao* has made one generalization that seems pertinent. He says, "absorption intensities of long wavelength bands of trans-isomers are always greater than those of corresponding cis-isomers."

e) Steric effects on absorption spectra have been observed in a number of instances. Just one example will be given here as an illustration. In diphenyl and substituted diphenyls, the strongest absorption bands are observed

* C. N. R. Rao, Ultraviolet and Visible Spectroscopy, Butterworths, London (1961).

when the two rings are co-planar. In ortho-substituted biphenyls, where steric effects prevent the two rings from lying in the same plane, the absorptivity is greatly reduced.

# 4.0 QUANTITATIVE ANALYSIS

The information presented up to this point makes it intuitively evident that the amount of light absorbed by a fixed quantity of an absorbing species will be dependent on the number of molecules of that species. This property forms the basis for quantitative analysis by spectrophotometry.

## 4.1 Laws of Light Absorption

### 4.1.1 Bouguer's Law

Consider the diagram of Figure 4.1 where a beam of monochromatic (one wavelength) light is incident on a cell filled with a transparent solution containing an absorbing substance. If 100 photons enter the cell and only 50 emerge from the other side, the transmittance is 0.5. If a series of identical cells, each containing a portion of the same solution, are placed in the light beam, the result shown in Figure 4.2 is obtained. This is an illustration which shows the effect

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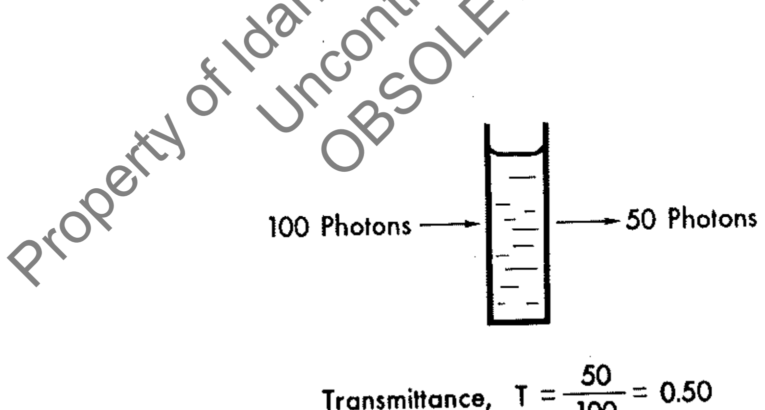


$$\text{Transmittance, } T = \frac{50}{100} = 0.50$$

Figure 4.1 Light Absorption

of increasing the path length of an absorbing solution. The first mathematical formulation of this effect is generally credited to Lambert (1760), although it now appears that Bouguer first stated it in 1729.

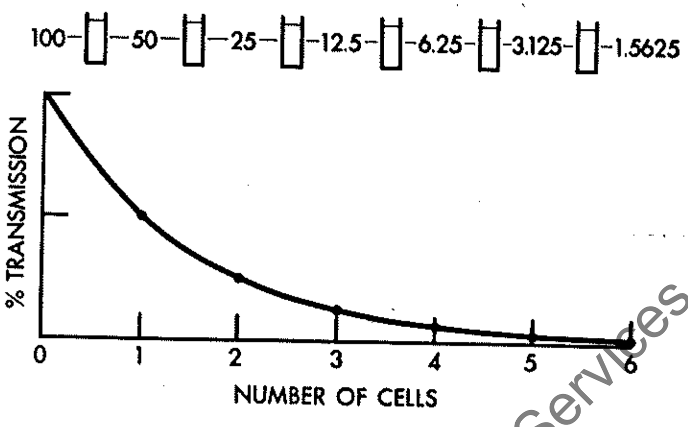


Figure 4.2 Bouguer's Law

The successive values of transmittance from Figure 4.2 are 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625. These correspond to numbers in the following sequence: $(0.5)^1$, $(0.5)^2$, $(0.5)^3$, $(0.5)^4$, $(0.5)^5$, $(0.5)^6$. Bouguer's Law can be formulated mathematically:

$$T = \frac{P}{P_o} = e^{-\alpha b}$$

or $$\log_e \frac{1}{T} = \alpha b$$

where

$P$ = the radiant power transmitted by the sample

$P_o$ = the radiant power incident on the sample

$b$ = path length (in centimeters)

$\alpha$ = absorption coefficient (characteristic of the sample)

$e$ = the base of natural logarithms

Bouguer's Law can thus be stated, "The absorbance of a homogeneous sample is directly proportional to the thickness of the sample in the optical path."

## 4.1.2 Beer's Law

Beer's Law is exactly analagous to Bouguer's Law, except that it is stated in terms of concentration. The amount of light absorbed is proportional to the number of absorbing molecules through which the light passes. The combined

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Beer-Bouguer Law can be formulated mathematically, using base 10 logarithms:

$$T = \frac{P}{P_o} = 10^{-abc}$$

or

$$A = -\log T = -\log \frac{P}{P_o} = \log \frac{P_o}{P} = abc$$

where $A$ = absorbance

$a$ = absorptivity

$b$ = path length (in centimeters)

$c$ = concentration (usually in grams/liter)

The Beer-Bouguer Law in the form $A = abc$ is widely used in quantitative analysis, because absorbance is directly proportional to concentration. Most modern spectrophotometers have scales calibrated in absorbance rather than in transmittance because of the convenience. Absorptivity, a, is a characteristic of a given substance only under precisely defined conditions of wavelength, solvent, temperature, and other parameters. If path length is given in terms of centimeters and concentration in grams solute per liter of solution, then the absorptivity unit is liters per gram centimeter. Another useful and well accepted term is molar absorptivity ($\varepsilon$), which is the product of the absorptivity, a, and the molecular weight of the substance.

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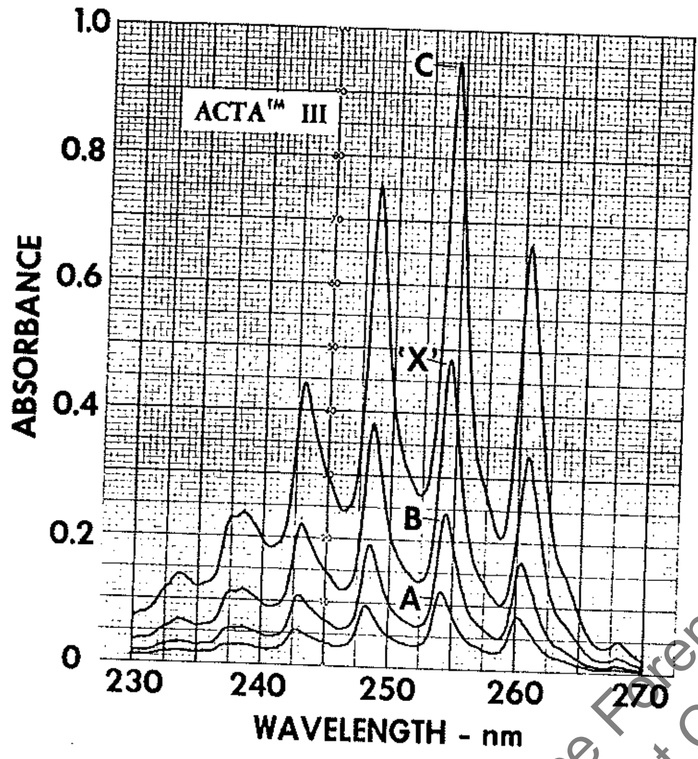
## 4.1.3 A Typical Quantitative Spectrophotometric Analysis

A typical analysis is the quantitative analysis for traces of benzene in ethanol. Since ethanol is a saturated alcohol, it does not contain a chromophore and is therefore non-absorbing in the near ultraviolet spectral region. Benzene, containing three double bonds, has several absorption bands with a very strong band at 254.5 nm. Samples containing known amounts of benzene are measured on the instrument and the absorbances at the wavelengths of maximum absorbance are determined. These are plotted on linear graph paper against concentration for use as a calibration graph. Concentration of the unknown sample can be determined from the graph by observing the concentration 0.177 g/l which corresponds to the absorbance of the unknown at 254.5 nm. In this type of analysis it is important to know that there is no interference caused by the presence of a second absorbing substance. Interferents can usually be detected by inspection and comparison of known and unknown spectra.




QUANTITATIVE ANALYSIS
Benzene in Ethanol

| SAMPLE | CONCENTRATION - g/l |
|---|---|
| A | 0.044 |
| B | 0.088 |
| C | 0.352 |
| "X" | Unknown |

Path Length: 10 mm (matched cells)
Reference: Ethanol

INSTRUMENT PARAMETERS
Scanning Speed: 0.2 nm/sec
Chart Expansion: 5 nm/in
Slit Program: 0.25 mm at 270 nm

Figure 4.3 Quantitative Analysis (Benzene in Ethanol)

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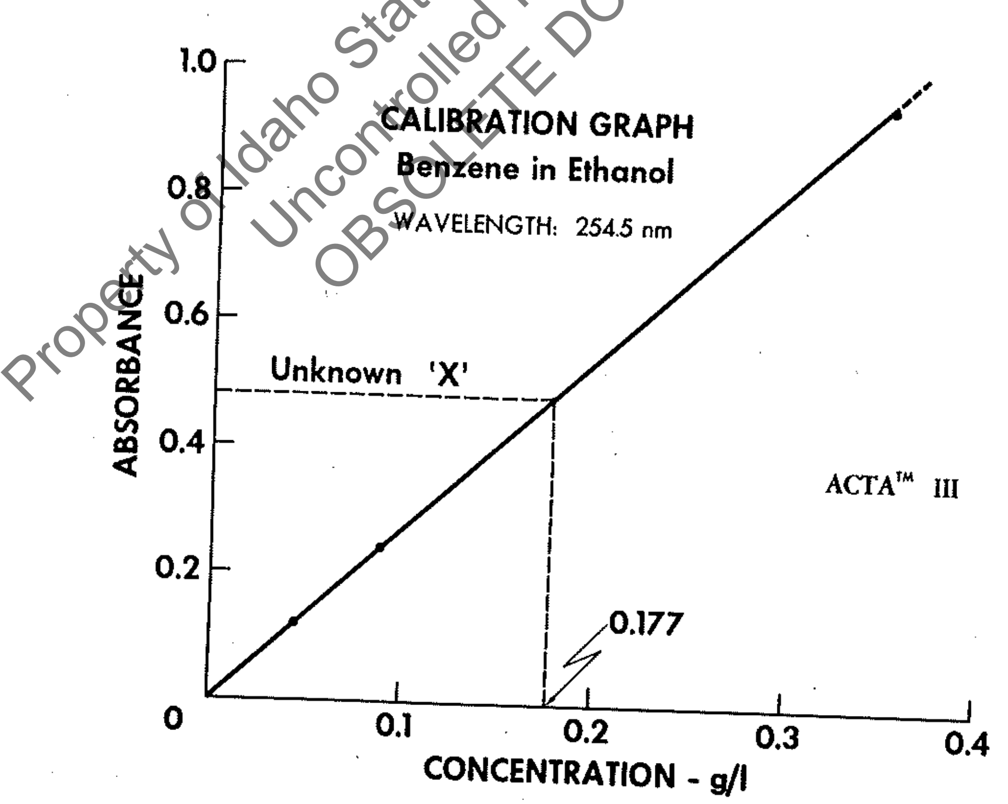


Figure 4.4 Calibration Graph

Use of a calibration graph is not absolutely necessary. Concentration can be determined directly from the Beer-Bouguer Law once the absorptivity has been evaluated.

$$c = \frac{A}{ab}$$

In the illustration, Figure 4.4, the path length, b, is 1.0 cm. Absorbances of 0.120, 0.242, and 0.950 for concentrations 0.044, 0.088, and 0.352 g/1 lead to calculated values of absorptivity, a, of 2.73, 2.75, and 2.70 l/g cm with the average value being 2.727 l/g cm. When this value of a is used with the measured absorbance, 0.483, the calculated concentration of the unknown sample is 0.177, the identical value obtained in the graphical solution of the problem.

## 4.1.4 Multi-Component Analyses

Sometimes more than one component in a mixture must be determined. As long as there is no overlapping of the absorption bands, each component can be treated exactly as if it were a single component. When there is interference because of overlapping absorption bands, the simple Beer-Bouguer's Law approach described above must be altered to minimize the effect of interference.

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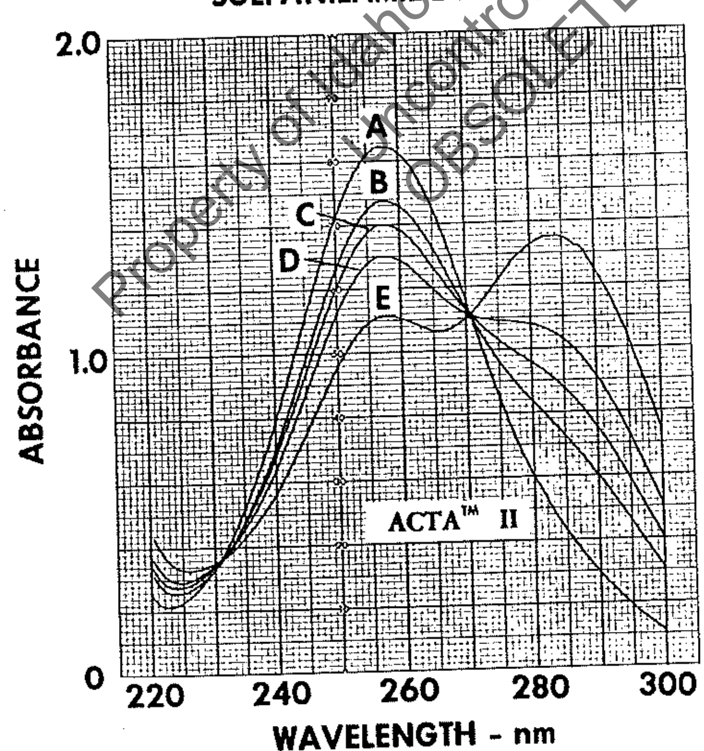


TWO-COMPONENT MIXTURE

| | Sulfanilamide - (g/l) | Sulfathiazole - (g/l) |
|---|---|---|
| A | 0.018 | 0 |
| B | 0.012 | 0.006 |
| C | 0.009 | 0.009 |
| D | 0.006 | 0.012 |
| E | 0 | 0.018 |

10 mm Path Length (Matched Cells)

INSTRUMENT PARAMETERS

Scanning Speed: 20 nm/min
Chart Speed: 2 in/min
Slit Program: 1.0 mm at 300 nm
Fast Response

Figure 4.5 Sulfanilamide and Sulfathiazole

Absorbances are additive, that is, at a specified wavelength, the absorbance of a mixture is equal to the sum of the absorbances of each component. In mathematical terms:

$$A_{total} = A_1 + A_2 + A_3 + \dots\dots\dots A_n$$

$$= a_1bc_1 + a_2bc_2 + a_3bc_3 + \dots\dots\dots a_nbc_n$$

In order to perform such an analysis for n components, select a separate wavelength for each component and solve n simultaneous equations. Usually this wavelength is the center of the absorption band which has the least interference from other components. Then determine the absorptivity of each component at each wavelength by measurements on pure solutions of a single component. Absorbance values of the unknown mixture are then measured at each wavelength and substituted in a set of n simultaneous equations:

$$A_1 = a_{11}bc_1 + a_{12}bc_2 + \dots\dots\dots a_{1n}bc_n$$

$$A_2 = a_{21}bc_1 + a_{22}bc_2 + \dots\dots\dots a_{2n}bc_n$$

$$\dots\dots\dots\dots\dots\dots\dots$$

$$\dots\dots\dots\dots\dots\dots\dots$$

$$A_n = a_{n1}bc_1 + a_{n2}bc_2 + \dots\dots\dots a_{nn}bc_n$$

where

$a_{11}$ = absorptivity of component 1 at wavelength 1

$a_{12}$ = absorptivity of component 2 at wavelength 1

$a_{1n}$ = absorptivity of component n at wavelength 1

etc.

For two components, the two simultaneous equations can be solved by simple arithmetic, but for larger numbers of equations the techniques of matrix inversion or successive approximations are normally used. These techniques are described in standard texts.

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## 4.1.5 Photometric Scales

Either the transmittance or the percent transmission scale is widely used on spectrophotometers, because these are the simplest for the instrument designer. They also have the advantage that they cover the entire range from no absorption to total absorption - that is, from 100% to 0% transmission or 1.0 to 0.0 transmittance. The T scale is inconvenient for quantitative work, because it is not a linear but a logarithmic function of sample concentration. For that reason the absorbance scale is now widely used. Other scales are also occasionally used. Some instruments are calibrated in percent absorption (100 - % T), particularly for use in atomic absorption work. The logarithm of absorbance (or log A) is sometimes used. It has the advantage that the shape of the absorption spectrum is independent of concentration. It is widely used by dye chemists for identification of dyes and pigments. Recently, general purpose spectrophotometers employing a concentration scale have become available. These instruments must be calibrated once for each particular analysis. They actually use the absorbance scale, but with a variable multiplication factor to make the scale read in concentration units directly.

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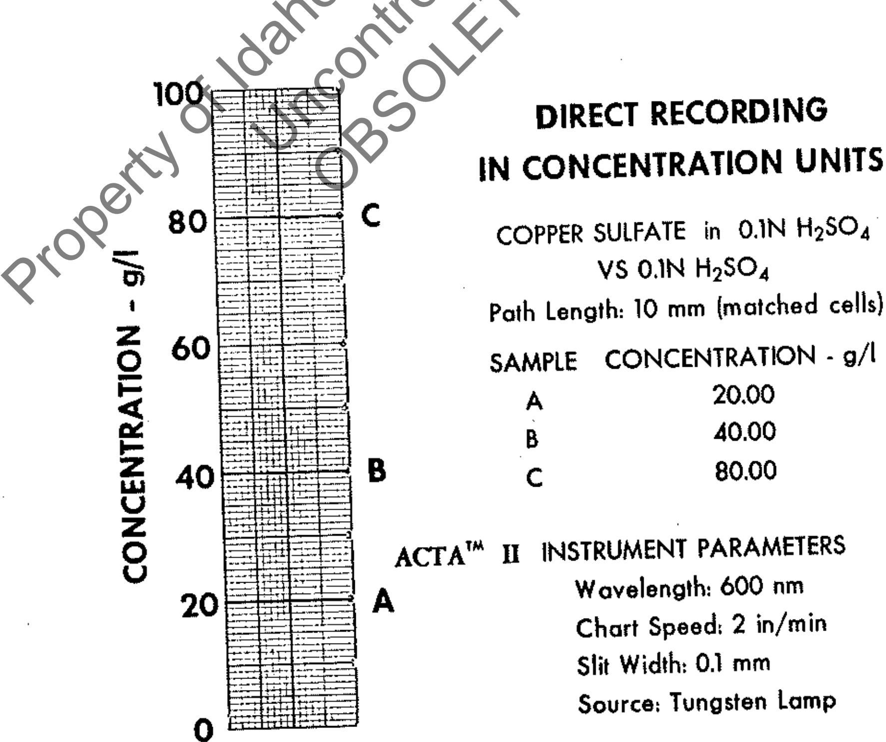


Figure 4.6 Direct Recording in Concentration Units

## 4.2 Absorptiometry

Absorptiometry is the term applied to quantitative analysis where the analyte (the substance being determined) is the absorbing species. The analysis for benzene in ethanol described above in paragraph 4.1.3 is a typical example of absorptiometry in an organic chemical system. Other examples are the determination of butadiene in a process gas stream, salicylic acid in an ointment, inhibitors in polymers, nicotine in tobacco smoke condensate solutions, acetone in isopropyl ether, xylene vapor in air, free salicylic acid in aspirin tablets, biphenyl in citrus fruits, and benzoic acid in soft drinks.

## 4.3 Colorimetry

Colorimetry is the term applied to quantitative analysis where a reagent is added which forms an absorbing compound with the substance being measured. Colorimetry is used when the substance being measured is non-absorbing, weakly absorbing, or beset with interferences. This technique has greatly extended the

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TABLE 4.1

| Analyte | Sample | Reagent |
|---|---|---|
| **Metals** | | |
| Al | Steel | Aluminon |
| Be | Rocks | Beryllon II |
| Bi | Tinning Baths | Thiourea |
| Cr | Laser Rubies | Diphenylcarbazide |
| Cu | Phosphors | Na Diethyldithiocarbamate |
| **Non-Metals** | | |
| As | Ores | Heteropoly Blue |
| B | Plants | Crystal-Violet |
| $Br^-$ | Brine | Fuchsin |
| $PO_4^{-3}$ | Organic | Mo-V-Phosphoric Acid |
| Se | Minerals | Dithizone |

Organics

| | | |
|---|---|---|
| Tetracyclines | Pharmaceuticals | HCl |
| Formic Acid | Formaldehyde | Bromphenol Blue |
| Ascorbic Acid | Tablets | Cacothelene |
| Thymols | Thyme | Sulfanilic Acid |
| Pectin | Apples | Carbazole |

scope of spectrophotometry, because a wide variety of reagents is available so that almost any type of compound can be measured. It can be applied to both organic and inorganic substances in an almost limitless variety of matrices.

Among inorganic materials, colorimetric methods have been worked out for all metallic elements and for most non-metallic elements. Additionally, various types of compounds such as sulfates, carbonates, borates, chlorates, perchlorates, etc., can be measured similarly. Among the organic materials, individual compounds as well as various classes of compounds can be determined. Examples include alcohols, acids, acid chlorides, aldehydes, sugars, nitrates, azides, acetylenes, barbiturates, alkaloids, salicylates, and almost any other type of compound desired. Typical examples of colorimetric analysis are listed in Table 4.1.

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## 4.4 Precision and Accuracy

### 4.4.1 Sources of Error in Quantitative Analysis

Certain instrumental sources of error will be discussed under the appropriate paragraphs on instrument operation.

Light Scattering - Light which is scattered by colloidal dispersions, dust, or other particulate matter in the sample appears to the instrument as absorption and can cause appreciable errors. Such samples should be filtered, centrifuged, or allowed to settle before measurement. Where turbid samples must be measured, a correction can sometimes be applied by measuring the scattered light at a wavelength where the sample does not absorb, and subtracting the reading from the reading at the center of the absorption band.

Fluorescence - Some samples fluoresce, that is, they emit light of one wavelength when irradiated with light of shorter wavelength. Under ordinary circumstances the instrument cannot distinguish between the desired light and the emitted light. The error due to fluorescence appears as a non-linearity in the plot of absorbance versus concentration. If the fluorescent substance is the substance being measured, the error can be eliminated by plotting a good calibration curve. Sometimes the effect of fluorescence can be minimized or eliminated by introducing a glass filter in the beam of the instrument between the sample and phototube. A filter is selected which passes the wavelength desired and absorbs the fluorescent light, which is always of a longer wavelength.

Temperature - Two different problems can be created when the temperature of the sample at the time of measurement is different from the temperature at the time of calibration. The volume of the solvent can change significantly, introducing a small error. The other source of error, the temperature coefficient of absorptivity, can, with some samples, be quite significant - amounting to as much as 1% to 2% per degree centigrade.

Sample Decomposition - Some samples are quite sensitive to photochemical reactions when exposed to ultraviolet light in the instrument or from fluorescent lights in the laboratory.

Solvent Evaporation - Volatile solvents such as ether, acetone, methylene chloride, and many others can evaporate rapidly from an open cell and cause a rapid change in sample concentration. Such solvents should always be used in stoppered cells only.

Dirty Sample Cells - Cells must always be kept scrupulously clean. The optical surfaces should be wiped clean with a soft tissue before each use. Particular care should be taken to avoid fingerprints, because skin oils and perspiration absorb strongly, especially in the ultraviolet regions of the spectrum.

Multiple Reflections - A rather insidious source of error is due to multiple reflections from cell windows and other flat optical elements in the light beam. Some of the light can be reflected, as shown in Figure 4.7, so that the measured absorbance is higher than the true absorbance.

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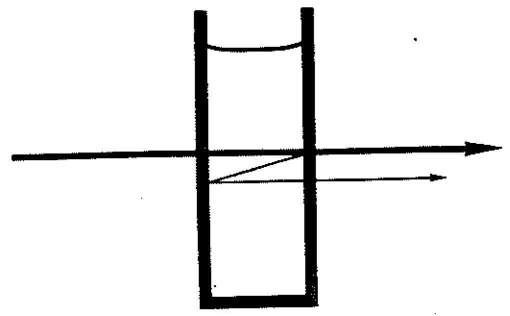


Figure 4.7 Reflection Losses in a Cell

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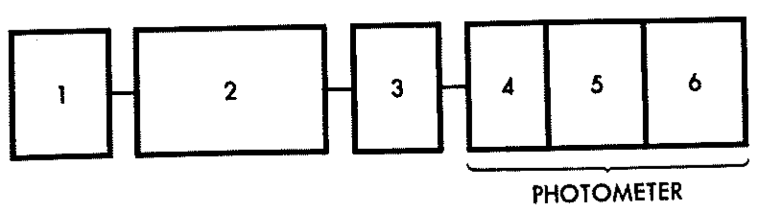
## 5.0 THE ULTRAVIOLET SPECTROPHOTOMETER

Spectroscopy is the measurement of electromagnetic radiation after it has been broken up into its spectral components.

A spectrophotometer is an instrument designed to be used for absorption spectroscopy. All spectrophotometers consist of components as shown in Figure 5.1. A source emits light of the desired wavelengths. This light passes through a monochromator which filters out all except a narrow band of monochromatic (one color) light. The monochromatic light is passed through the sample where some is absorbed, and what passes through the sample strikes the light-sensitive detector. The detector generates an electrical signal proportional to light intensity. This electrical signal is amplified and read out on a meter or other

COMPONENTS OF A SPECTROPHOTOMETER




1—SOURCE
2—MONOCHROMATOR
3—SAMPLE
4—DETECTOR
5—AMPLIFIER
6—READOUT

Figure 5.1 Components of a Spectrophotometer

device. In order to understand just how a spectrophotometer works, it is necessary to understand the function of each of the major components. These will be explained in detail in the following paragraphs.

## 5.1 Source

In an ultraviolet-visible spectrophotometer the ideal source would have the following desirable characteristics:

1) High intensity
2) Wide spectral range
3) Stable output
4) Optimum size
5) Dimensional stability
6) Output is a continuum without sharp emission lines
7) Long life
8) Low cost

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Three different types of lamps are commonly used in ultraviolet-visible spectrophotometers.

The tungsten lamp (typically about 30 watts) satisfies most of the requirements listed above except for wide spectral range. It is a poor source for ultraviolet radiation below about 300 nm. The short wavelength limit of the tungsten lamp depends on the envelope. Glass envelopes begin absorbing below 350 nm, but with fused silica, the lamp is useful to wavelengths somewhat below 300 nm. A tungsten lamp will be found in most UV-visible spectrophotometers, but it will be interchangeable with a hydrogen or deuterium arc lamp, which is used in the UV region below 350 nm.

The deuterium arc lamp (again typically about 30 watts) has a strong continuum from about 370 nm to 190 nm and below. Again, the short wavelength limit is a function of the envelope. In order to get appreciable energy below 190 nm, the lamp must have a special envelope of very high purity fused silica and then it is good to at least 160 nm. The continuum extends to wavelengths longer than

370 nm, but it has an increasingly complex line spectrum superimposed on it which makes it unsuitable for most work.

Either hydrogen or deuterium can be used as the gas in the lamp, but deuterium seems to have a very slight advantage in energy. Arc lamps have a low voltage filament which must be turned on for about a one-minute warm-up before the arc discharge is started by applying high voltage. It is very important for lamp life that the lamp be warmed up before high voltage is applied.

Xenon arc lamps in fused silica envelopes are used in some spectrophotometers, because they have a continuous output throughout the entire UV-visible spectral region. They also have a high intrinsic brightness superior to both W and $D_2$ lamps. The reason they are not more widely used is the instability of the arc, which has a tendency to "wander" from point to point on the electrodes.

Stability of lamp output is important with all lamps, and a highly regulated power supply for the lamps is a standard part of the instrument.

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## 5.2 Monochromator

The purpose of the monochromator is to provide a narrow spectral band of monochromatic light. Desirable characteristics of a monochromator include:

1) High efficiency or throughput
2) Easily selected wavelength
3) Good wavelength accuracy
4) High spectral purity
5) Wide wavelength range
6) Mechanical stability

In a monochromator, white light from the source, comprised of a wide range of wavelengths, is "dispersed" or spread out into a spectrum according to wavelength with the shortest wavelengths at one end and the longest at the other. By rotation of the dispersing element this spectrum can be moved across a narrow slit which permits only a narrow band of wavelengths to leave the monochromator. Wavelength spread of the light passing through the slit is determined by the width of the slit and by dispersion of the dispersing element.

## OPTICAL DIAGRAM OF A SINGLE MONOCHROMATOR

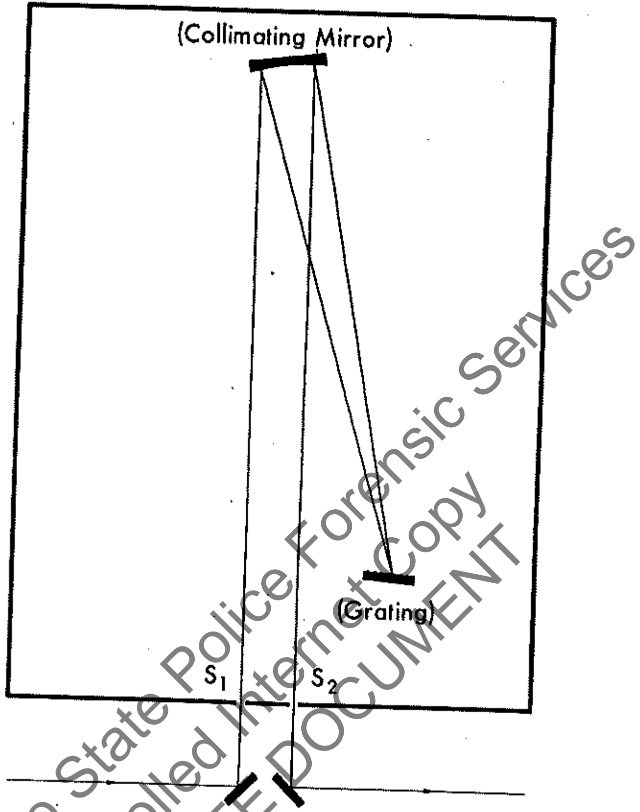


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Figure 5.2 Optical Diagram of a Single Monochromator

A typical monochromator is diagrammed in Figure 5.2. Light from the source passes through an entrance slit, $S_1$, and then to a collimating mirror, which sends a parallel beam of light rays to the dispersing element - in this illustration a diffraction grating. Dispersed light is reflected back to the collimator and then out the exit slit, $S_2$. The dispersing element can be a prism, a variable interference filter, or a diffraction grating.

Variable interference filters, sometimes called wedge filters, are used primarily in low performance, inexpensive instruments. Higher quality spectrophotometers use either prisms or diffraction gratings. Many thousands of prism instruments have been manufactured in the past, but the present trend is toward the exclusive use of gratings as dispersing elements for two major reasons -- gratings have higher dispersion than prisms, and the dispersion is linear over the entire wavelength region in contrast to the non-linear dispersion of prisms. Improved

manufacturing techniques have also improved quality of diffraction gratings while significantly reducing manufacturing costs.

### 5.2.1 Diffraction Gratings

A diffraction grating is essentially a front surface aluminized mirror in which a large number of parallel grooves has been cut. A typical grating for the UV-visible spectral region has about 600 grooves per millimeter, or 15,000 grooves per inch. Dispersion of light by a grating is due to the diffraction of light by the grooves of the grating such that each groove acts as a separate source of diffracted light. When light is reflected at an angle $\theta$ to the normal of the grating, path difference (P.D.) between rays coming from successive grooves of the grating is:

$$P.D. = d \sin \theta$$

where d is the distance between grooves. If the grating is illuminated with monochromatic light (e.g., a sodium vapor lamp) a bright spot occurs at certain fixed angles where:

$$n \lambda = d \sin \theta$$

where n is an integer (1, 2, 3, . . . . . n) and $\lambda$ is the wavelength of the light. This is a condition where the path difference of the rays from succeeding lines of the grating is exactly one wavelength or multiples of one wavelength. At these points the light rays are in phase and combine constructively (constructive interference) to produce a bright spot. At all other angles, there is destructive interference.

If the grating is now illuminated with polychromatic (white) light, a complete spectrum will be observed near each angle. Each angle corresponds to a separate "order" of the grating. The dispersion is proportional to the order number. Efficiency of the grating can be controlled somewhat by controlling shape and angle of the grooves in the grating. It is possible to concentrate up to 75% of the energy into a single order.

The overlapping orders of a grating create one problem in their use. If a grating is being used in the first order, there will also be light of succeeding orders passing through. For example, if the wavelength is set for 600 nm, then 300 nm second order radiation and 200 nm third order radiation will also pass through the exit slit and be seen by the detector.

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Some kind of order sorting device is required to eliminate the unwanted orders. The usual device is a filter which transmits light of the desired wavelength, but is opaque to light from the higher orders. Several filters are built into a rotating device which is programmed automatically to insert the proper filter for each wavelength region.

### 5.2.2 Double Monochromator

Some instruments are built with two monochromators in series (Figure 5.3). In this case, dispersion of the instrument is approximately doubled. Probably more important, however, is the reduction in stray light which is afforded by the double monochromator. Stray light is light of wavelengths other than the desired wavelength. In a well-designed, modern, ultraviolet monochromator, the intensity of stray light will typically comprise less than 0.1% of total output of the monochromator. Even this small amount, however, can cause significant errors in measurements involving strongly absorbing samples. Addition of a second monochromator reduces intensity of stray light by about three orders of magnitude to 0.0001% or less, which is negligible in nearly all applications.

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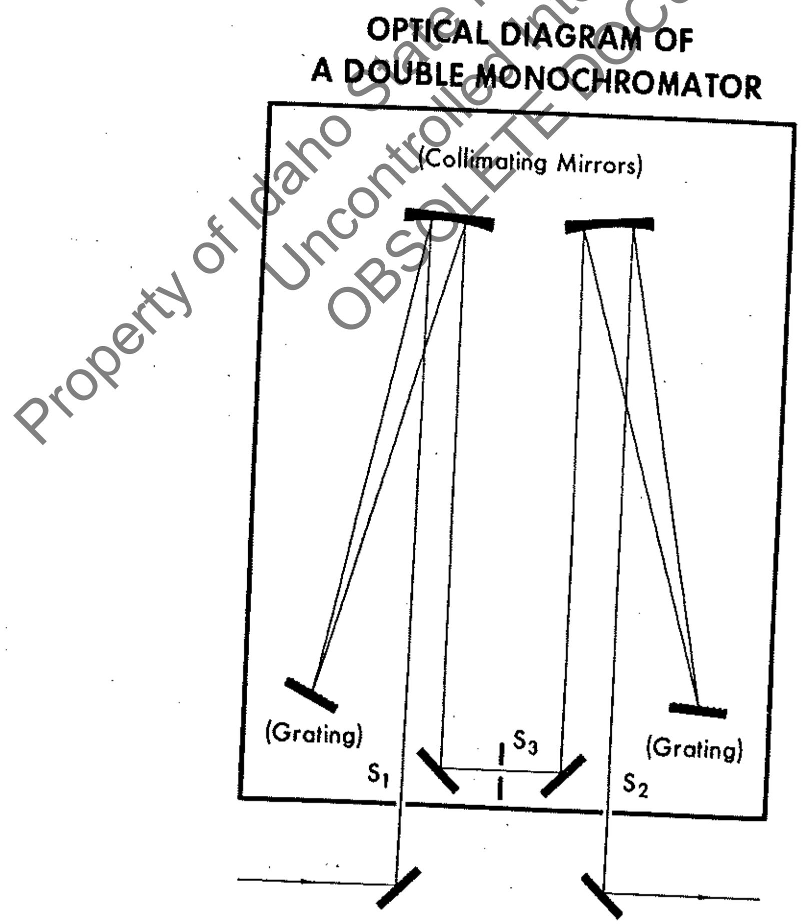


Figure 5.3 Optical Diagram of a Double Monochromator

## 5.3 Sample Compartment

A spectrophotometer should have a large and roomy sample compartment with wide separation between sample and reference beams and working space above and below the beams. A large sample compartment provides space for many different types of sample holders and accessories. Automatic sample changers, cryogenic flasks, titration flasks, and fluorescence accessories are typical of the bulky apparatus that must frequently be accommodated.

## 5.4 Photometer

Light passing through the instrument and sample is measured quantitatively by the photometer. The photometer consists of a detector, amplifier, and signal processing electronics and readout device.

### 5.4.1 Detector

The detector is a device which converts a light signal into an electrical signal. In ultraviolet-visible spectrophotometry the phototube is the detector that is most widely used. A particular type of phototube, called a photomultiplier tube, combines signal conversion with nine or more stages of amplification within the body of the tube. Photomultipliers produce a large electrical signal which is directly proportional to intensity of light striking the photocathode. Sensitivity of the photomultiplier tube can be varied by varying dynode voltage, the bias voltage which is applied across the tube. "Noise" or random variation of the electrical signal from a photomultiplier tube is a function of dynode voltage and signal level. "Shot noise" in a phototube is proportional to the square root of light level. Modern, solid-state electronics circuits contribute very little noise compared with this "shot noise", and the instruments are said to be "shot-noise" limited.

### 5.4.2 Amplifier and Signal Processing Electronics

The electronics sections of modern spectrophotometers are compact, with all solid-state components mounted on interchangeable, plug-in, printed circuit boards. The amplifier raises the signal from the phototube to the desired voltage. By electronic techniques, the amplified signal can be converted to units of transmittance, absorbance, or concentration, and modified by scale expansion or

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contraction and zero suppression. Output signal can be either an analog voltage or a binary-coded decimal (BCD) signal which is fed to some sort of a readout device.

### 5.4.3 Readout Device

The readout device is the component of a spectrophotometer on which results of the measurement are presented. It can be a meter, a digital display, a recorder, a printer, or other type of presentation. An electrical meter makes a convenient readout device, but it is limited in accuracy to about ± 0.5%. A digital voltmeter combines a very readable display with accuracy to 0.1% or 1 digit. Printers are usually used in conjunction with a digital display in order to make a permanent, printed record of a reading. A strip-chart recorder is also frequently used as an adjunct to a meter or digital display, although in some instruments it is the only readout device. The recorder is almost a necessity for determining the shape of an absorption spectrum of a sample in which absorbance or transmittance is plotted versus wavelength. A recorder also offers a convenient means of reading a very "noisy" signal, because the signal can be recorded over a section of chart paper and the average value estimated by inspection.

## 6.0 OPERATION OF A SPECTROPHOTOMETER

This section contains a description of the operation of a simple, double-beam spectrophotometer such as the Beckman ACTA II, ACTA III, or ACTA V. The availability of clean, matched cells of the proper path length is assumed. Because actual operating instructions are contained in the instrument instruction manual, this section will concentrate more on the philosophy of operation and will offer suggestions for optimizing operating parameters.

### 6.1 Selection of Cells

Clean, high quality cells are necessary for precise spectrophotometry. They must be transparent at the wavelength of the measurement. Glass cells are adequate for wavelengths longer than about 350 nm, but fused silica cells should be used at shorter wavelengths. Optical surfaces should be flat, parallel, and free of bubbles and striae. Test tubes are not recommended. High quality plastic cells can be used, but care must be exercised in handling them to avoid scratching the windows and only inert solvents can be used.

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Matched sets of cells should be frequently checked to insure that the cells remain matched. Scratches and deposits on the windows and etching by corrosive chemicals can cause cells to become mismatched. Rectangular cells of 10 mm pathlength are the standard cells, and therefore usually the least expensive. Other path lengths should be used only when necessary.

## 6.2 Absorbance, Concentration, and Transmittance

### 6.2.1 Measurement of Absorbance

This mode is most widely used for both qualitative and quantitative analysis. It has the advantage that the absorbance value is a linear function of sample concentration. This simplifies preparation of a calibration graph, because it is a straight line with one end at the origin.

For quantitative analysis, where possible, the concentration of the sample solution should be such that the absorbance reading is in the range of 0.5 A to 2.0 A. It can be shown mathematically that the instrumental accuracy for a "shot-noise" limited instrument is greatest at an absorbance of about 1.0 A. The relative error curve is as shown in Figure 6.1. It should also be noted that the relative error rises rapidly at low absorbances. Figure 6.2 illustrates how the noise level, and therefore the uncertainty of reading, increases at the higher absorbance levels and higher dynode voltage levels. Sample concentrations can be controlled by dilution or else cells of other path lengths can be used to assure that the absorbance is in the region of optimum accuracy.

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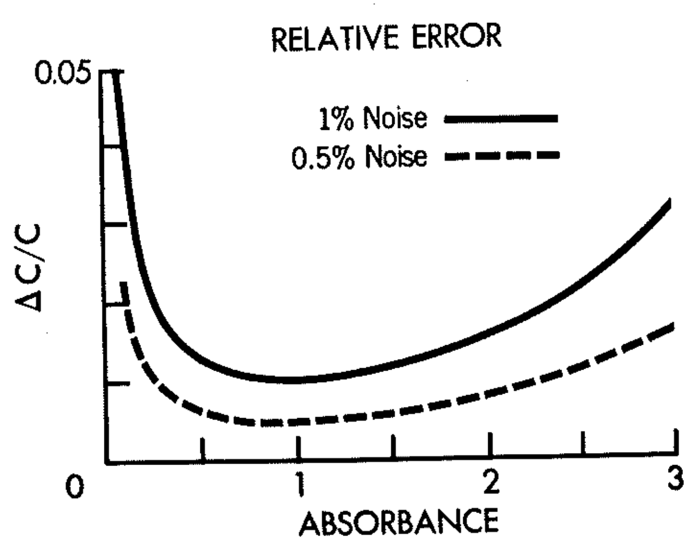


Figure 6.1 Relative Error

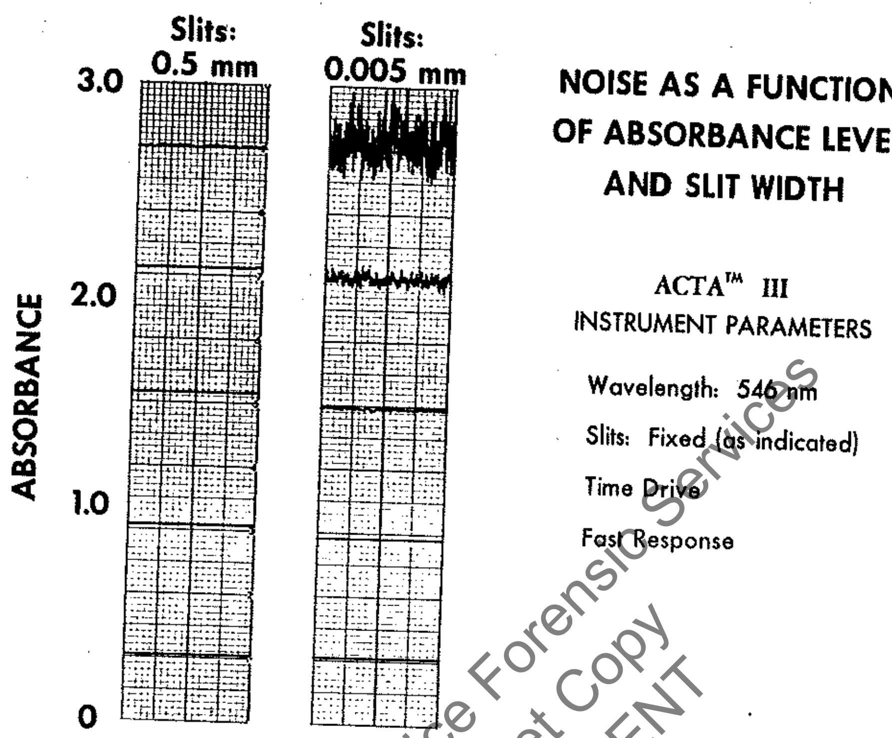


Figure 6.2 Noise as a Function of Absorbance Level and Slit Width

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Use of an expanded scale such as 0 - 1.0 A instead of the 0 - 3.0 A scale for recording absorbance versus wavelength may be desirable when all of the absorption bands lie below 1.0 A.

## 6.2.2 Measurement of Concentration

The concentration mode is extremely useful for quantitative analysis, because it eliminates computation. Analytical results are read from the instrument directly in the desired units. This mode of operation will be used primarily with the digital readout, although it can be used, within limitations, with the recorder as well.

Precision will be improved if the calibration is made at the high end of the scale. For example, if samples are expected to lie in the range 50 to 200 g/liter, a calibration sample should be made up at about 200 g/liter rather than 50 g/liter so that more digits are used in the calibration. When there is a choice, the readout should be set to use the greatest number of digits. For example, when possible, the 200 g/liter calibration sample should be set to read 2000 rather than 200.

## 6.2.3 Measurement of Transmittance

Transmittance has the advantage that all points will be on-scale, although some information may be lost because of inherent scale compression existing near zero transmittance. The transmittance scale is most useful for qualitative scans. It is also quite useful for single beam recording of emission, e.g., in fluorescence analysis or in checking the wavelength scale using a mercury vapor lamp as a source of emission lines.

## 6.3 Resolution, Slit Width, and Band Width

Resolution is a measure of the spectral detail that can be separated by the instrument. It is a function of the dispersion and slit width of the instrument. Very narrow slit widths must be used in order to see as much of the spectral detail as possible in a gas sample. Slit width effects are not limited to resolution of fine structure of absorption bands, however.

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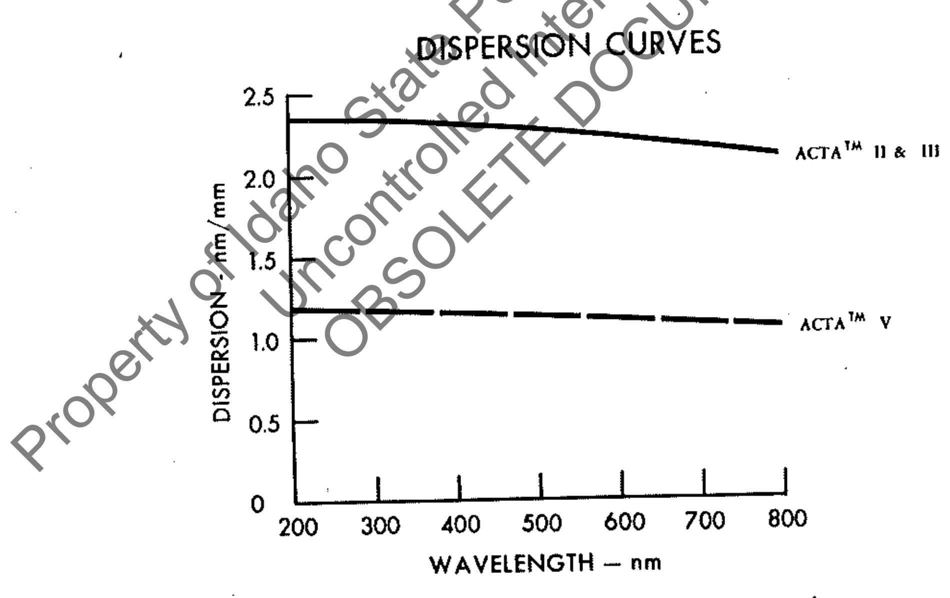


Figure 6.3 Dispersion Curves

Quantitative analysis of relatively broad bands can be significantly affected by slit width. This subject is important enough to deserve a few paragraphs of explanation.

Because the subject of band width can be confusing, the various terms must be defined precisely. If the intensity of the light emerging from the exit slit

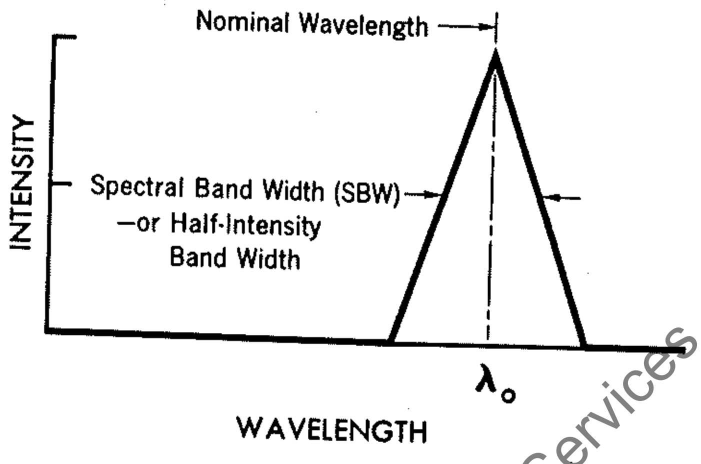


Figure 6.4 Slit Function

of the instrument is plotted against wavelength, it will have a triangular shape when the wavelength is set at $\lambda_o$, as shown in Figure 6.4. In order to determine the effect of slit width, it is necessary to define a similar term for the sample absorption band. The natural band width (NBW) of the sample absorption band is defined as the band width half-way between the background and the peak. The observed band width (OBW) is the width of the absorption band at half its maximum absorbance as graphed by the spectrophotometer. The observed band width may be wider but never narrower than the natural band width. The narrower the spectral band width, the closer the observed band width will be to the natural band width, and the closer the instrument will come to recording the true band shape and height. This is shown graphically in Figure 6.5. This graph indicates that the SBW should be much narrower than the NBW for the best precision. Actually, this is usually the case for most samples in liquid solutions, because the absorption bands are relatively wide compared with the spectral band width. Typical natural band widths are 5 nm to 50 nm. Typical spectral band widths are 0.1 nm to 1 nm. Much sharper natural band widths are encountered in gases and some solids. Solutions of some of the rare earth salts have sharp bands with NBW's of 1 nm to 5 nm.

## 6.4 Stray Light Effects

Stray light is light contributed to the signal by wavelengths other than the desired wavelength. In instruments with double monochromators (ACTA V) stray

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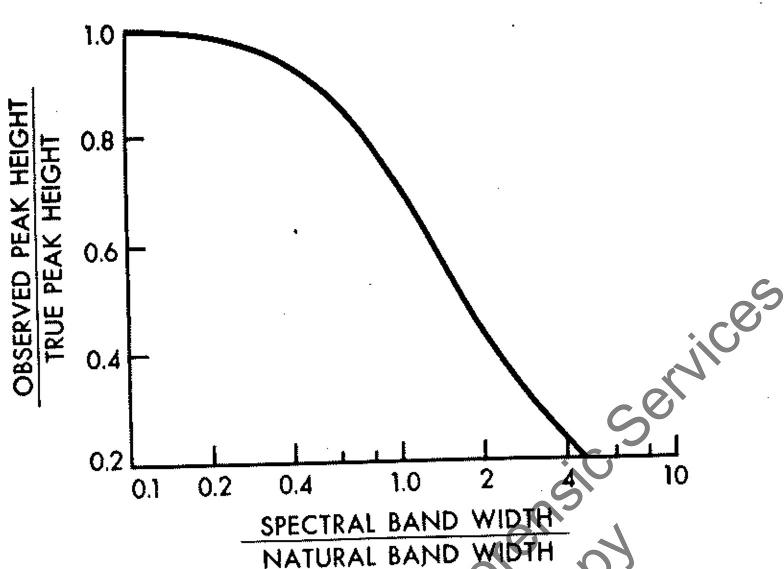


Figure 6.5 Observed Peak Height/True Peak Height vs. SBW/NBW

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light is typically less than 0.0001%, but in instruments with single monochromators it may reach as much as 0.1% at some wavelengths. In most work, the effect of stray light is negligible, but at higher absorbance levels it can become significant. For example, a stray light level of 0.01% causes errors in measured absorbance of 0.2% at an absorbance of 2.0 and 1.4% at an absorbance of 3.0. See Figure 6.6. In a given analysis, measurement of stray light values is difficult, because stray light level is affected by sample absorption at wavelengths contributing stray light. Stray light effects in a given analysis can be precisely evaluated only by plotting actual absorbance readings against concentration and observing departure from linearity. Stray light effects can be minimized by:

a) Sample dilution to reduce absorbance values

b) Optical scale expansion by using an absorbing reference of known absorbance

c) Insertion of a stray light filter in the instrument

Stray light filters can be quite effective and are of two types, depending on the wavelength of the measurement. So-called "notch" filters transmit only a narrow band of wavelengths, including the wavelength of interest, and absorb essentially all other wavelengths (which contribute the stray light). "Sharp-cut" filters absorb all wavelengths shorter than the "cut-off" wavelength and

transmit the longer wavelengths. These filters are useful at the red light end of the wavelength scale where most of the stray light is due to the shorter wavelengths.

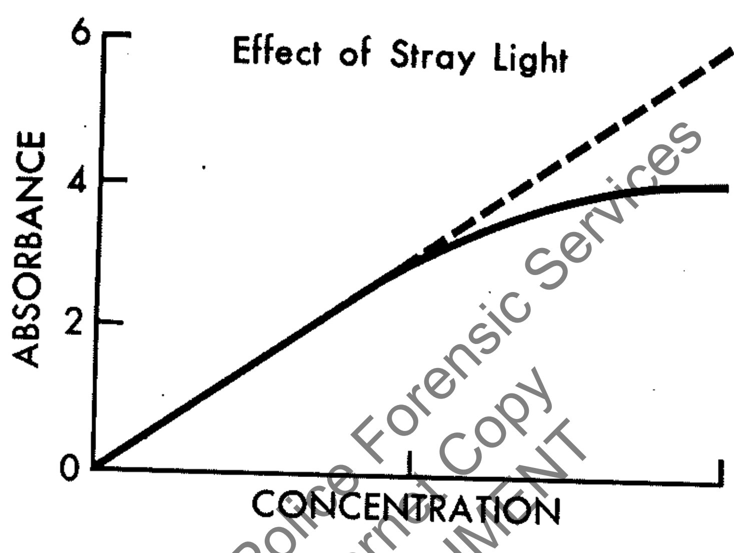


Figure 6.6 Effect of Stray Light

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## 6.5 How to Set the Controls

### 6.5.1 Setting Zero in Absorbance and Concentration Modes

Matched cells containing solvent only should be placed in the sample and reference beams of the instrument. With the wavelength set at the center of the absorption band, adjust the zero control (OA) until the instrument reads exactly 0.00. Setting zero in this manner will automatically compensate for any cell mismatch and will assure maximum accuracy. This procedure should be followed for both static measurements at a fixed wavelength and for scanning through a wavelength region.

Note: In % T operation, follow exactly the same procedure for setting the 100% control.

### 6.5.2 Setting the Wavelength

For most samples, once the wavelength of maximum absorbance has been determined, manual selection of that wavelength usually suffices. Always approach the

setting from the same direction (preferably moving from long toward shorter wavelengths) for maximum repeatability. For samples with very sharp absorption bands, it may be necessary to scan back and forth manually through the region of the absorption band in order to locate the exact wavelength of maximum absorption. When the measurement is to be made at an inflection point or in a mixture with overlapping absorption bands, the procedure of scanning through the region of interest and recording the information is usually preferable.

### 6.5.3 Setting the Slit

a) Fixed or Programmed Slit - Quantitative measurements at a single wavelength or over a short wavelength span (< 20 nm) should be made with a fixed slit. All other double-beam measurements should use the programmed slit mode.

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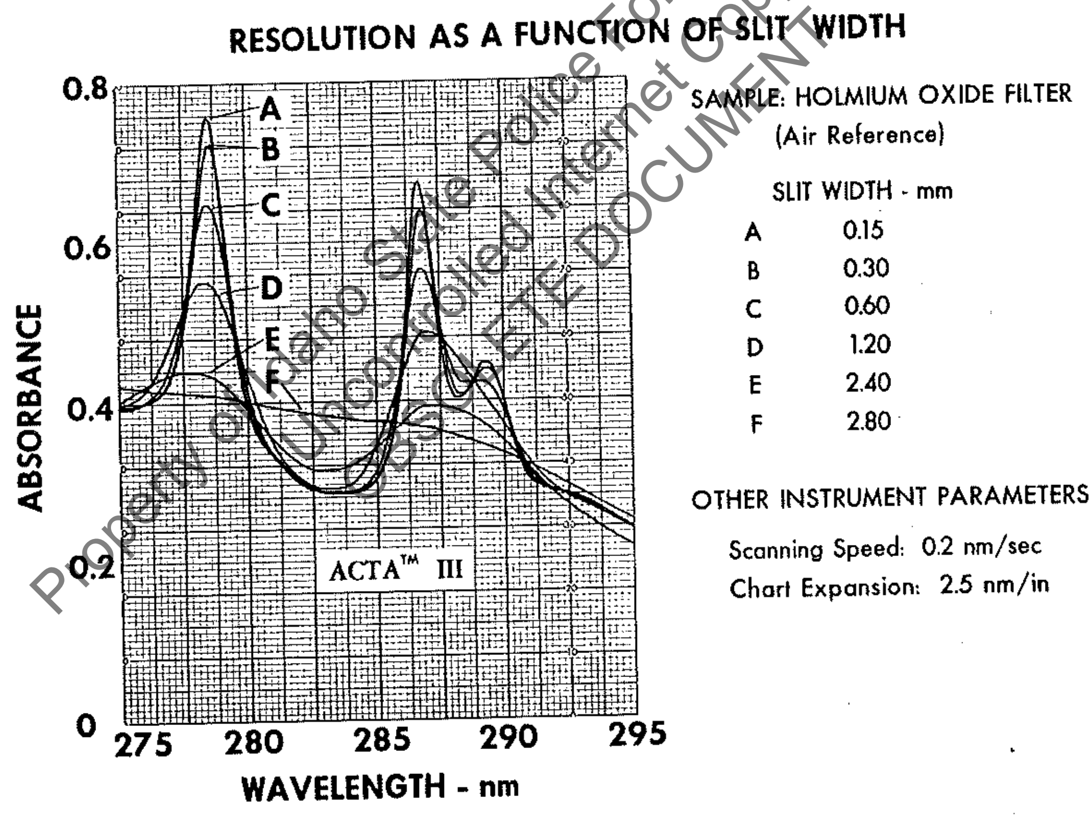


Figure 6.7 Resolution as a Function of Slit Width

b) Selecting the Proper Slit Width - Slit width determines band width and, as discussed in Section 6.3, the narrower the spectral band width, the closer the measurement will be to the true shape and intensity of the

sample absorption band. It must be remembered, however, that the slit width also determines the amount of energy passing through the monochromator and therefore the size of the signal. When light reaching the detector is of low intensity, the instrument will automatically increase dynode voltage. As dynode voltage increases, noise increases and therefore places a limit on reduction of slit width. Slit width and resolution must always be balanced against noise. In an instrument which is "shot-noise" limited, noise is proportional to resolution.

NOTE: Resolution varies directly with slit width, while energy varies with the square of slit width. In the "shot-noise" limited instrument, noise is proportional to the square root of the energy, and therefore noise is proportional to resolution:

$$R \propto S$$

$$E \propto S^2 \qquad \text{Therefore: } N \propto \sqrt{E} \propto S^2 \propto R$$

$$N \propto \sqrt{E}$$

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In actual practice, using the Beckman ACTA instruments, it is customary to set the slit width on the basis of dynode voltage, which is the controlling factor in determining noise level. Trial scans through the absorption band are then made at the selected slit width and at one-half the selected slit width; if there is no significant difference in peak height between the two scans, then the selected slit width is satisfactory. If a significant difference is observed, then another run should be made at one-fourth the selected slit width and the process repeated until no further change is observed. If it is necessary to go to a very narrow slit width with consequent high noise level, it may be necessary to increase response time of the instrument in order to reduce noise level. Fortunately, the high resolution of the grating monochromator assures that nearly all of the absorption bands of liquid solutions can be completely resolved with relatively wide slit widths and consequent low noise levels. Only in gases and some solids will resolution capabilities of the ACTA instrument be taxed.

## 6.5.4 Setting the Scanning Speed

Since time is precious, scanning speed should usually be as fast as possible. However, if the instrument scans too fast, it loses information because the pen cannot follow the energy changes in the system. This loss of information shows

up in three ways:

a) reduced resolution
b) shift in wavelength of absorption maxima
c) reduced absorbance values

When the instrument is scanning at the rate of one natural band width (NBW) per period, the observed peak height will be only about 70% of true peak height (Figure 6.5). In order to get within 1% of true peak height, it is necessary to reduce scanning speed to about 1/5 to 1/10 NBW per period. Spectral band width of the instrument is controlled by slit width (Paragraph 6.3). If the approximate natural band width of the sample is known, it is possible to use the dispersion curve of the instrument (Figure 6.3) to select the appropriate slit width and scanning speed to achieve the desired results. Tables 6.1 and 6.2 list recommended settings for various types of samples. For quantitative analyses it is imperative that all instrument operating parameters (slit width, scanning speed and period) be identical for both calibration and measurement runs.

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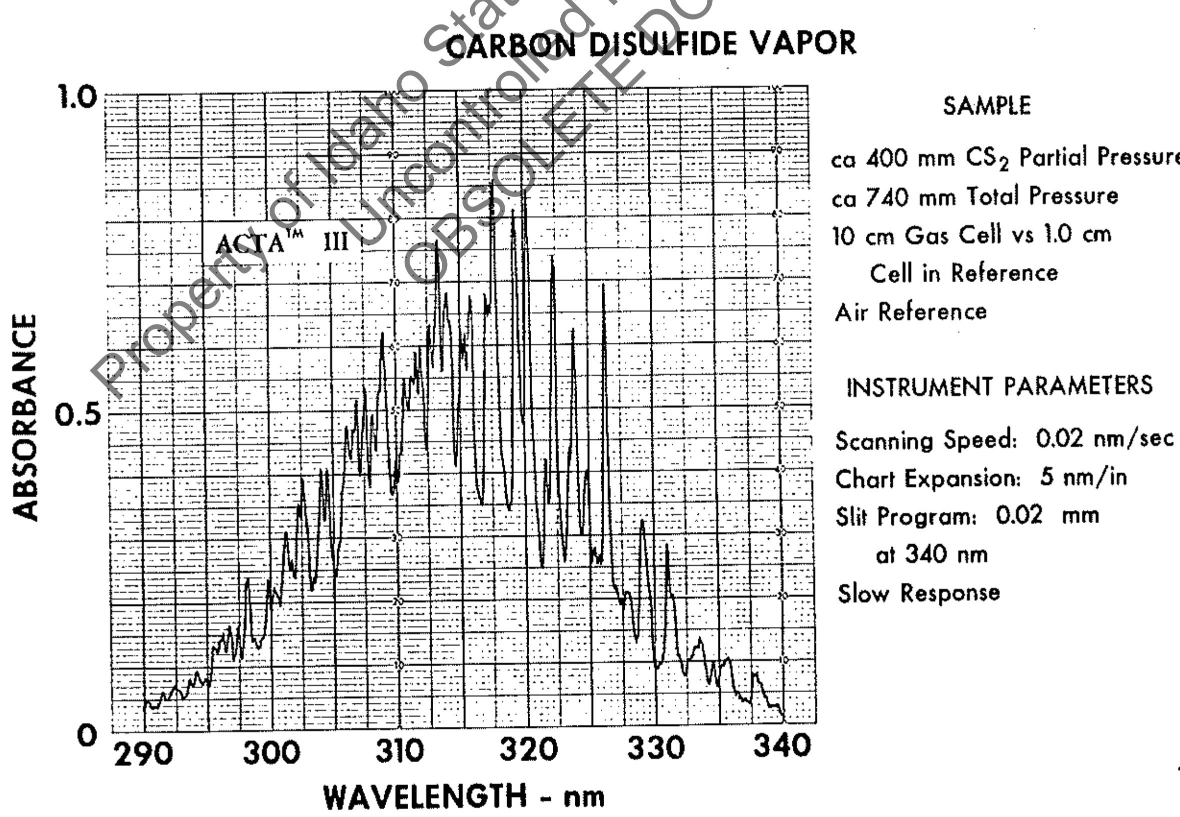


Figure 6.8 Carbon Disulfide Vapor

TABLE 6.1

Recommended Operating Conditions for ACTA CIII

| If minimum NBW is (nm) | SBW required (nm) | Maximum Slitwidth (mm) | Scanning Speed (nm/sec) | Period* (sec) |
|---|---|---|---|---|
| For quantitative analysis | | | | |
| 0.5 | 0.1 | 0.045 | 0.1 | 0.5 |
| 1 | 0.2 | 0.09 | 0.2 | 0.5 |
| 5 | 1 | 0.45 | 1.0 | 0.5 |
| 10 | 2 | 0.9 | 2.0 | 0.5 |
| 50 | 10 | 4.5 | Maximum | 0.5 |
| For qualitative analyses | | | | |
| 0.5 | 0.2 | 0.09 | 0.2 | 0.5 |
| 1 | 0.4 | 0.18 | 0.5 | 0.5 |
| 5 | 2 | 0.9 | 4 | 0.5 |
| 10 | 4 | 1.8 | 4 | 0.5 |

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TABLE 6.2

Recommended Operating Conditions for ACTA CV

| If minimum NBW is (nm) | SBW required (nm) | Maximum Slitwidth (mm) | Scanning Speed (nm/sec) | Period* (sec) |
|---|---|---|---|---|
| For quantitative analyses | | | | |
| 0.5 | 0.1 | 0.09 | 0.1 | 0.5 |
| 1 | 0.2 | 0.18 | 0.2 | 0.5 |
| 5 | 1 | 0.9 | 1.0 | 0.5 |
| 10 | 2 | 1.8 | 2.0 | 0.5 |
| 50 | 10 | 7 | Maximum | 0.5 |
| For qualitative analyses | | | | |
| 0.5 | 0.2 | 0.18 | 0.5 | 0.5 |
| 1 | 0.4 | 0.36 | 1 | 0.5 |
| 5 | 2 | 1.8 | 4 | 0.5 |
| 10 | 4 | 3.5 | Maximum | 0.5 |

*Note: All of the settings in Tables 6.1 and 6.2 assume a period of 0.5 sec. If noise is too great under these conditions, it can be reduced by increasing the period of the instrument. An increase in period should be compensated by a proportionate decrease in speed. If the period is increased to 2 seconds, the speed must be decreased by a factor of 4.

# 7.0 OTHER MEASUREMENTS

## 7.1 Tristimulus Color Measurement

### 7.1.1 Introduction

". . . . while serving as the basis for color specification, a spectrophotometric curve (or table of data) is quite inadequate as a color specification in itself, particularly if colorimetric tolerances are desired as part of the specification. For adequate color specifications with tolerances, the spectrophotometric data must be converted to some form of tristimulus specification by means of methods and data defining a standard observer, a standard coordinate system, and a standard illuminant." (Spectrophotometry, National Bureau of Standards Circular 484)

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The color response of the human eye may vary from person to person. Colored objects, as paint chips, dyed swatches of cloth, ceramic tiles, and colored solutions sometimes used as color standards, may fade or change with age. In order to avoid these difficulties and to place measurement and specification of color on a scientific basis, the International Commission on Illumination*, in a series of international agreements, adopted a set of standards which make it possible to define color in absolute terms. These standards, used in conjunction with instrumental measurements, provide a highly precise method for evaluating and controlling color systems and for correlating results obtained in different laboratories.

### 7.1.2 CIE System of Color Specificaton

The color vision of a normal human observer is tridimensional, i.e., there are at least three independent excitations corresponding to three primary colors, red, blue, and green, which are necessary for normal color vision. The CIE has defined a "standard observer" and standard illuminants from which are derived the tristimulus specifications now widely used in color work. Any given color

* More commonly known as the Commission Internationale d'Eclairage (CIE)

can be specified in terms of the relative amounts of the three CIE primaries required to produce that color (Figure 7.1). The amounts of red, green, and blu primaries are designated by X, Y, and Z. The green (Y) primary was chosen so that it carries all of the luminosity, or brightness. Thus, the Y value is a measure of the luminous reflectance or the luminous transmittance.

The X, Y, and Z values can be calculated from a spectrophotometric curve. The spectral curve can be obtained from transmittance measurements of transparent samples such as solutions, plastic sheets or film, glass plates, and other similar samples. By means of special attachments, the reflectance of solid samples such as colored paper, leather, painted surfaces, and similar materials can be measured.

The instrument produces a spectrophotometric curve, but more information is needed for a complete color evaluation. Results depend not only on the curve, but also on the type of illumination under which the sample is to be viewed. The standard illuminants, A, B, and C, correspond to a tungsten lamp, average noon sunlight, and average daylight. Illuminant C, corresponding to average daylight, is most widely used.

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### 7.1.3 Selected Ordinate Method

A number of methods are available for calculating tristimulus values. The selected ordinate method will be described here, since it is much less tedious than the other methods. Depending on accuracy required and shape of the spectrophotometric curve, up to 300 separate readings are required. Ten selected ordinates can give significant information, but 30 selected ordinates are recommended for most work. In the selected ordinate method, tristimulus values X, Y, and Z are obtained by merely adding measured reflectances (or transmittances) at specified wavelengths and multiplying by a factor.

The X, Y, and Z tristimulus values are seldom used directly. They are normally converted to the chromaticity coordinates x, y, and z, which give the interrelationship among the three values.

$$x = \frac{X}{X + Y + Z} \quad (1) \qquad y = \frac{Y}{X + Y + Z} \quad (2) \qquad \text{and} \qquad x + y + z = 1 \quad (3)$$

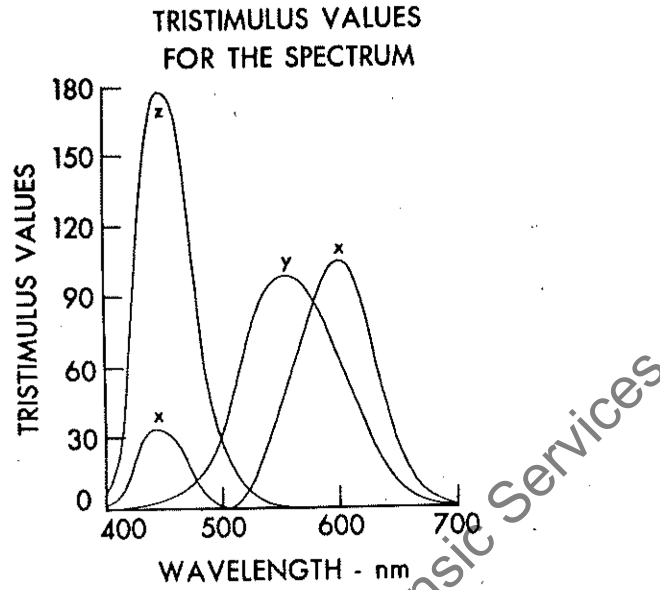


Figure 7.1 Tristimulus Values for the Spectrum

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The desired factors are usually the Dominant Wavelength or hue, the Color Purity or saturation, and the Visual Efficiency or brightness, all terms which can be easily understood. Color Purity is a measurement of the saturation of the Dominant Wavelength with respect to the illuminant. In the sample given below, the Dominant Wavelength is 488.9 nm and the Color Purity is 20.9%. This means that 79.1% of the response is due to the illuminant and 20.9% is due to spectral light of the Dominant Wavelength. Brightness, or Visual Efficiency, is a measure of transmission or reflection efficiency and is independent of hue and purity. Dominant Wavelength and Color Purity are obtained from an (x,y)-chromaticity diagram (Figure 7.2), and Visual Efficiency is equal to Y. Large scale (x,y)-chromaticity diagrams for Illuminant C are contained in Hardy's "Handbook of Colorimetry". Single diagrams and complete sets are available from the publisher*

## 7.1.4 Calculation of Trichromatic Coefficients

Blue ceramic tile has been chosen as a sample to illustrate the procedure used in determination of color characteristics. Figure 7.3 is the reflectance curve of this tile using magnesium oxide as a reference. Reflectance for each specified wavelength ordinate is read from the curve. For example, at 435 nm the

* The Technology Press of MIT, Cambridge 39, Mass.

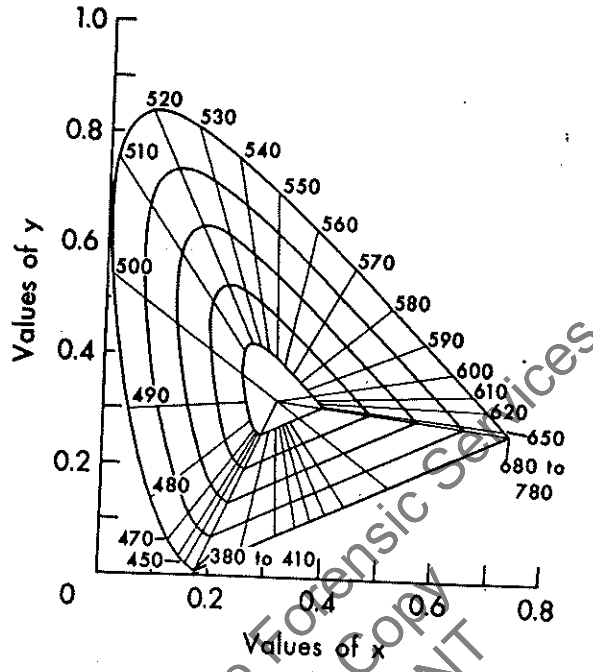


Figure 7.2 Chromaticity Diagram

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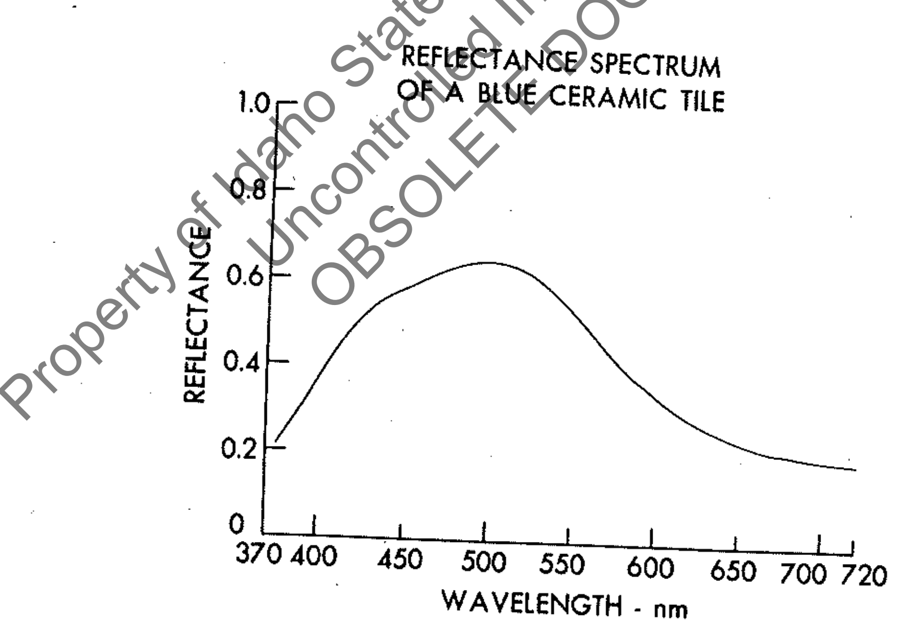


Figure 7.3 Reflectance Spectrum of a Blue Ceramic Tile

reflectance is 55% or 0.550. Reflectance values are entered on a chart similar to Figure 7.4. All of the reflectance values in the X column are then added, and the sum is multiplied by the factor 0.03269 (a proportionality factor based

on the tristimulus values of the illuminant) to give the value X. The values Y and Z are obtained in a similar manner. The trichromatic coefficients are calculated using formulas (1), (2), and (3). Visual Efficiency is equal to Y, 48.4%. By using the trichromatic coefficients x and y, and referring to the chromaticity diagrams in Hardy's "Handbook of Colorimetry", the Dominant Wavelength of 488.9 nm and Color Purity of 20.9% are obtained.

## THIRTY SELECTED ORDINATES FOR ILLUMINANT C

| X λ | X R or T | Y λ | Y R or T | Z λ | Z R or T |
|---|---|---|---|---|---|
| 424.4 | .511 | 465.9 | .613 | 414.1 | .465 |
| * 435.5 | .551 | * 489.4 | .642 | * 422.2 | .503 |
| 443.9 | .570 | 500.4 | .645 | 426.3 | .520 |
| 452.1 | .584 | 508.7 | .640 | 429.4 | .532 |
| * 461.2 | .602 | * 515.1 | .633 | * 432.0 | .538 |
| 474.0 | .624 | 520.6 | .622 | 434.3 | .549 |
| 531.2 | .597 | 525.4 | .613 | 436.5 | .553 |
| * 544.3 | .550 | * 529.8 | .599 | * 438.6 | .560 |
| 552.4 | .520 | 533.9 | .583 | 440.6 | .565 |
| 558.7 | .488 | 537.7 | .570 | 442.5 | .568 |
| * 564.1 | .467 | * 541.4 | .565 | * 444.4 | .570 |
| 568.9 | .442 | 544.9 | .548 | 446.3 | .575 |
| 573.2 | .429 | 548.4 | .534 | 448.2 | .579 |
| * 577.3 | .411 | * 551.8 | .520 | * 450.1 | .582 |
| 581.3 | .399 | 555.1 | .506 | 452.1 | .585 |
| 585.0 | .383 | 558.5 | .492 | 454.0 | .588 |
| * 588.7 | .370 | * 561.9 | .478 | * 455.9 | .592 |
| 592.4 | .364 | 565.3 | .464 | 457.9 | .600 |
| 596.0 | .351 | 568.9 | .442 | 459.9 | .601 |
| * 599.6 | .339 | * 572.5 | .430 | * 462.0 | .604 |
| 603.3 | .330 | 576.4 | .415 | 464.1 | .610 |
| 607.0 | .317 | 580.5 | .400 | 466.3 | .614 |
| * 610.9 | .308 | * 584.8 | .382 | * 468.7 | .618 |
| 615.0 | .297 | 589.6 | .368 | 471.4 | .621 |
| 619.4 | .288 | 594.8 | .355 | 474.3 | .626 |
| * 624.2 | .276 | * 600.8 | .334 | * 477.7 | .632 |
| 629.8 | .266 | 607.7 | .314 | 481.8 | .637 |
| 636.6 | .257 | 616.1 | .295 | 487.2 | .640 |
| * 645.9 | .240 | * 627.3 | .270 | * 495.2 | .644 |
| 663.0 | .222 | 647.4 | .239 | 511.2 | .638 |
| TOTAL | 12.353 | TOTAL | 14.511 | TOTAL | 17.509 |
| FACTOR | .03268 | FACTOR | .03333 | FACTOR | .03938 |

$X = .03268\Sigma_X = .4037$ $\qquad x = \frac{X}{X+Y+Z} = .2560$

$Y = .03333\Sigma_Y = .4836$ $\qquad y = \frac{Y}{X+Y+Z} = .3067$

$Z = .03938\Sigma_Z = .6895$ $\qquad z = \frac{Z}{X+Y+Z} = .4373$

Dominant Wavelength 488.9 nm
Purity 20.9%
Visual Efficiency 48.4%

Method of computation for 10 selected ordinates (Illuminant C) using ordinates designated with asterisks.

| X | Y | Z |
|---|---|---|
| Total 4.114 | Total 4.853 | Total 5.843 |
| Factor .09804 | Factor .1000 | Factor .11812 |

$X = .09804\Sigma_X = .4033$ $\qquad x = \frac{X}{X+Y+Z} = .2554$

$Y = .1000\Sigma_Y = .4853$ $\qquad y = \frac{Y}{X+Y+Z} = .3074$

$Z = .11812\Sigma_Z = .6903$ $\qquad z = \frac{Z}{X+Y+Z} = .4372$

Dominant Wavelength 489.0 nm
Purity 21.2%
Visual Efficiency 48.5%

Figure 7.4 Thirty Selected Ordinates for Illuminant C

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These results are based on use of the 30 selected ordinate method of calculatio Also, included in Figure 7.4 are results based on the 10 selected ordinate method. Results from the two methods obviously agree very well. Choice of a method must depend on accuracy required. As the number of selected ordinates is increased, proportionately smaller color differences can be reliably measured.

The method described here can be used with either transparent or opaque samples. Selected ordinates for illuminants A and B are provided in Hardy's "Handbook of Colorimetry". Typical uses for this type of color measurement are to be found in the ceramic, textile, plastics, paint, glass, dye, paper, ink, and leather industries - in fact, in any industry in which uniformity of color is important.

## 7.2 Measurement of Film Thickness by Reflectance

### 7.2.1 Introduction

The infrared reflectance method of Spitzer and Tannenbaum* has proved most suitable for determination of semiconductor thin film thickness. This method which uses the interference fringes produced when the sample is examined in the 10 - 35 µm region with an infrared spectrophotometer, is primarily useful for deposits of silicon on silicon and germanium on germanium. A limitation of this method is its application to very thin films (< 1 µm thickness) since the number of fringes is diminished, thus impairing accuracy of the determination.

In some semiconductor devices, a thin (~ 1 µm) layer of silicon dioxide is applied to a polished silicon wafer. Since certain electrical properties of the device are dependent on this film thickness, it becomes desirable from both a research and a control viewpoint to be able to determine this thickness quickly, accurately, and non-destructively. Thicknesses of this order are readily determined by an ultraviolet-visible reflectance technique using a spectrophotometer and specular reflectance accessory.

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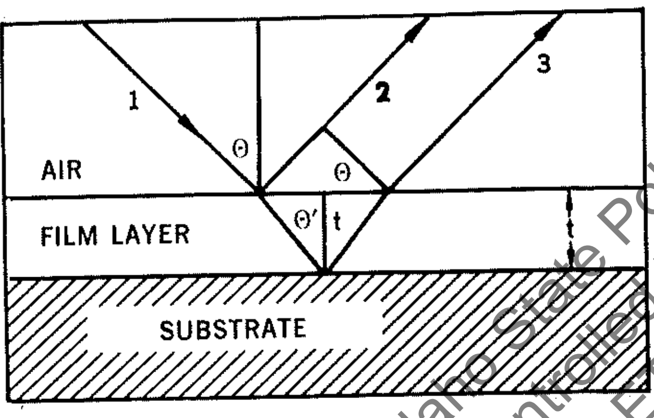
* W. G. Spitzer and M. Tannenbaum, J. Applied Physics 32, 744 (1961).

The method described here is based on that of Corl and Wimpfheimer* and may be carried out on sample areas as small as 1 mm diameter (0.78 $mm^2$ area) by reference beam attenuation and/or recorder scale expansion.

## 7.2.2 Theory

The interference fringe method has been employed for many years for measuring infrared cell path lengths. A similar phenomenon occurs for the case of a film deposited on a reflecting surface. An understanding of the phenomenon which gives rise to these fringes may be acquired by reference to Figure 7.5.

LIGHT REFLECTION IN A THIN FILM




An incident beam of light, 1, strikes the surface of the epitaxial film at an angle θ from the normal. Part of the beam is reflected at the front surface as beam 2, while a portion of the remainder is refracted by the film layer, traverses the film, is reflected at the interface of film and substrate, and finally emerges from the film as beam 3. While beams 2 and 3 both enter the spectrometer, the combined intensity of the net resultant ray at any given wavelength is a function of the phase difference, if any, between the two at that wavelength.

Figure 7.5 Light Reflection in a Thin Film

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If the wavelength of incident light is continuously varied, as when scanning a spectrum, the result will be a continuous series of maxima at wavelengths at which constructive interference occurs (reinforcement), and minima at wavelengths at which destructive interference occurs (cancellation). The reflection spectrum obtained is the familiar "fringe pattern", as shown in Figure 7.6.

For constructive interference to occur, with a subsequent reinforcement of intensity, film thickness and wavelength must be related by the expression:

$$2t \cos \theta' n_f = M \lambda \qquad (1)$$

* E. A. Corl and H. Wimpfheimer, Solid State Electronics 7, 755 (1964).

where $t$ = thickness of film in microns

$\theta'$ = internal angle of the beam from the normal after refraction by the film

$n_f$ = refractive index of film material at wavelength $\lambda$

$M$ = any positive integer

$\lambda$ = wavelength of the light ray in microns

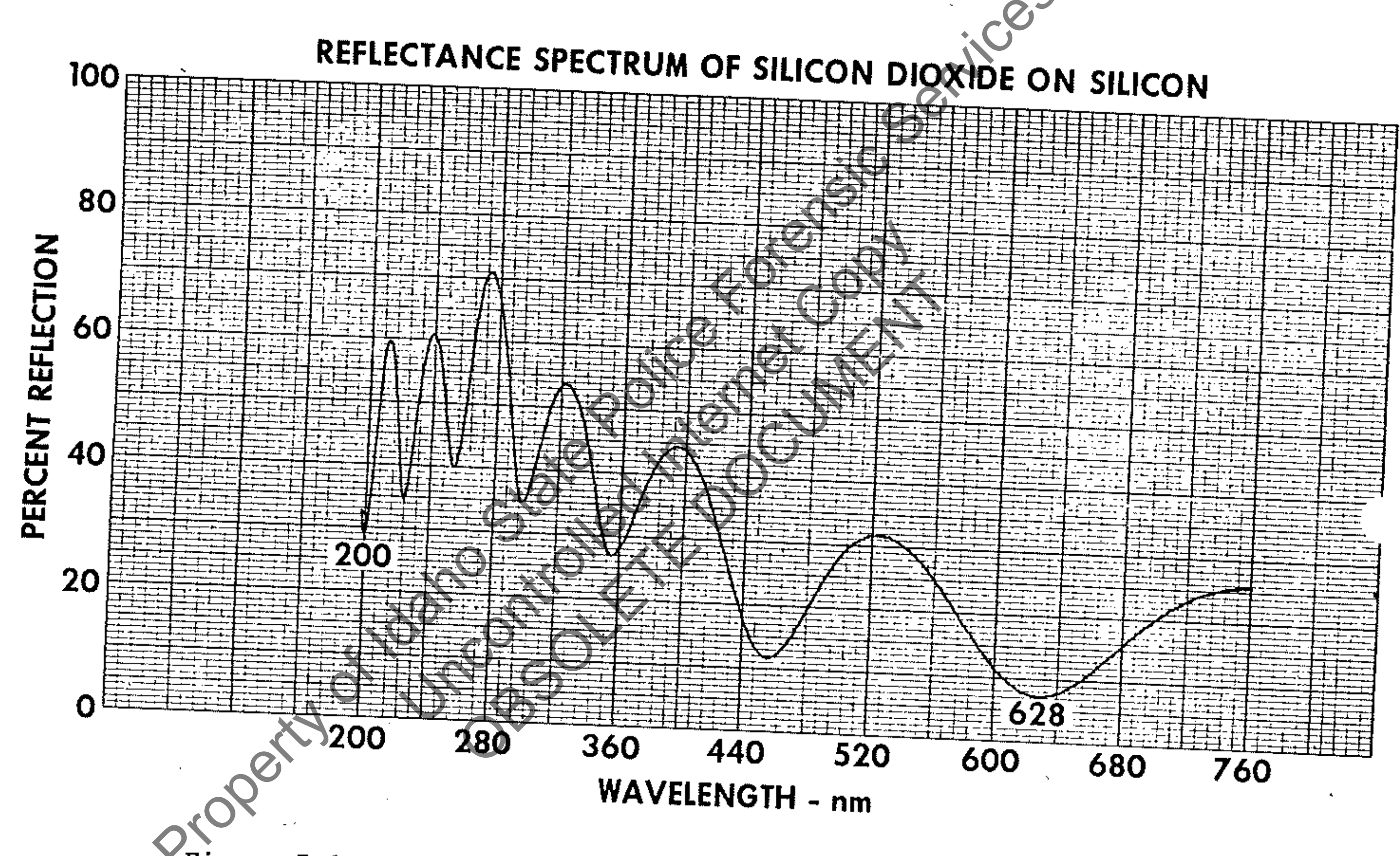

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Figure 7.6 Reflectance Spectrum of Silicon Dioxide on Silicon

Since M may be any whole number, there will be a series of values of $\lambda$ for which constructive interference may occur. While it is not possible to determine any given M value, the difference between any two M values is represented by the number of complete cycles in the reflection curve between two chosen wavelengths. Thus, at some convenient wavelength, $\lambda_1$, at which a maximum is found:

$$2t \cos \theta' n_f = M_1 \lambda_1 \tag{2}$$

and at another maximum at $\lambda_2$:

$$2t \cos \theta' n_f = M_2 \lambda_2 \qquad (3)$$

Subtraction of Equation (3) from Equation (2) and the expression of $\cos \theta'$ in terms of $1-\sin^2 \theta$ leads to the following expression, which may be used for calculation of film thickness.*

$$t = \frac{M'}{2\sqrt{n_f^2 - \sin^2 \theta}} \cdot \frac{(\lambda_1 \lambda_2)}{(\lambda_1 - \lambda_2)} \qquad (4)^{**}$$

where $M'$ is the number of fringes counted.

## 7.2.3 A Typical Example

As an illustration of the calculation, the reflectance spectrum of silicon dioxide on silicon in Figure 7.6 exhibits six complete fringes between 628 and 200 nm. The incident angle in the specular reflectance accessory is 20°, and $\sin \theta = 0.342$. The index of refraction for $SiO_2$ in this region is taken as 1.50.

Thus, from Equation (4)

$$t = \frac{6}{2 \times \sqrt{(1.5)^2 - (0.342)^2}} \times \frac{0.628 \times 0.200}{0.628 - 0.200} = 0.604 \ \mu m$$

## 7.2.4 Advantages, Limitations, and Sources of Error

Minimum oxide thicknesses which may be determined by this method are of the order of 1000 Å (0.1 µm). Oxide purity, condition of substrate surface, and accuracy of refractive index data also pose limitations as to the absolute accuracy of this procedure. In addition, accuracy in reading wavelength maxima and minima from the recorder chart places limitations on the overall accuracy. However, additional accuracy in locating wavelength maxima on broad bands may be obtained by using ordinate scale expansion, as suggested by Sloane and Ulrich*.

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* H. Sloane and W. Ulrich, Appl. Spectroscopy 18, 65 (1964).

** The complete derivation of this expression is given in the papers by Spitzer and Tannenbaum and by Sloane and Ulrich. For the sake of brevity it has been omitted from this discussion.

This method of film thickness determination has been found to be as accurate for mechanically polished samples - and more accurate for chemically polished samples - than either the weighing or double beam interferometric methods. The relative speed of the spectrophotometric method makes this procedure readily adaptable to production control testing.

Note that this procedure can be used with instruments that record either absorbance or transmittance, since it is only the wavelength of the maxima and not the amplitude that is of importance.

## 7.3 Kinetic Studies

Studies of reaction rates can frequently be accomplished using a spectrophotometer. If either a product or one of the reactants has a distinctive absorption band, the change in concentration can be followed during the course of the reaction. In a typical analysis, absorbance is plotted as a function of time. The shape and slope of the plot can be used to determine the order of the reaction or the concentration of one of the reactants. For example, a first order reaction is one in which the rate of reaction is directly proportional to the concentration of the reacting substance. It can be shown mathematically that a reaction is first order if a straight line is obtained when the logarithm of the concentration is plotted against time, and a reaction is third order if a straight line is obtained when $\frac{1}{C^2}$ is plotted against time.

Temperature control is very important in measuring reaction rates, because the rate is markedly influenced by temperature. A good rule of thumb is that an increase of 10°C will cause the reaction rate to double.

The special field of enzyme kinetics has grown very rapidly in recent years. Enzymes are natural catalysts which are involved in nearly all biochemical reactions. Normally a given enzyme is very specific and catalyzes only one particular reaction, e.g., lactate dehydrogenase catalyzes the oxidation of lactic acid to pyruvic acid. Any study of biochemical reactions almost always involves a study of enzyme kinetics. Spectrophotometric methods are frequently the most convenient means of following these reactions, particularly since the discovery of biochemical oxidation-reduction indicators such as DPNH (reduced diphosphopyridine nucleotide).

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Thermostatically controlled cell holders, automatic sample changers, and other special accessories simplify and increase accuracy of kinetic measurements.

## 7.4 Thermal Denaturation Studies ($T_m$ Analysis)

Investigation of thermal denaturation properties of nucleic acids has become a very important field. The double-stranded, helical configuration of DNA (deoxyribonucleic acid) can be ruptured by elevated temperatures. The temperature at which this transition takes place is characteristic of the particular DNA and of the ionic strength of the solution. The transition temperature (melting temperature or $T_m$) can be detected by the change in light transmission characteristics.

The mechanics of inducing these transitions thermally are simple. The temperature of the nucleic acid solution is systematically raised and the absorbance of the solution is plotted against temperature. The resultant plot is S-shaped with temperature as the abscissa and absorbance as the ordinate. Absorbance may be expressed either directly or as percentage increase over the absorbance at the starting temperature, or as a ratio $A_t/A_{25°C}$. A generalized $T_m$ is defined as the midpoint of the total absorbance change. For example, if the initial absorbance is 0.2 and final absorbance is 0.8, $T_m$ is the temperature at which A = 0.5. Temperature programming rates are normally less than 10°C/min, and 1°/min is a typical rate. Because transition normally occurs within a very narrow temperature range, too rapid programming would tend to distort the curve and produce an erroneous $T_m$ value. Initial and final temperatures generally fall within the 25 - 100°C range. There is a fairly wide variation in $T_m$, depending on the nature of the preparation, but values seldom exceed 100°C.

Instrumentation for $T_m$ analysis requires a variable temperature cell holder and some means of programming temperature. The simplest system utilizes an electrically heated cell holder and a linear temperature programmer. Circulating baths which pump the heat exchange fluid through a special cell holder are also used. The use of an X-Y recorder or a dual-pen recorder is most useful, but with an accurately linear temperature programming device a standard strip chart recorder is sufficient.

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## 7.5 Measurements with Flow Cells

Flow cells are very useful for:

a) Monitoring large-volume external reactions

b) Monitoring the effluent of a liquid chromatographic column

c) Measuring sequential samples

Results obtained with flow cell measurements depend greatly on design of the flow cell. The design must minimize entrapment of bubbles in the light path and must avoid "channeling" due to poor flow characteristics. Small volume and ease of cleaning are also desirable. These desirable characteristics mean that the best flow cells usually have a short path length, typically one or two millimeters rather than the usual 10 mm. This compromise is worthwhile, even if it forces use of expanded scales to get sufficient sensitivity for measurement.

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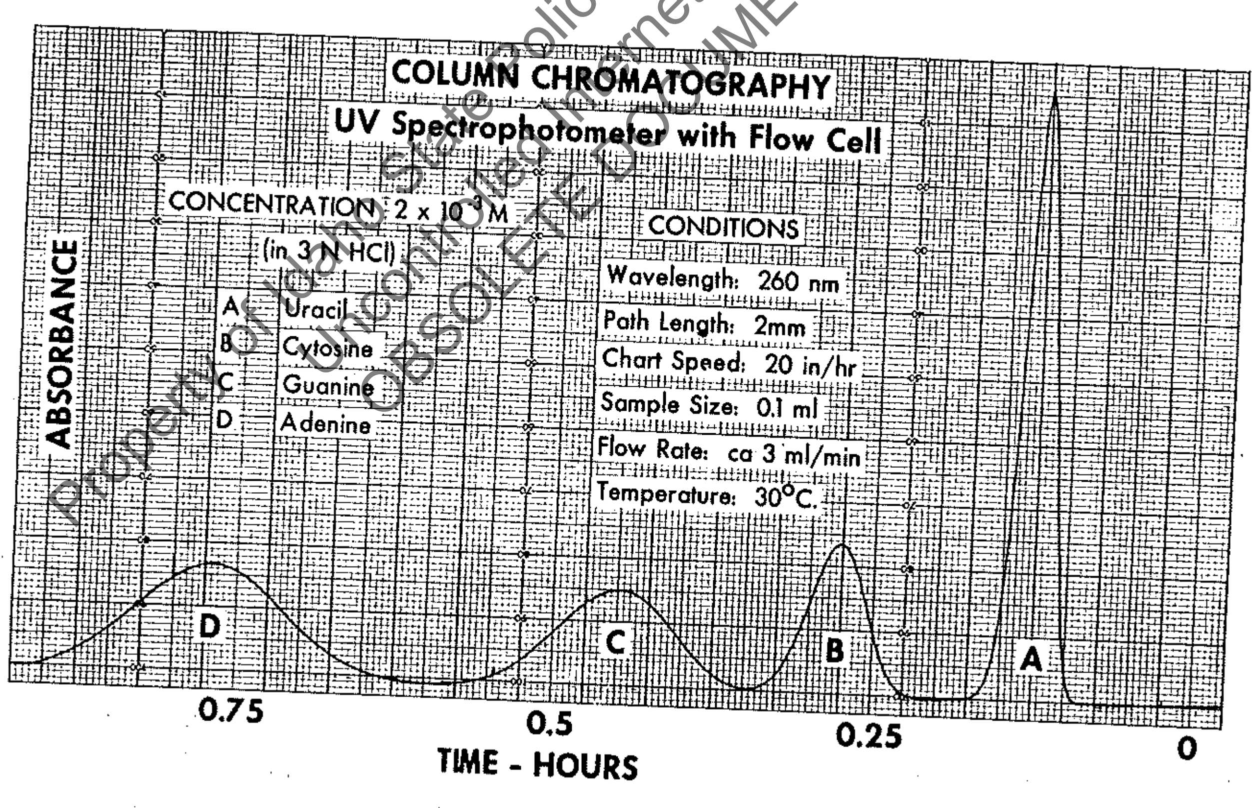


Figure 7.7 Column Chromatography

In monitoring the effluent of a liquid chromatographic column, the instrument should always be used in the absorbance mode, because variations in flow rate

will not affect quantitative accuracy based on integration of area under the curve, which is directly proportional to concentration of the component being measured. A recording in transmittance involves a logarithmic function which is very difficult to quantitate.

An occasional source of error in the use of flow cells is scattered light which is carried into the instrument through the connecting tubing which can act as a "light pipe". Black tubing, light shields, a black cloth over the tubing entrances, and other similar techniques may be used to minimize the problem.

## 7.6 Solid Samples

Studies of the spectra of opaque or highly scattering solid samples were discussed under reflectance techniques in Section 7.1. Many other occasions arise where it is desirable to measure the transmission properties of various types of solid samples. The techniques are sufficiently different to deserve special discussion.

### 7.6.1 Crystals, Glasses, and Films

The ultraviolet-absorbing properties of glass optical filters, wine bottles, plastic films for food packages, and lenses for welding goggles must be measured and controlled in order to meet manufacturing specifications.

Thin glass plates and optical filters present few problems other than reflection losses at the air-glass surfaces, which typically amount to about 5% per surface. Thicker glasses, even with parallel, polished surfaces can cause defocusing and beam shifting, which can cause significant errors. A thick glass with parallel surfaces should always be carefully positioned exactly perpendicular to the light beam in order to minimize the problem.

Plastic films can range from completely amorphous to highly oriented molecular arrangements which can affect the transmission properties through polarization. Sample holders are available which stretch the film in only one direction or in all directions, as desired, to study these effects.

Crystals must be very carefully oriented in the light beam in order to avoid shifting the light beam on the detector. Again, special holders and apertures

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are available to define the light beam and position the crystal. Usually several measurements must be made, because transmission properties of a crystal can vary depending on the particular axis which is being viewed.

### 7.6.2 KBr Pellets

The term "KBr Pellet" is descriptive of a technique very widely used in infrared spectroscopy, which is also applicable in the UV-visible spectral region. There are many powdered materials which are difficult to dissolve in common solvents. Spectra of these materials can frequently be obtained by grinding a small portion of the sample with a much larger portion of potassium bromide, and pressing the mixture in a hydraulic press at about 100,000 psi to form a transparent disc. This technique will frequently provide information which is not obtainable in any other way. A typical example is shown in Figure 7.8.

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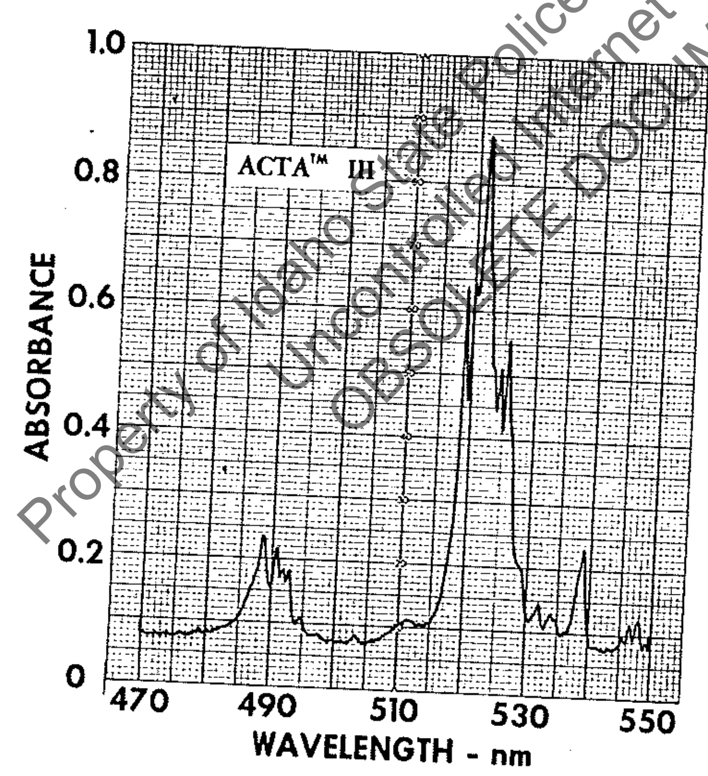


ERBIUM OXIDE IN KBr PELLET

3% $Er_2O_3$ in 0.3 g KBr

INSTRUMENT PARAMETERS

Scanning Speed: 0.2 nm/sec

Chart Expansion: 10 nm/in

Slit Program: 0.2 mm at 550 nm

Fast Response

Figure 7.8 Erbium Oxide in KBr Pellet

## 8.0 DEFINITIONS

Absorbance - The logarithm to the base 10 of the reciprocal of transmittance. Generally, pure solvent is the reference material.

$$A = \log_{10} (1/T)$$

Absorption Band - A region of the absorption spectrum in which the absorbance passes through a maximum.

Absorption Spectrum - A plot of absorbance, or any function of absorbance, against wavelength, or any function of wavelength.

Absorptivity, a - The absorbance divided by the product of the concentration of the substance and the sample path length, $a = A/bc$. The units of b and c, or of a, shall be specified.

Absorptivity, Molar, $\epsilon$ - The product of absorptivity, a, and molecular weight of the substance.

Accuracy - The extent to which a measured or enumerated value agrees with the assumed or accepted value.

Analysis - Ascertainment of the identity and/or concentration of constituents or components of a sample. (The term "analysis" is also employed in referring to the method by which analyses, or determinations which make up an analysis, are made. The following examples are chosen to illustrate correct and incorrect uses for analysis and determination:

1) Determination of Mn, Cu, and Ni in steel; not analysis of Mn, Cu, and Ni in steel.
2) Analysis of phosphorus. This should be interpreted only as the examination of a sample of phosphorus for its constituents. The statement should not be applied, for example, to mean determination of phosphorus as a constituent of steel.)

Analyte - The component or constituent of a sample identified and/or determined in an analysis. (Term is not officially recognized.)

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Analytical Curve - The graphical representation of a relation between some function of radiant power and concentration or mass of the substance emitting or absorbing it.

Analytical Wavelength - Any wavelength at which an absorbance measurement is made for the purpose of determination of a constituent of a sample.

Angstrom, Å - A unit of length equal to $10^{-10}$ m.

Background - Apparent absorption caused by anything other than the substance for which the analysis is being made.

Beer's Law - Absorbance of a homogeneous sample containing an absorbing substance is directly proportional to concentration of the absorbing substance. See also Absorptivity

Bouguer's Law - Absorbance of a homogeneous sample is directly proportional to thickness of the sample in the optical path.

Chromophore - An atom or group of atoms or electrons in a molecule which is chiefly responsible for an absorption band.

Concentration, c - Quantity of substance contained in a unit quantity of sample.

Determination - Ascertainment of the quantity or concentration of a specific substance in a sample. See Analysis.

Diffraction - When a light wave strikes the edge of an obstacle, a part of the wave travels straight ahead without change of direction. By interaction between the light wave and the edge, another portion of the wave changes its direction. For this reason, the edges of opaque objects do not form absolutely sharp shadows, as some of the light is directed into the edge of the shadow. This phenomenon is caused by diffraction and basically arises from the finite wavelength of light. Diffraction plays a part in all image formation in optics. Diffraction gratings used in spectroscopy depend upon diffraction as the basis of their action.

Dispersion - The separation of light into its component wavelengths. Dispersion is usually produced by a prism or a diffraction grating.

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Filter - A substance that attenuates radiant power reaching the detector in a definite manner with respect to spectral distribution.

Filter, Neutral - A filter that attenuates radiant power reaching the detector by the same factor at all wavelengths within a prescribed wavelength region.

Fluorescence - The emission of light of longer wavelengths excited by the absorption or radiation of shorter wavelengths in certain materials.

Frequency, $\nu$ - The number of cycles per unit time.

Hertz, Hz - The unit of frequency equal to one cycle per second.

Infrared - Pertaining to the region of the electromagnetic spectrum from approximately 0.78 to 300 μm.

Isoabsorptive Point - A wavelength at which the absorptivities of two or more substances are equal.

Isosbestic Point - A wavelength at which the absorptivities of two substances, one of which can be converted into the other, are equal.

Linear Dispersion - The derivative, $dx/d\lambda$, where x is the distance along the spectrum, in the plane of the exit slit, and $\lambda$ is the wavelength.

Micrometer, μm - A unit of length equal to $10^{-6}$ m.

Micron, μ - A unit of length equal to $10^{-6}$ m. Term now obsolete; see Micrometer.

Millimicron, mμ - A unit of length equal to $10^{-3}$ μ. Term now obsolete, see Nanometer.

Monochromator - A device or instrument that, with an appropriate energy source, may be used to provide a continuous calibrated series of electromagnetic energy bands of determinable wavelength or frequency range.

Nanometer, nm - A unit of length equal to $10^{-9}$ m.

Natural Band Width - The natural band width is the width at half height of a sample absorption peak. It is independent of instrument band width, being an intrinsic sample characteristic.

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Observed Band Width - The width of the absorption band at half its maximum absorbance, as graphed by the spectrophotometer.

Photometer - A device so designed that it furnishes the ratio, or a function of the ratio, of the radiant power of two electromagnetic beams. These two beams may be separated in time, space, or both.

Photometric Linearity - The ability of a photometric system to yield a linear relationship between radiant power incident on its detector and some measurable quantity provided by the system.

Precision - The extent to which a set of observations deviate from their own mean as frequently measured by the standard deviation. See Repeatability and Reproducibility.

Radiant Energy - Energy transmitted as electromagnetic waves.

Radiant Power - The rate at which energy is transported in a beam of radiant energy.

Repeatability - A measure of deviation of test results from their mean value, all determinations being carried out by one operator and without change of apparatus where apparatus can be significant.

Reproducibility - A measure of deviation of test results from their mean value, the determinations being carried out by different operators using apparatus generally understood to be located in different laboratories. See Accuracy and Precision.

Resolution, Spectral - The ratio, $\lambda/\Delta\lambda$, where $\lambda$ is the wavelength of the region examined, and $\Delta\lambda$ is the separation of two absorption bands that can just be distinguished. Resolution can also be defined as $\nu/\Delta\lambda$, where $\nu$ and $\Delta\nu$ refer to the wavenumber scale.

Sample Path Length, b - Internal cell or sample length. The symbol for sample path length is b.

Spectral Band Width - The wavelength or frequency interval of radiation leaving the exit slit of a monochromator between limits set at a radiant power level half way between the continuous background and the peak of an emission line or an absorption band of negligible intrinsic width.

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Spectral Position - The effective wavelength or wavenumber of an essentially monochromatic beam of radiant energy.

Spectral Slit Width - The mechanical width of either the exit or the entrance slit, whichever is the larger, divided by the linear dispersion in the exit slit plane.

Spectrophotometer - A spectrometer with associated equipment, so designed that it furnishes the ratio, or a function of the ratio, of the radiant power of two beams as a function of spectral position. The two beams may be separated in time, space, or both.

Standard Sample - A material of definite composition that closely resembles in chemical and physical nature the materials with which the analyst expects to deal, and which is employed for calibration.

Stray Radiant Energy - All radiant energy that reaches the detector at wavelengths that do not correspond to the spectral position under consideration.

Transmittance - The ratio of radiant power transmitted by the sample to radiant power incident on the sample.

Ultraviolet - Pertaining to the region of the electromagnetic spectrum from approximately 10 to 380 nm. The term ultraviolet without further qualification usually refers to the region from 200 to 380 nm.

Visible - Pertaining to radiant energy in the electromagnetic spectral range visible to the normal human eye (approximately 380 to 780 nm).

Wavelength, $\lambda$ - The distance, measured along the line of propagation, between two points that are in phase on adjacent waves.

Wavenumber, $cm^{-1}$ - The number of waves per centimeter.

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# 9.0 LABORATORY EXPERIMENTS

## 9.1 Experiment 1 - SPECTROPHOTOMETRIC UNITS

### 9.1.1 Wavelength, Frequency, and Energy

Purpose - To gain familiarity with the various units and their interrelationships.

Wavelength - The recommended unit of wavelength is the nanometer, nm, (formerly called millimicron), but the terms angstrom, Å, and micrometer, μm (formerly micron), are occasionally encountered.

Nanometer, nm = $10^{-9}$ meter = 10Å = .001 μm
Angstrom, Å = $10^{-10}$ meter
Micrometer, μm = $10^{-6}$ meter

Frequency - Frequency and wavelength are related by the expression:

$$c = \nu \lambda$$

where $\nu$ = frequency in Hertz (cycles per second)
$\lambda$ = wavelength in cm
$c$ = speed of light, $2.998 \times 10^{10}$ cm/sec

A more convenient frequency unit is the wavenumber, the number of waves per centimeter, $cm^{-1}$.

$$\bar{\nu} \text{ (in } cm^{-1}) = \frac{10^7}{\lambda \text{ (in nm)}}$$

Energy - If energy per molecule equals the photon energy, $h\nu$, then energy per mole, E, equals $Nh\nu$ where:

N = Avogadro's number, $6.023 \times 10^{23}$ molecules/mole
h = Planck's constant, $6.624 \times 10^{-27}$ erg sec

or, since one kilocalorie equals $4.184 \times 10^{10}$ ergs,

$$E = \frac{Nhc}{4.184 \times 10^{10} \lambda}$$

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TABLE 9.1

| nm | Å | µm | $cm^{-1}$ | V | kcal/mole | ν (Hertz) |
|---|---|---|---|---|---|---|
| 1 | 10 | $1 X 10^{-3}$ | $10 x 10^{6}$ | $1.24 X 10^{3}$ | $28.59 X 10^{3}$ | $2.997 X 10^{1}$ |
| $10 X 10^{6}$ | $10 X 10^{7}$ | $10 X 10^{3}$ | 1 | $1.24 X 10^{-4}$ | $2.859 X 10^{-3}$ | $2.997 X 10^{1}$ |
| 1240 | 12,400 | 1.240 | $8.066 X 10^{3}$ | 1 | 23.06 | $2.417 X 10^{1}$ |
| $2.997 X 10^{17}$ | $2.997 X 10^{18}$ | $2.997 X 10^{14}$ | $3.33 X 10^{-11}$ | $4.14 X 10^{-15}$ | $9.54 X 10^{-14}$ | 1 |
| 1000 | ______ | ______ | ______ | ______ | 28.593 | $2.997 X 10^{1}$ |
| 500 | ______ | ______ | 20,000 | ______ | ______ | $5.994 X 10^{1}$ |
| ______ | 4000 | ______ | ______ | 3.10 | ______ | ______ |
| ______ | ______ | .25 | ______ | ______ | ______ | 11.988 X 10 |
| 200 | ______ | ______ | ______ | 6.20 | 142.965 | ______ |
| ______ | ______ | ______ | 33,333 | ______ | ______ | 9.982 X 10 |
| ______ | 5720 | ______ | 17,487 | ______ | 50.000 | $5.24 X 10^{1}$ |
| 229 | ______ | ______ | 43,719 | 5.42 | 125.000 | 13.10 X 10 |
| ______ | 3101 | ______ | ______ | 4.00 | ______ | 9.668 X 10 |

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or

$$E = \frac{28,593}{\lambda \text{ (in nm)}} \text{ kcal/mole}$$

Energy can also be expressed in units of the electron volt, V.

$$V = \frac{300hc}{e\lambda} = \frac{300h\nu}{e}$$

where:

$e$ = charge on electron = $4.803 \times 10^{-10}$

$\lambda$ = wavelength in cm

Procedure - Fill in the blank spaces in Table 9.1.1.

## 9.1.2 Light Absorption vs Concentration

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Discussion - The Beer-Bouguer Law relates sample concentration to the amount of light absorbed by the sample.

$$A = abc = \log_{10} \frac{1}{T}$$

where:

$A$ = absorbance

$a$ = absorptivity - a characteristic of the sample

$b$ = sample thickness (path length)

$c$ = concentration

$T$ = transmittance - fraction of light transmitted

Other terms sometimes used are percent transmission, percent absorption, and the logarithm of absorbance (log A)

Percent transmission, $\%T = 100\ T$

Percent absorption, $\%A = 100\ (1 - T)$

Procedure - Fill in the blank spaces in Table 9.1.2.

TABLE 9.1.2

| %T | T | A | Log A | %A |
|---|---|---|---|---|
| 100.0 | 1.000 | ______ | - ∞ | ______ |
| 90.0 | ______ | ______ | - 1.337 | ______ |
| 50.0 | .500 | ______ | - 0.521 | 50.0 |
| ______ | 0.315 | 0.501 | - 0.300 | ______ |
| ______ | .234 | ______ | - 0.200 | ______ |
| ______ | ______ | ______ | 0 | 90.0 |
| 5.0 | ______ | ______ | 0.114 | ______ |
| ______ | ______ | 2.00 | ______ | ______ |
| 0.5 | ______ | 2.301 | ______ | ______ |
| ______ | ______ | 0.0044 | - 2.356 | ______ |
| 0 | ______ | ______ | ∞ | ______ |

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## 9.2 Experiment 2 - WAVELENGTH CALIBRATION

### 9.2.1 Wavelength Calibration with a Holmium Oxide Glass Filter

Purpose - To check the accuracy of the wavelength scale of a spectrophotometer.

Discussion - Holmium oxide glass has a number of sharp absorption bands which occur at precisely known wavelengths in the ultraviolet-visible region of the spectrum. These bands can be used to check the accuracy of the wavelength scale of a spectrophotometer by noting the wavelength at which the maximum absorption occurs for each band.

Materials and Equipment - Holmium oxide glass filter (Beckman Part Number 96157).

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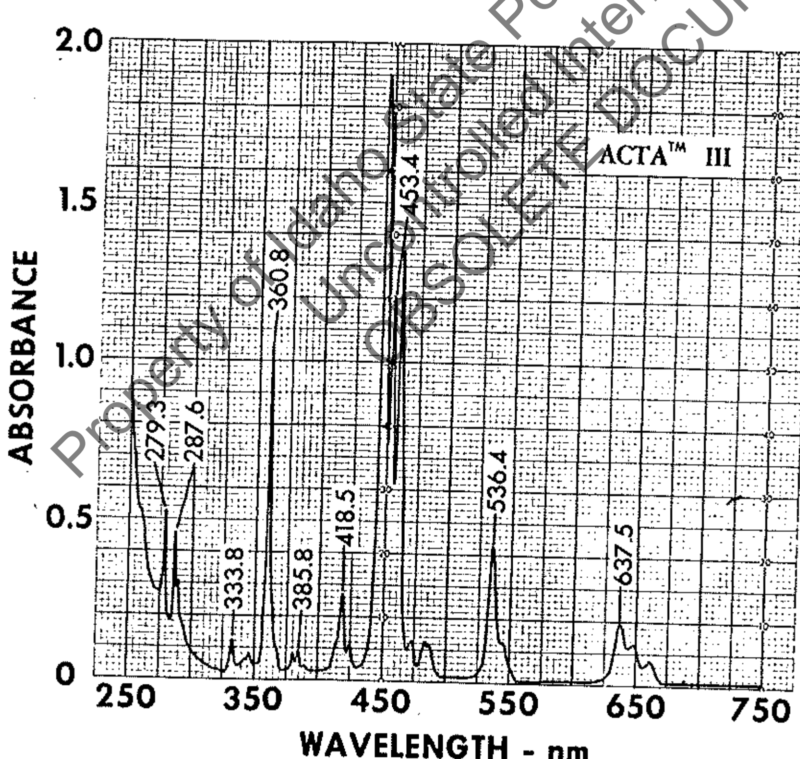


HOLMIUM OXIDE GLASS FILTER

INSTRUMENT PARAMETERS

Scanning Speed : 1 nm/sec

Chart Expansion: 50 nm/in

Slit Program : 0.1 mm at 750 nm

Fast Response

Figure 9.1 Spectrum of Holmium Oxide Glass Filter

Procedure - Set up the instrument for double-beam operation. Use a slit program which will give a dynode voltage of 500-600 volts. Insert the filter

in the sample beam of the instrument. Set the wavelength near one of the absorption bands identified in Figure 9.2.1. Manually scan slowly back and forth through the absorption band and note* the exact wavelength at which the maximum absorbance occurs. The difference between the measured wavelength and that shown in Figure 9.2.1 is the wavelength error. Repeat for each of the listed wavelengths and fill in the values in Table 9.2.1.

TABLE 9.2.1

| True λ | Measured High to Low | Measured Low to High | Error High to Low | Hysteresis |
|---|---|---|---|---|
| 637.5 | ________ | ________ | ________ | ________ |
| 536.4 | ________ | ________ | ________ | ________ |
| 453.4 | ________ | ________ | ________ | ________ |
| 418.5 | ________ | ________ | ________ | ________ |
| 385.8 | ________ | ________ | ________ | ________ |
| 360.8 | ________ | ________ | ________ | ________ |
| 333.8 | ________ | ________ | ________ | ________ |
| 287.6 | ________ | ________ | ________ | ________ |
| 279.3 | ________ | ________ | ________ | ________ |

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## 9.2.2 Wavelength Calibration with a Mercury Vapor Lamp

Purpose - To check the accuracy of the wavelength scale of a spectrophotometer.

* Note: The reading should always be taken as the wavelength is being scanned from long toward shorter wavelengths, as this is the normal scanning direction and it is the way the wavelength scale is initially calibrated at the factory. It is instructive to record wavelengths of the peaks as obtained by scanning in both forward and reverse directions. The difference between the two readings is called "wavelength hysteresis".

Discussion - Radiation emitted by a mercury vapor lamp is confined to very narrow bands of wavelengths known as "emission lines". The wavelengths of these lines can be used to calibrate the wavelength scale of a spectrophotometer. Some of the important mercury emission lines with their wavelengths in nanometers:

579.0 and 577.0, a pair of lines of about equal intensity
546.1
435.8
404.7
365.0, strongest of a triplet, two minor lines at 365.5 and 366.3
313.2 and 312.6, the first is stronger
253.6, a very strong line

Materials and Equipment

189313 mercury lamp

or

181150 mercury calibrating lamp (11SC-1)

181160 mercury lamp power supply (SCT-1) (required for use with 181150 mercury calibrating lamp)

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Procedure

1. Install the mercury lamp in the source compartment of the instrument.
2. Set up the instrument for single-beam operation with a slit width of 0.1 mm or less. Set the dynode voltage at about 500 volts. Since the intensity of the individual emission lines varies greatly, it may be necessary to vary the dynode voltage and/or the slit width to keep the signal at a usable level.
3. Manually scan wavelength slowly back and forth across one of the emission lines and note the wavelength* at which the minimum absorbance** occurs.
4. Enter the measured values in Table 9.2.2.

* See note page 69.

** If the instrument is equipped for measurement of percent transmission then that scale should be used. In the %T mode, the instrument or recorder will read close to zero except at the emission lines, where it will give some finite readings. If it goes off scale beyond 100%T, the signal should be reduced by narrowing the slit width or reducing the dynode voltage.

TABLE 9.2.2

| True λ nm | Measured λ High to Low | Measured λ Low to High | Error High to Low | Hysteresis |
|---|---|---|---|---|
| 579.0 | ______ | ______ | ______ | ______ |
| 577.0 | ______ | ______ | ______ | ______ |
| 546.1 | ______ | ______ | ______ | ______ |
| 435.8 | ______ | ______ | ______ | ______ |
| 404.7 | ______ | ______ | ______ | ______ |
| 365.0 | ______ | ______ | ______ | ______ |
| 313.2 | ______ | ______ | ______ | ______ |
| 312.6 | ______ | ______ | ______ | ______ |
| 253.6 | ______ | ______ | ______ | ______ |

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## 9.3 Experiment 3 - SPECTROPHOTOMETER CELLS

Purpose - To study the various properties of spectrophotometer cells.

Discussion - The quality of spectrophotometric work depends to a significant degree on the quality of the sample container. In this experiment the absorption properties of various types of cells will be measured.

Materials and Equipment - Spectrophotometric cells made of glass, Pyrex, Vycor, and different types of quartz or fused silica.

Procedure

1. Select one cell of each type. Fill each one with distilled water and obtain an absorption spectrum between 350 and 200 nm. Do not use anything in the reference beam.

   Which material is best (most transparent)? ______________________
   What is the limiting wavelength for Pyrex cells? ______________
   What is the limiting wavelength for Vycor cells? ______________
   Are there significant differences between the various grades of quartz and silica cells? ______________________________________________
   ______________________________________________________________
   ______________________________________________________________

2. Measure the transmission of an empty cell. Repeat with cell filled with water. Explain the difference. ________________________________________
   ______________________________________________________________
   ______________________________________________________________

3. Using a pair of unmatched cells filled with distilled water, one in each beam, record the absorption spectrum between 350 and 200 nm. Reverse the cells and repeat. Explain any differences. ______________________________
   ______________________________________________________________
   ______________________________________________________________
   ______________________________________________________________
   ______________________________________________________________

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## 9.4 Experiment 4 - SOLVENTS FOR UV SPECTROSCOPY

Purpose - To investigate the properties of common solvents to determine their utility for UV spectroscopy.

Discussion - Spectrophotometric solvents must be almost completely transparent at the wavelength of interest. Ultraviolet-absorbing impurities are quite common, and even reagent grade solvents may have impurities which render the solvent unfit for use. This problem can usually be avoided by using "spectro" grade solvents which are specially purified to remove UV-absorbing impurities. Small amounts of residual absorption are automatically compensated in double-beam operation. Polarity effects and pH effects can be important and will be demonstrated in this experiment.

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Materials and Equipment

1. A set of matched 10 mm silica absorption cells.
2. A selection of common solvents such as water, ethanol, methanol, carbon tetrachloride, chloroform, acetone, acetonitrite, cyclohexane, and others. If possible, various grades of solvents such as reagent, pure, technical and spectrograde should be available.
3. Solution of phenol in water, 0.1 g/1.
4. Solution of concentrated potassium hydroxide in water.

Procedure

1. Set up the instrument for double-beam operation between 350 and 200 nm.
2. Using only a single cell in the sample beam, obtain absorption spectrum of each solvent between 350 and 200 nm.

   Which solvents are suitable for use at 350 nm? ________________________

   ________________________________________________

   ________________________________________________

   At 300 nm? ________________________________________

   ________________________________________________

   at 220 nm? ________________________________________

   At 200 nm? ________________________________________
3. Fill one of a pair of matched cells with cyclohexane and obtain an absorption

spectrum between 350 and 200 nm. Now fill the second cell and place in reference beam. Compare the two records and explain results. ____________

____________________________________________________________

4. Fill a cell with water, add 1 drop of acetone, and record the absorption spectrum between 350 and 200 nm. Repeat, adding acetone to other solvents such as hexane, acetonitrile, methanol, chloroform, and others. How do the curves differ? ____________________________________________

____________________________________________________________

____________________________________________________________

5. Obtain absorption spectra of two or more grades of several solvents and compare. Are there significant differences? ____________________

____________________________________________________________

Are the differences of sufficient magnitude to affect their use as solvents for ultraviolet spectrophotometry? ________________________

____________________________________________________________

____________________________________________________________

____________________________________________________________

6. Fill a 10 mm silica cell with the phenol solution and record the absorption spectrum between 320 and 240 nm. Add two drops of concentrated potassium hydroxide solution, mix and record the spectrum again. Compare the two records. How do they differ? ______________________________

____________________________________________________________

____________________________________________________________

Explain ____________________________________________________

____________________________________________________________

____________________________________________________________

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## 9.5 Experiment 5 - NOISE: CAUSE AND EFFECTS

Purpose - To gain an understanding of the cause and effects of noise in a spectrophotometer.

Discussion - Noise is the random variation of the signal with time. It will vary with the size of the signal, the dynode voltage, and other factors. This experiment is designed to illustrate how the noise level varies under different instrumental conditions.

Materials and Equipment - A solution having a broad absorption band and a maximum absorbance of about 3.0 A. A solution of 0.12 g/l potassium dichromate has an absorbance of about 3.0 A at 372 nm.

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Procedure

1. Set up the instrument for double-beam operation. Use the "fast" response setting.
2. Manually adjust the slit until the dynode voltage is 300 volts.
3. Adjust zero absorbance and record signal (in "Time Drive") for about 30 to 60 seconds. With non-recording instruments, observe the signal spread.
4. The difference between the maximum and minimum signal values is the noise (peak-to-peak noise). Insert this value in the appropriate blank space in Table 9.5.1.
5. Insert sample in the beam and adjust wavelength until the absorbance is about 0.5 A.
6. Record or observe the noise level for 30 to 60 seconds and put the value in Table 9.5.1.
7. Repeat steps 2 to 6 for dynode voltages of 600 and 900 and absorbances of 1.0, 1.5, 2.0, 2.5, and 3.0 A.
8. On linear graph paper, plot noise versus absorbance for each value of dynode voltage.
9. Repeat steps 1 through 8 using the "slow" response setting. What conditions of dynode voltage, absorbance, and response would appear to be most suitable for maximum precision in quantitative analysis? ______________________

______________________________________________

______________________________________________

Suggest a suitable set of conditions for practical quantitative analysis.

________________________________________

________________________________________

________________________________________

TABLE 9.5.1

| Dynode Voltage Response | 300 Volts | | 600 Volts | | 900 Volts | |
|---|---|---|---|---|---|---|
| Absorbance | Fast | Slow | Fast | Slow | Fast | Slow |
| 0 | ___ | ___ | ___ | ___ | ___ | ___ |
| 0.5 | ___ | ___ | ___ | ___ | ___ | ___ |
| 1.0 | ___ | ___ | ___ | ___ | ___ | ___ |
| 1.5 | ___ | ___ | ___ | ___ | ___ | ___ |
| 2.0 | ___ | ___ | ___ | ___ | ___ | ___ |
| 2.5 | ___ | ___ | ___ | ___ | ___ | ___ |
| 3.0 | ___ | ___ | ___ | ___ | ___ | ___ |

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# 9.6 Experiment 6 - RESOLUTION AND BANDWIDTH

Purpose - To observe the effect that changing spectral band width has on resolution and intensity of sample absorption bands.

Discussion - The methanical slit width determines the spectral band width of the radiation leaving the monochromator of a spectrophotometer. This will affect the amount of spectral detail that can be observed in sample absorption bands.

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Materials and Equipment

1. A pair of matched 10 mm silica absorption cells.
2. One 100 mm glass-stoppered silica absorption cell.
3. Solution of benzene in methanol, 0.1 ml/l.
4. Carbon disulfide.

Procedure

Part I. Resolution vs. Slit Width

1. Prepare a sample of carbon disulfide vapor by putting one drop of liquid $CS_2$ in the 100 mm cell and replacing the cell stopper.
2. Set up the instrument for double-beam operation in the region below 340 nm. Adjust slits for a dynode voltage of 300 at 340 nm. Select the 0 - 1 A range and a wavelength scale of 5 nm/in. on the recorder chart.
3. Insert the vapor-filled cell in the sample beam. Record the absorption spectrum over the range 340 to 290 nm, scanning at the speed recommended in Table 6.1.
4. Repeat steps 2 and 3 at various other slit widths selected to give dynode voltage readings of 500, 700, and 900 volts. Remember to select the proper scanning speed for each run from Table 6.1. Describe how the spectrum changes as band width is decreased. ______________________________

   ______________________________

   What are the approximate spectral band widths for each curve? Hint: see dispersion curves, Figure 6.3. ______________________________

   ______________________________

   ______________________________

What is the wavelength separation between the two most closely spaced absorption bands? ______________________

Part II. Band Width and Absorption Intensity

1. Set up the instrument for double-beam operation below 300 nm. Adjust slits so that the dynode voltage is approximately 300 volts at 270 nm. Select the 0 - 1.0 A range.
2. Fill one of the 10 mm matched cells with the benzene solution and the other with methanol.
3. Insert the benzene cell in the sample beam and the other in the reference beam. Record the absorption spectrum in the range 270 - 240 nm, scanning at the speed recommended in Table 6.1.
4. Repeat steps 2 and 3 at various other slit widths selected to give dynode voltages of 400, 500, 600, and 800 volts. Remember to select the proper speeds from Table 6.1. Describe how the spectrum changes as band width is decreased. ______________________

______________________

______________________

Does the wavelength of the center of the strongest absorption band change with band width? ______________________

______________________

Why is it necessary to specify band width in reporting the value of absorptivity, a,? ______________________

______________________

How much of an error would be introduced if an analytical calibration curve was made from data at 300 dynode volts and the unknown sample was measured at 600 volts? ______________________

______________________

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## 9.7 Experiment 7 - SINGLE-BEAM AND DOUBLE-BEAM OPERATION

Purpose - To compare results obtained in single-beam and double-beam operation of a spectrophotometer.

Discussion - The signal in single-beam operation of a spectrophotometer is a function of many different factors including detector sensitivity, lamp output, mirror reflectivity, grating efficiency, and filter transmission. Since these factors change with wavelength, the signal changes continuously as wavelength is scanned. The single-beam spectrum includes all of these variations plus the absorption spectrum of the sample. Double-beam operation compensates for all factors that are common to both sample and reference beams, and only the differences are measured.

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Materials and Equipment

1. A pair of matched 10 mm silica absorption cells.
2. A solution potassium permanganate ($KMnO_4$) in water, 0.1 g/l.

Procedure -

1. Set up the instrument for single-beam operation. Turn on both lamps. Select 0 - 2A range.
2. Place a cell filled with the potassium permanganate solution in sample beam.
3. Set the wavelength of 600 nm.
4. Set dynode voltage at 400 and adjust slit until absorbance is in the range 0 - 0.1 A. Lock slit in "program" position.
5. Record the spectrum over the range 700 to 200 nm. Remember to change sources at 350 nm.
6. Remove the sample cell and repeat the 700 to 200 nm scan over the same piece of chart paper.
7. Now set up the instrument for double-beam operation over the range 700 to 200 nm.

8. Put the permanganate solution in the sample beam and distilled water in the reference beam, and scan from 700 to 200 nm.

9. Remove both cells and repeat scan over same chart. Compare the two baseline curves and the two sample curves. Explain the features observed in the curves. ____________________________________________

____________________________________________

____________________________________________

____________________________________________

____________________________________________

____________________________________________

____________________________________________

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## 9.8 Experiment 8 - BEER-BOUGUER LAW AND QUANTITATIVE ANALYSIS

Purpose - To demonstrate the use of a spectrophotometer for quantitative analysis.

Discussion - The Beer-Bouguer Law states that the absorbance of a solution is directly proportional to the path length and the concentration of the absorbing substance. It can be written:

$$A = abc \qquad (1)$$

where
- A = absorbance
- a = absorptivity
- b = path length
- c = concentration

This law forms the basis for quantitative analysis, because once the absorptivity has been evaluated from standard solutions, the concentration of an unknown solution can be determined by solving equation (1) for c:

$$c = \frac{A}{ab}$$

Either an arithmetical or a graphical solution to the equation is valid. Both methods will be demonstrated.

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Materials and Equipment

1. A pair of matched 10 mm absorption cells.
2. A solution of nickel nitrate hexahydrate, $Ni(NO_3)_2 \cdot 6H_2O$, approximately 0.35 molar, made by dissolving 51.0 g of the salt in distilled water in a 500 ml volumetric flask. This is solution A.

   Solution B - 10 ml of A diluted to 100 ml (0.035 molar)
   Solution C - 20 ml of A diluted to 100 ml (0.070 molar)
   Solution D - 40 ml of A diluted to 100 ml (0.14 molar)

Solution E - 75 ml of A diluted to 100 ml (0.26 molar)

Solution X - 50 ml of A diluted to 100 ml (0.175 molar)

Procedure

1. Set up the instrument for double-beam operation over the range 430 to 360 nm. Be particularly careful in setting the zero controls for quantitative analysis.
2. Record the absorption spectrum of each solution using distilled water in the reference beam.
3. Read the maximum absorbance value of each solution from the recorder traces.

   A ____________ B ____________ C ____________

   D ____________ E ____________ X ____________

4. Graphical Solution
   4a) Plot the absorbance values of solutions A, B, C, D, and E versus the corresponding concentrations on linear graph paper* with concentration along the X-axis and absorbance along the Y-axis. A straight line will result if all steps have been followed carefully.
   4b) Consider solution X as an unknown. Determine its concentration from the graph by finding the concentration which corresponds to the measured absorbance value.

   Concentration of solution X ____________________
5. Arithmetical Solution
   5a) For each solution, A, B, C, D, and E, determine a value of the absorptivity, a, from the equation:

$$a = \frac{A}{bc}$$

   A ____________ B ____________ C ____________

   D ____________ E ____________ Average ____________

* A piece of recorder chart paper is convenient to use as graph paper.

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5b) Get an average value of the absorptivity and solve the following equation for the concentration of solution X.

$$c = \frac{A}{ab}$$

Concentration of X ______________

Concentrations determined by the graphical and arithmetical solutions should agree.

True value ______________

Graphical ______________ Error ______________

Arithmetical ______________ Error ______________

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## 9.9 Experiment 9 - MULTICOMPONENT ANALYSIS

Purpose - To become familiar with the procedure for performing a multi-component quantitative analysis.

Discussion - Absorbances are additive. At a given wavelength, the absorbance of a mixture is the sum of the absorbances of the individual components. Mathematically:

$$A_{total} = A_1 + A_2 + A_3 + \ldots A_n$$
$$= a_1bc_1 + a_2bc_2 + a_3bc_3 + \ldots a_nbc_n$$

In an actual analysis, an optimum wavelength is selected for each component and the absorptivity of each component at each wavelength is determined by running pure compounds. The concentration of each component of a mixture can be determined by solving a set of simultaneous equations of the type:

$$A_1 = a_{11}bc_1 + a_{12}bc_2 + \ldots \ldots a_{1n}bc_n$$

$$A_2 = a_{21}bc_1 + a_{22}bc_2 + \ldots \ldots a_{2n}bc_n$$

$$\ldots \ldots \ldots \ldots \ldots \ldots$$

$$\ldots \ldots \ldots \ldots \ldots \ldots$$

$$A_n = a_{n1}bc_1 + a_{n2}bc_2 + \ldots \ldots a_{nn}bc_n$$

Where: $A_1$ = absorbance of mixture at wavelength 1

$a_{11}$ = absorptivity of component 1 at wavelength 1

$a_{12}$ = absorptivity of component 2 at wavelength 1

$a_{1n}$ = absorptivity of component n at wavelength 1

etc.

The two component mixture of nickel and cobalt nitrates will be studied. Although cobalt interferes significantly at the nickel absorption band, accurate analyses are still possible.

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Materials and Equipment

1. Matched 10 mm absorption cells.
2. Make 0.35 molar solutions of nickel nitrate hexahydrate, $Ni(NO_3)_2 \cdot 6H_2O$, and cobalt nitrate hexahydrate, $Co(NO_3)_2 \cdot 6H_2O$. Since the molecular weights of the two compounds are nearly identical, the same amount of each salt is required. Dissolve 10.2 g of salt in distilled water to make 100 ml of solution. Make up two mixtures as described below.

Solution P - 0.35 molar $Ni(NO_3)_2$

Solution Q - 0.35 molar $Co(NO_3)_2$

Solution R - 30 ml p + 20 ml Q

Solution S - 10 ml P + 20 ml Q

Procedure

1. Set up the instrument for double-beam operation in the range 600 to 350 nm.
2. Record absorption spectra of solutions P, Q and R by scanning over the range 600 to 350 nm using distilled water as a reference. It will be easier to visualize the interference effect if all three curves are run on the same section of chart paper by rerolling the chart after each run and starting the next run on the same starting line.
3. From the curve of solution P determine wavelength #1, the position of the maximum absorbance of the nickel solution. Wavelength #2 is the position of maximum absorbance for cobalt as obtained from the curve of solution Q.

$\lambda_1$ ____________ $\lambda_2$ ____________

4. Determine the absorptivity of component #1, nickel, and component #2, cobalt, at each of the two wavelengths by substituting values from the curves into the equation:

$$a = \frac{A}{bc}$$

For example:

$$a_{11} = \frac{\text{Absorbance of nickel at 395 nm}}{\text{Path length (1.0 cm) X concentration (.035M)}}$$

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Similarly, obtain $a_{12}$, $a_{21}$, and $a_{22}$.

$a_{11}$ ______ $a_{12}$ ______ $a_{21}$ ______ $a_{22}$ ______

5. From the curve of solution R, determine $A_1$ and $A_2$.

$A_1$ ______________ $A_2$ ______________

6. Substitute the values of $A_1$, $A_2$, $a_{11}$, $a_{12}$, $a_{21}$, and $a_{22}$ in the following equations and solve for $c_1$ and $c_2$.

$$A_1 = a_{11}bc_1 + a_{12}bc_2$$

$$A_2 = a_{21}bc_1 + a_{22}bc_2$$

$c_1$ = ______________

$c_2$ = ______________

How do the values compare with true values (0.21M for nickel and 0.14M for cobalt)?

Nickel error ________% Cobalt error ________%

7. Obtain absorption spectrum of solution S and repeat steps 5 and 6.

$c_1$ = ______________ Error ______________ %

$c_2$ = ______________ Error ______________ %

True values are 0.117 M for nickel and 0.233 M for cobalt.

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## 9.10 Experiment 10 - USE OF THE CONCENTRATION READOUT

Purpose - To illustrate use of an instrumental feature by which a spectrophotometer may be made to give answers directly in concentration units.

Discussion - The object of quantitative analysis in determination of the concentration of an analyte in some matrix. Normally this is done by using graphical or arithmetical solutions of Beer-Bouguer Law equations as illustrated in Experiment 8, Section 9.8. It matters little what actual sample absorbance is, as long as the value relates to analyte concentration in a logical way. In recognition of this, ACTA instruments are provided with circuitry by which data may be displayed digitally in arbitrary units, the size of which may be adjusted to coincide with the concentration units of his analysis.

This is done with a concentration converter, a device with which the operator can multiply the absorbance value by a variable scaling factor so that the absorbance units are converted into any desired concentration units. A sample of known concentration is placed in the instrument, and the scaler ("concentration" control) is adjusted to display its concentration. Placing similar subsequent samples in the beam results in the visible display of their concentration.

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Materials and Equipment

1. Pair of matched 10 mm absorption cells.
2. Potassium hydroxide, KOH, 0.05N. Dissolve 3.3 g of 85% KOH in 1.0 liter distilled water.
3. Solution A - potassium chromate - $K_2CrO_4$, 0.0400 g/l in 0.05N KOH.
4. Dilutions of solution to give:

    Solution B - 80% of A
    Solution C - 50% of A
    Solution D - 20% of A

Procedure

1. Set up the instrument for double-beam operation at 372 nm. Use the "concentration" scale. For quantitative analysis be particularly careful in making

the "calibrate" and "zero" adjustments.

2. Fill one cell with solution A and place it in sample beam; fill the other with 0.05N KOH solution and place in reference beam. Adjust the "concentration" control until the digital display reads 0400. If the true concentration is not exactly 0.0400 g/l (say 0.0395), adjust the display to read exactly the true concentration.

| Sample | Measured | True | % Error |
|---|---|---|---|
| B | _____ | _____ | _____ |
| C | _____ | _____ | _____ |
| D | _____ | _____ | _____ |

Cite two advantages of direct concentration display.

_______________________________________________

_______________________________________________

_______________________________________________

_______________________________________________

_______________________________________________

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_______________________________________________

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# 9.11 Experiment 11 - COLORIMETRIC ANALYSIS

Purpose - To illustrate the principles of colorimetric analysis based on the development of a colored complex between the analyte and a color-producing reagent.

Discussion - Colorimetric analysis is based on the formation of a colored solution as a result of the reaction of the analyte with another substance. The colored product usually has a very high absorptivity. This is one of the factors- sensitivity to traces of analyte - that makes colorimetry particularly attractive as an analytical technique. Desirable attributes of a colorimetric reagent are:

a) Sensitivity to trace concentrations of analyte.
b) Selectivity for the analyte of interest.
c) Stability of developed color.

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Materials and Equipment

1. Ferrous ammonium sulfate, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, in aqueous solutions as follows:

   Solution A - 1.404 g/1; 1 ml = 0.200 mg $Fe^{++}$ (200 ppm)
   Solution B - 25 ml A diluted to 100 ml (50 ppm)
   Solution C - 15 ml A diluted to 100 ml (30 ppm)
   Solution D - 5 ml A diluted to 100 ml (10 ppm)
   Solution X - 20 ml A diluted to 100 ml
   Solution Y - 10 ml A diluted to 100 ml

2. Solution of orthophenanthroline (1,10-phenanthroline monohydrate) made by dissolving 0.1 g of reagent in 100 ml distilled water to which 2 drops concentrated hydrochloric acid have been added. Gentle warming may aid dissolution. One ml of this solution is sufficient to complex 0.1 mg ferrous iron.
3. Linear graph paper. A section of recorder chart paper is convenient to use.

Procedure

1. Pipet 10 ml of solution B into a 25 ml volumetric flask.

2. Add 5 ml of orthophenanthroline solution and dilute to volume.
3. Repeat steps 1 and 2 for solutions C, D, X, and Y.
4. Allow the solutions to stand 30 minutes while color develops.
5. Set up the instrument for double-beam operation at 510 nm. Use scale 0 - 2A.
6. Measure the absorbance of each solution at 510 nm using distilled water as a reference.

   B __________ C __________ D __________

   X __________ Y __________

7. Plot absorbance values of B, C, and D against concentration on linear graph paper.
8. From the calibration graph determine the concentrations of solutions X and Y.

   X __________ Y __________

   What is your estimate of the minimum detectable iron concentration using this procedure ? ____________________

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# 9.12 Experiment 12 - SPECTRAL COLOR AND COLORED SOLUTIONS

Purpose - To relate visible color with wavelength and to measure the absorption spectrum of colored solutions.

Discussion - A colored solution has color because some of the wavelengths in the visible spectrum are absorbed and some are transmitted by the solution. For example, a blue solution transmits blue light but absorbs red and yellow light. In this experiment the color associated with the different wavelengths of the spectrum will be investigated and the color of colored solutions will be measured.

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Materials and Equipment

1. Liquid food colors in green, blue, yellow, and red.
2. A strip of stiff, white paper or cardboard about 1" x 5" in size.

Procedure

1. Set up the instrument for double-beam operation in the region 800 - 350 nm. Close the shutter in the sample beam and adjust the slits to their widest opening.
2. Set the wavelength to 550 nm, open the sample compartment and insert the strip of white paper into the sample beam. A colored spot of light will be observed on the card. What is the color of 550 nm radiation? ____________

   410 nm ________________ 450 nm ________________

   475 nm ________________ 590 nm ________________

   650 nm ________________

   What is the longest wavelength at which you can still see the spot of light? ______________________ What is the shortest wavelength? ____________
3. Use the food colors to make solutions of colored water. The concentrations of the colors should be adjusted so that the strongest absorption band of each solution is between 1.5 and 2.0 A.
4. Insert the green solution in the sample beam. Insert the white paper strip in the beam between the sample and the shutter.

Which colors are transmitted and show up as bright spots on the card?
__________ __________ __________ What wavelength regions are
brightly visible? __________nm to __________nm. Repeat with the remaining solutions. Red solution transmits __________ to __________nm.
Blue solution transmits __________ to __________ nm. Yellow solution
transmits __________ to __________nm.

5. Now obtain absorption spectra of the colored solutions by scanning over the region 800 to 350 nm. How do the spectral curves compare with the visual measurements made above? ______________________________

6. Describe in words what you think the spectrum of a purple solution would look like. ______________________________

7. Mix red and blue solutions to make a purple solution, and obtain a spectrum of the resulting solution. Compare with your description in 6. __________

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# ESSENTIALS OF THE ULTRAVIOLET ANALYSIS OF DROGS

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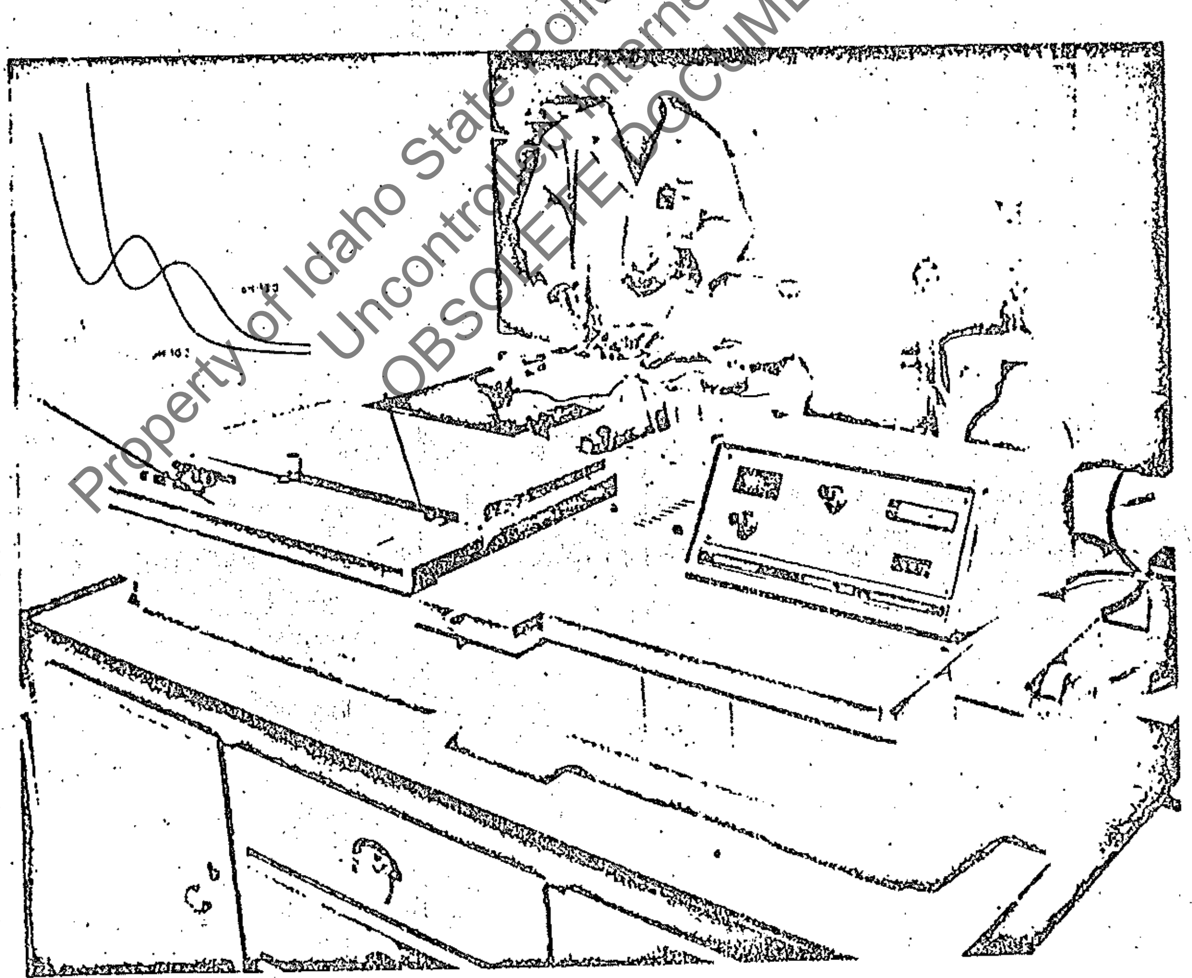

BIOMEDICAL REPORTS

Applications Research · Scientific Instruments Division · Fullerton, California 92634

# ESSENTIALS OF THE ULTRAVIOLET ANALYSIS OF DRUGS

by J. A. Miller, Vina Spiehler, and C. W. Keller

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## Foreword

This publication first appeared as Section IV in the Beckman Clinical Technical Report TR-579 - Clinical Procedures for Drug Screening. Ultraviolet spectrophotometry is such an important analytical tool in the analysis of drugs that we decided to publish this chapter and the accumulated ultraviolet drug spectra as a separate report.

Some references are made throughout this section to analytical procedures and other sections for drug screening which also appeared in the larger volume TR-579, but for the most part the material is complete and covers some of the fundamentals of ultraviolet spectrophotometry, the essentials of a typical ultraviolet drug analysis, and some important ultraviolet spectrophotometer instrument performance checks.

Beckman

# Section 4.0

# ULTRAVIOLET SPECTROPHOTOMETRY

THE ELECTROMAGNETIC SPECTRUM

WAVENUMBER · cm⁻¹

4 X 10² | 25 | 400 | 4000 | 12.5 X 10³ | 25 X 10³ | 50 X 10³ | 10⁷ | 10⁸

SPIN ORIENTATIONS (in magnetic fields) NMR · ESR | MOLECULAR ROTATIONS | MOLECULAR VIBRATIONS | VALENCE ELECTRONIC TRANSITIONS | INNER SHELL ELECTRONIC TRANSITIONS | NUCLEAR TRANSITIONS

Infrared Region | Visible | Ultraviolet | X-rays

RADIO WAVES | MICRO-WAVES radar | FAR INFRARED | FUNDA-MENTAL REGION | OVERTONE REGION | NEAR UV | VACUUM UV | "SOFT" X-RAYS | GAMMA RAYS

25 cm | 0.04 cm 400 μm | 25 μm | 2.5 μm | 800 nm 0.8 μm | 400 nm | 200 nm | 10 A | 1 A

WAVELENGTH

Figure 4.1. The Electromagnetic Spectrum

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## 4.0 ULTRAVIOLET SPECTROPHOTOMETRY

### 4.1 THE ELECTROMAGNETIC SPECTRUM*

Ultraviolet and visible radiation is a manifestation of just one very small portion of the electromagnetic spectrum, which includes other forms of radiation such as radio, radar, infrared, X-rays, and cosmic rays (Figure 4.1).

Electromagnetic radiation can be considered to be an oscillating electric field, with an associated magnetic field, which travels through space with a wave motion. In order to explain the various properties of electromagnetic radiation, a dualistic nature must be assigned to it. It is both an oscillating electromagnetic field and a stream of photons, particles having energy but no mass.

#### 4.1.1 WAVELENGTH AND FREQUENCY OF ELECTROMAGNETIC RADIATION

Since radiation acts as a wave, it can be classified in terms of either wavelength or frequency. As illustrated in Figure 4.2, wavelength is the distance measured along the line of propagation, between the crests of two adjacent waves. Frequency is the number of waves passing a given point per unit time.

Wavelength, $\lambda$, and frequency, $\nu$, can be related by the equation:

$$\nu = \frac{c}{\lambda}$$

where:

c is the speed of light (approximately $3 \times 10^{10}$ cm/sec)

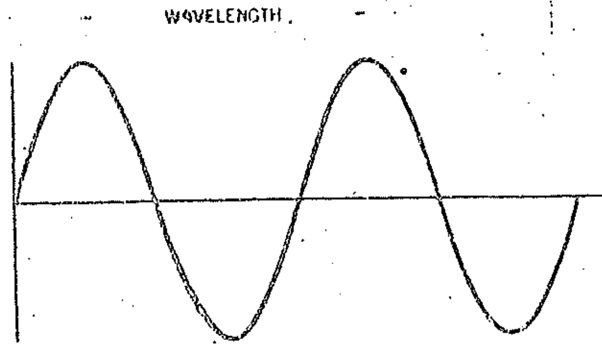


Figure 4.2. Wavelength (Definition)

[illegible], the micrometer

*Portions of this section have been reprinted from "An Introduction to Ultraviolet Spectrophotometry" (Beckman Part No. 82058) with permission of R. J. Manning, the author.

(m = $10^{-6}$ meter), and the angstrom (Å = $10^{-10}$ meter). In this manual, the term nanometer will be used exclusively in designating wavelength. When working in the ultraviolet region, wavelength is not normally expressed in its corresponding frequency, because such large numbers would be involved. The common term for frequency is wavenumbers ($cm^{-1}$)—or the number of wavelengths in a centimeter. The ultraviolet-visible range (190-780 nm) corresponds to 52,631 to 12,800 $cm^{-1}$. Note that wavenumber is an inverse function of wavelength, that is, the longer the wavelength, the lower the frequency.

The ultraviolet region is considered to be the region from 190 nm to 340 nm; it is this region with which we will be concerned for these drug determinations.

## 4.2 ORIGIN OF SPECTRA

### 4.2.1 ABSORPTION OF RADIATION

When light passes through a material such as a drug solution, certain wavelengths of light may be absorbed. If the intensity of the transmitted light is plotted as a function of wavelength, an absorption spectrum of the material is obtained. It is this selective absorption of radiation that forms the basis for application of absorption spectrophotometry to qualitative and quantitative analysis.

Since light is a form of energy, [illegible] a photon of light by a molecule causes an increase in the energy content of the molecule. The amount of this increase is equal to the energy of the photon. Remember that a photon is an indivisible particle having energy but no mass. The amount of energy possessed by a particular photon is related to its wavelength — (or more specifically, its frequency).

$$[illegible] = h\nu$$

where:

[illegible]

If the molecule is in its normal or ground state before the interaction, the absorption raises its energy content to a higher or "excited" state. In order for a photon to be absorbed by a molecule, energy of the photon

MOLECULAR ENERGY LEVELS

I — Electronic
II — Electronic + Vibrational
III — Electronic + Vibrational + Rotational

Figure 4.3. Molecular Energy Levels

must correspond precisely to the difference between two characteristic energy states of the molecule.

### 4.2.2 ENERGY STATE OF MOLECULES

The total potential energy of a molecule (excluding nuclear energy) can be considered to be the sum of its electronic, vibrational, and rotational energies.

$$E_{total} = E_{electronic} + E_{vibrational} + E_{rotational}$$

Energy differences between rotational states of a molecule are quite small—very much smaller than between electronic states—while vibrational transitions are intermediate between the two. Rotational transitions will have absorption bands in the low frequency or long wavelength (or infrared) region of the spectrum. Vibrational spectra will be in a different infrared region of the spectrum, the electronic spectra involve higher energies and occur almost entirely below 1000 nm—1.0 μm—wavelength (Figure 4.1). Ultraviolet determinations are concerned with electronic energy transitions.

The various types of transitions are not independent but are interrelated. Rotational energy levels are superimposed on vibrational levels, and both are superimposed on electronic levels as shown in Figure 4.3.

The lengths of the different arrows correspond to the energies required to effect the transitions. The short arrows represent rotational energies, and the intermediate arrows and long arrows indicate vibrational and electronic energies, respectively. These diagrams also illustrate why the electronic absorption spectra of molecules are not sharp "lines" occurring at a single wavelength, but are rather broad bands spread out over a range of wavelengths.

## 4.3 ABSORPTION SPECTRA OF ORGANIC MOLECULES

### 4.3.1 UNSATURATION

Unsaturation (multiple bonds) has long been recognized as characteristic of ultraviolet absorbing molecules. Saturated compounds are transparent in the useful ultraviolet region. In molecular orbital theory, electrons forming single bonds are called sigma (σ) electrons and those forming double bonds are called pi (π) electrons. In the near ultraviolet spectral region, transitions of the π electrons give rise to most of the observed absorption bands. Nonbonded or unshared electrons in molecules containing atoms like oxygen or nitrogen are called n electrons, and interactions between π and n electrons are responsible for some important ultraviolet absorptions. Organic groups can be classified according to their effect on the ultraviolet absorption characteristics of the molecules to which they are attached.

Figure 4.4 illustrates the different types of electrons described above.

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TABLE 4.1

| Chromophore | System | Example | Max (nm) | ε* |
|---|---|---|---|---|
| Carbonyl (ketone) | RR'C=O | Acetone | 271 | 16 |
| Carbonyl (aldehyde) | RHC=O | Acetaldehyde | 293 | 12 |
| Carboxyl | RCOOH | Acetic Acid | 204 | 60 |
| Amide | $RCONH_2$ | Acetamide | 208 | — |
| Azo | –N=N– | Azomethane | 347 | 5 |
| Nitroso | –N=O | Nitrosobutane | 300<br>665 | 100<br>20 |
| Nitro | $-NO_2$ | Nitromethane | 271 | 19 |
| Nitrate | $-ONO_2$ | Ethyl Nitrate | 270 | 12 |
| Thiocarbonyl | >C=S | Thiobenzophenone | 620 | 70 |
| Sulfoxide | >S=O | Cyclohexylmethyl Sulfoxide | 210 | 1,500 |

* ε = Molar absorptivity in liters/mole cm.

ELECTRON TYPES

H, H, C = Ö, π, n, σ

Figure 4.4. Electron Types

### 4.3.2 CHROMOPHORES

A chromophore is a group which, when introduced into a saturated hydrocarbon (one having no multiple bonds), produces a compound which has a selective absorption somewhere between 185 nm and 1000 nm. For example, n-octane is a saturated hydrocarbon which is quite transparent at all wavelengths between 185 nm and 1000 nm. If a nitrite group is introduced into the octane molecule, a compound, octyl nitrite, is produced which has a strong absorption band centered at 230 nm. Therefore, the nitrite group is classified as a chromophore. Table 4.1 lists typical simple chromophoric groups. Note that each of the groups has at least one multiple bond.

The intensity of absorption of the simple chromophore varies widely from one group to the next. However, all members of a class of compounds containing a single chromophore will normally have absorption bands that have approximately equal intensity and that lie within a narrow spectral range. Since each series has its own absorption characteristics, the presence or absence of a particular chromophore may frequently be determined from a study of the absorption spectrum of a compound. If two or more chromophores occur in a single molecule, their relative positions within that molecule determine the ultimate absorption effect. These effects involving multiple chromophores are called conjugation effects. More detail and discussion is provided in "An Introduction to Ultraviolet Spectrophotometry," which was cited earlier.

### 4.3.3 pH AND SOLVENT EFFECTS

Whenever the absorption bands of compounds are discussed, it is necessary to specify the solvent used, because the position and intensity of a band will vary with the solvent. For example, the absorption band of acetone can vary from 259 nm to 270 nm, depending on the solvent (Figure 4.5). These effects are due to:

1. The nature of the solvent.
2. The nature of the absorption band.
3. The nature of the solute.

In general, the magnitude of the solvent effect can be correlated with polarity of the solvent. Polar solutes such as drugs are affected more by changes of solvent than are non-polar solutes.

As evidenced by the classical changes in barbiturate spectra, pH effects can be quite striking. Most of the effects are due to shifting equilibria between two different forms such as keto-enol and amino-imino isomers. And, of course, everyone is familiar with the dramatic color changes effected by pH changes on solutions of indicator dyes such as methyl orange and phenolphthalein.

Solvent effects can often be used to great

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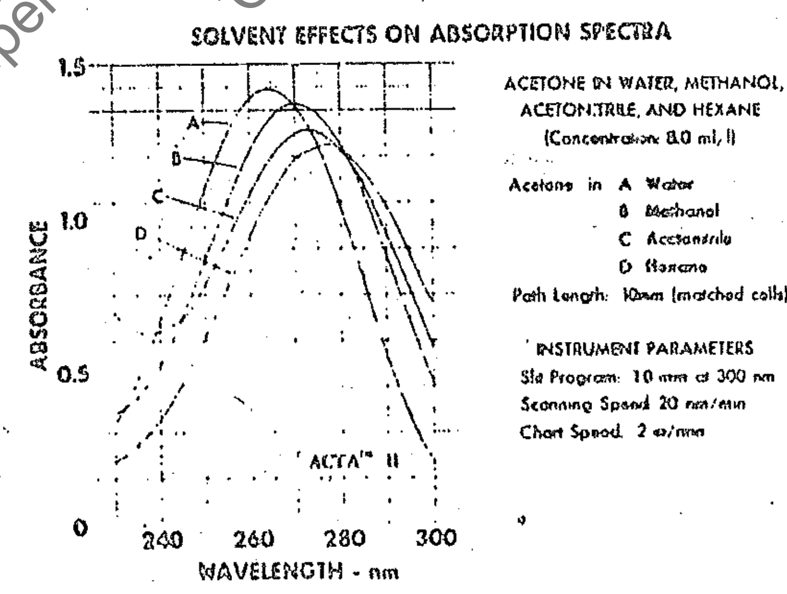


Figure 4.5. Solvent Effects on Absorption Spectra

advantage in establishing the nature of a given group by noting the effect on a given band as solvents or pH are changed. An excellent example of pH effect is illustrated in Figure 4.6.

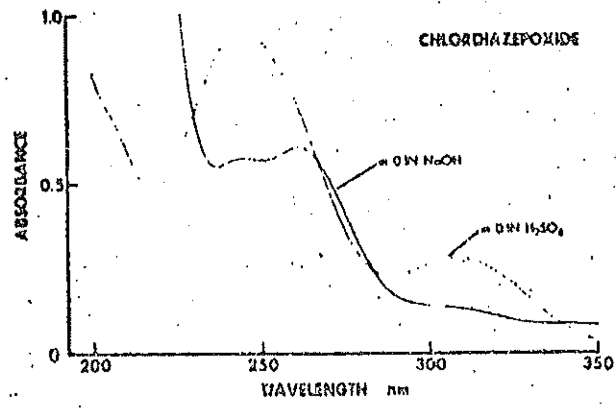


Figure 4.6. Effect of pH on Absorption Spectra

The most widely used solvents are water, ethanol, cyclohexane, iso-octane, and acetonitrile. For use below 220 nm, it is usually necessary to use the highly purified "spectrophotometric grade" solvents which are now widely available.

Other solvents which are useful include glycerol, dioxane, ethyl ether, butyl ether, n-hexane, n-heptane, and saturated alcohols from methanol to the pentanols. Chloroform is very useful down to about 250 nm, but it absorbs strongly at shorter wavelengths.

For pH adjustment and control, acetic and sulfuric acids, sodium hydroxide, and the phosphate buffers can be used. The example spectra provided in Appendix II were prepared using either 0.1N NaOH or 0.1N $H_2SO_4$. If no differences resulted in the spectra for these drugs between the acidic or basic solutions, then only one (either the base or the acid) was included. Where spectral differences were noted, both were included.

NOTE

These same acidic or basic solutions are specified in the procedures for drug identification by ultraviolet spectrophotometry in Section 2.0.

## 4.4 QUALITATIVE ANALYSIS—ULTRAVIOLET IDENTIFICATION OF DRUG COMPOUNDS

While ultraviolet—visible spectrophotometry is not normally considered to be a good qualitative tool, UV—visible methods may aid in confirming an identification.

Ultraviolet spectrophotometric identifica-

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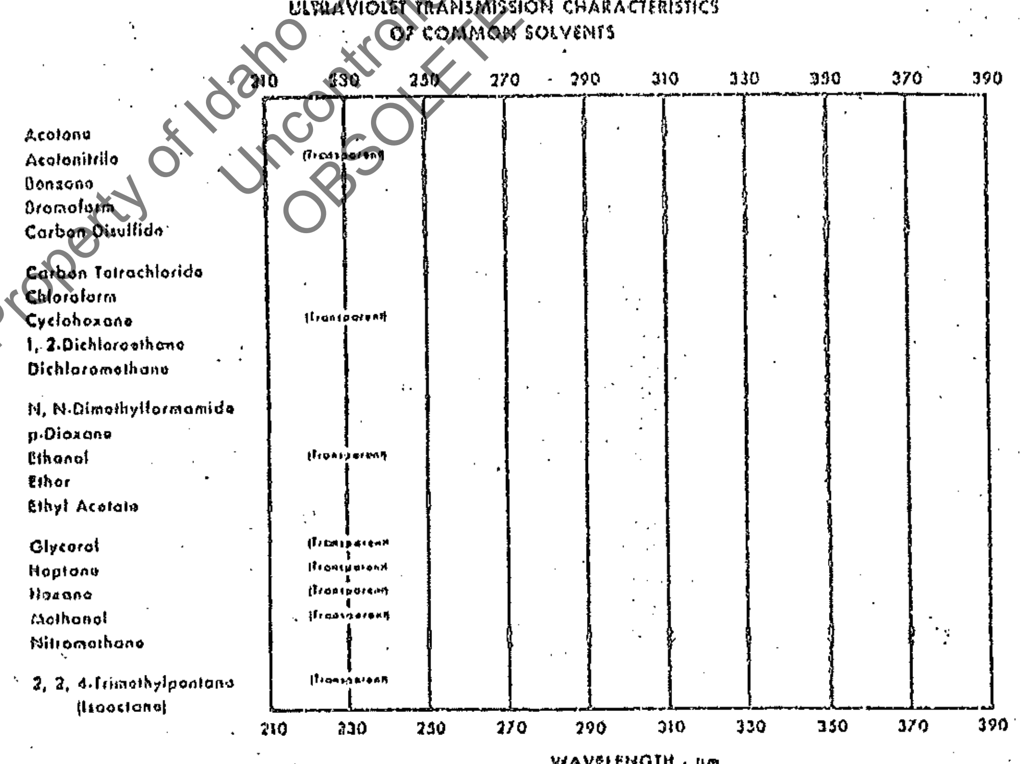


Figure 4.7. Ultraviolet Transmission Characteristics of Common Solvents

tions of organic compounds are usually accomplished by matching the spectra of the unknown compounds with the spectra of known compounds. The conditions under which the spectra of the unknown compounds and the known compounds are prepared must be exactly the same. Factors which must be considered in making spectral comparisons are the general shape of the curves, wavelengths where maxima and minima occur, and the ratio of the absorbance values obtained at maxima and minima. Although a mixture of unknown compounds can sometimes be identified, the identification of more than one unknown is most difficult, in fact most often impossible. Possibly with extractive techniques the unknowns in a mixture can be separated so that spectra can be determined on individual components.

One advantage of the drug screening systems employed in this manual is that the extractions help to eliminate compound interferences which would make the ultraviolet spectra hard to interpret. Another advantage of these techniques is that the drug residues remaining after the chloroform evaporation may be dissolved in the solvent that is ideal for the ultraviolet examination.

The choice of solvent is very important, because the absorption of a sample may be strongly affected by the solvent. Both the maximum and minimum wavelengths, as well as the absorptivity (amount of absorbance per unit of sample) can be changed by changing the solvent. Often similar compounds can be distinguished by running the sample in different solvents or at different pH values and noting the differences in the spectra.

Appendix II contains a number of drug compounds which exhibit marked changes in their spectra depending on whether the sample was dissolved in 0.1N $H_2SO_4$ or 0.1N NaOH. Among the examples shown are: methaqualone, salicylic acid, quinine sulfate, the barbiturates, benzocaine, and chlordiazepoxide.

Some drug compounds do not characteristically change their spectra with changes in solvent or pH. Benzphetamine, ephedrine, mephentermine, and glutethimide exhibit similar spectra in acidic or basic media.

If the residue from a given chloroform extract is found to have no significant absorption in the region 220 nm to 340 nm, this fact immediately eliminates all drug compounds which normally have absorption in this region. THIS, IN ITSELF, IS IMPORTANT CONFIRMATORY INFORMATION. There are a few compounds which do not have analytically useful ultraviolet spectra; ethinamate and diphenylhydantoin are examples which do not (Appendix II). However, some of these compounds can interfere with other UV determinations—as diphenylhydantoin is said to interfere with the determination of barbiturates.

### 4.4.1 BARBITURATES—pH CHANGES—RESONANCE FORMS

The characteristic changes which occur in the spectra of the barbiturates, when observed in alkali solutions of varying pH, have been the basis for their analysis by ultraviolet spectrophotometry. Changes in their spectra are associated with changes in resonance forms of the substituted barbiturates (Figure 4.8). These spectral changes have been used to identify and to quantitate barbiturates in biological samples. The UV methods outlined in Section 2.0 of this manual define the most straightforward methods for these compounds—simply a comparison of spectra at different pH values (Figure 4.8).

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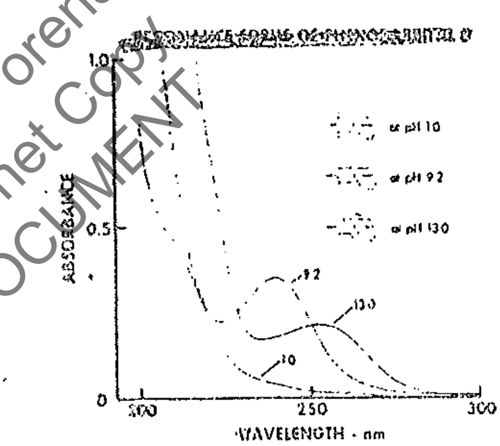


Figure 4.8. Resonance Forms of Barbiturates

If barbiturates are present, the absorbance of the solution at pH 13 will have a maximum at 260 nm and possibly a minimum at 240 nm. The maximum absorbance of the solution at pH 10 will shift to 240 nm. If the absorbance curves for the solutions cross and the maxima shift as illustrated in Figure 4.8, the drug present is probably a barbiturate.

### 4.4.2 BARBITURATES—HYDROLYSIS TECHNIQUE

Ultraviolet methods may be used to do more than indicate the presence of a barbiturate—other spectral changes, introduced by more than pH change, may be used to classify the type of barbiturate present. In the procedures (Section 2.0) of this manual, the gas chromatographic analysis is used to identify the specific barbiturate, but the following ultraviolet technique might occasionally be helpful and provide additional confirmatory evidence.

[illegible] barbiturate ingested is based on a [illegible] of the alkaline extract to hydrolysis at [illegible]°C (457). If a long acting barbiturate is present, hydrolysis easily occurs and less than one third of the original barbiturate remains. Short acting barbiturates are very resistant to hydrolysis under these conditions and about [illegible]% of the activity remains. Medium acting barbiturates are intermediate experiencing about 50% destruction. A curve illustrating hydrolysis of a typical medium-acting barbiturate is shown in Figure 4.9.

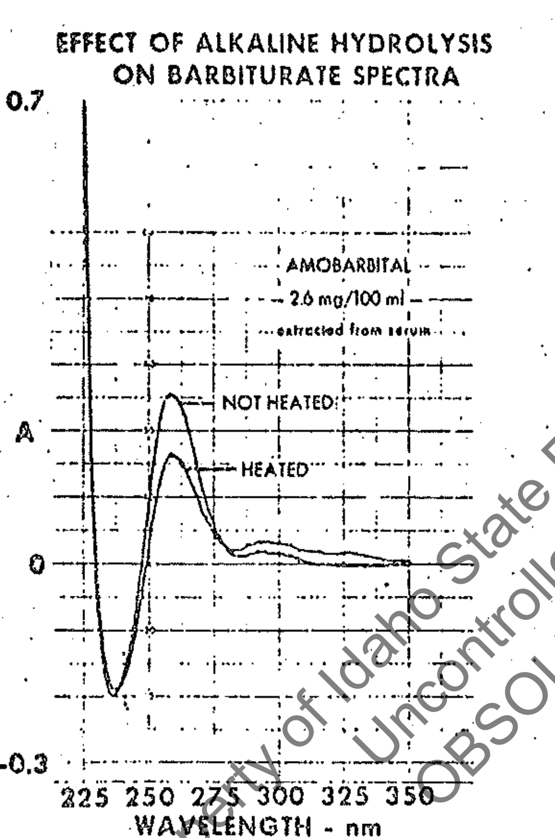


Figure 4.9. Effect of Alkaline Hydrolysis on Barbiturate Spectra (457)

To determine the type of barbiturate present using hydrolysis techniques, dissolve the residue from the chloroform extraction in 5.0 ml of 0.45N NaOH. Three 1200 $\mu$l microcells (Beckman Part No. 130774 – a set of 4) are needed in this analysis.

1. Using a serological pipet, transfer 1.2 ml of the sample extract in 0.45N NaOH to Cuvettes 1 and 2. Also, to Cuvette 1 add 0.2 ml additional 0.45N NaOH, and to Cuvette 2 add 0.2 ml of a 30% $NH_4Cl$ solution (w/v).
2. Transfer 2.0 ml of the remaining sample extract to a test tube, accurately marking the sample level in the test tube. Stopper the test tube and place it in a boiling water bath for 15 minutes.
3. After 15 minutes remove the tube, cool it rapidly in an ice bath, and allow the solution to return to room temperature.

NOTE

Check the volume of the solution and if there is any evaporation loss, add 0.45N NaOH to bring the level to the 2.0 ml marks made on the test tube. This solution is now a hydrolyzed alkaline aliquot of the original sample extract.

4. Take 1.2 ml of this solution and 0.2 ml of 0.45N NaOH and place in Cuvette 3.
5. Operate the Beckman DB-GT as described in Section 4.6.4.
6. Turn on the Differential Absorbance Scale Accessory (switch is located at the rear of the instrument next to the recorder outlet). Scan from 340 nm to 220 nm. The Differential Absorbance Scale Accessory automatically sets the absorbance scale on the recorder from -0.3 A to +0.7 A, with zero now being at 30 divisions on the chart.
7. Place Cuvette 1 in the sample beam and Cuvette 2 in the reference beam and scan the spectrum from 340 nm to 220 nm.
8. Replace Cuvette 1 in the sample beam with Cuvette 3 containing the hydrolyzed sample. To make comparison of the spectra easier, roll the chart paper back to the starting point of the previous spectrum and superimpose the spectrum of the hydrolyzed sample on that of the non-hydrolyzed sample.

The type of barbiturate is determined by calculating the percent activity remaining (% R) after alkaline hydrolysis.

$$\%R = \frac{\text{Absorbance at 260 nm (Hydrolyzed Sample)}}{\text{Absorbance at 260 nm (Unhydrolyzed Sample)}} \times 100$$

when:

% R is 30 or less, a long acting barbiturate is indicated; when

% R is about 50, a medium acting barbiturate is present; and when

% R is 90 or higher, a short acting barbiturate is present.

Phenobarbital [illegible] barbiturate; % R is less than [illegible]

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Amobarbital is a medium duration barbiturate; % R is approximately equal to 50.

Pentobarbital and secobarbital are short duration barbiturates; % R is greater than 90.

4.4.3 NONBARBITURATE SEDATIVES

Extracted along with the barbiturates are the following non-barbiturate sedatives: methyprylon, glutethimide, meprobamate, ethinamate, and diphenylhydantoin.

Methyprylon cannot be easily analyzed by ultraviolet spectroscopy because its ring opens in 0.45N NaOH, and the resulting compound does not have useful absorption. In Appendix II we show the spectrum resulting when methyprylon is dissolved in 0.45N NaOH (that is, at pH 13). This breakdown product has an absorbance maximum at 287 nm and a minimum at 259 nm, but the absorbance peak does not shift as pH is changed from 13 to 10.

Glutethimide is also extracted with the barbiturates and can be determined quantitatively by ultraviolet spectroscopy. Because glutethimide decomposes rapidly in alkaline solution, the decrease of absorption at 235 nm, as a function of time, is indicative of the amount of glutethimide present. This decrease in absorbance is reportedly due to hydrolysis of the piperidine ring. The glutethimide spectrum in Appendix II was obtained in 0.1N NaOH. In the procedures in this manual, this would only act as a confirmatory test because the gas chromatographic analysis of our barbiturate fracfraction would indicate the presence or absence of glutethimide, as well as provide a quantitative value (138, 430).

Meprobamate, ethinamate, and diphenylhydantoin do not have analytically useful UV absorption in NaOH solution. Their spectra (though exhibiting no analytically useful absorptions) are available in Appendix II.

4.4.4 SALICYLATES

Salicylic acid has a maximum absorbance at 260 nm. The maximum is shifted by pH change (to 237 nm in 0.1N $H_2SO_4$). Salicylates can give false positive results for barbiturates. To determine salicylates or to correct for their presence with barbiturates, use the procedure of Williams, Linn, and Zak (461), employing the Differential Absorbance Accessory on the DB-GT.

4.4.5 OTHERS (ACID FRACTION)

Some tranquilizers (the chlordiazepoxide and diazepam group) and narcotics (for example, methadone) also are extracted at pH 6.4 and absorb radiation in the UV region. See UV Spectra (Appendix II) and Drug Profiles (Appendix III) for further guides on identification.

NOTE

Combinations of drugs will give ambiguous spectra in the UV analysis. UV determinations must be confirmed by GC and TLC when combinations of drugs are present.

4.4.6 BASIC DRUGS

The residue from the chloroform extracted at a pH of 9–10 contains the alkaloid and amine types of drugs. This residue should be dissolved in 3.0 ml 0.1N $H_2SO_4$ and the ultraviolet spectrum determined from 340 to 220 nm (Section 2.2.9 or 2.7.2). Any absorption in this region indicates the presence of alkaloid or amine drugs, as listed under Bases in Figure 2.0–Flow Chart by Generic Name.

Some drugs in this fraction yield different spectra in acidic and in basic solutions. Consequently, when comparison of the sample and standard spectra indicates the possible presence of a drug, it may be desirable to obtain the spectrum in a basic as well as in an acidic solution. Comparison of these spectra with appropriate standard spectra may yield additional confirmatory information.

4.5 QUANTITATIVE ANALYSIS

The amount of light absorbed by a fixed quantity of an absorbing species such as a drug in solution will be dependent on the number of molecules of that species in the solution. This property forms the basis for quantitative analysis by spectrophotometry. As noted earlier, ultraviolet–visible spectrophotometry is a better quantitative than qualitative tool.

4.5.1 LAWS OF LIGHT ABSORPTION: BOUGUER'S LAW

Consider the diagram of Figure 4.10 where a beam of monochromatic (one wavelength) light is incident on a cuvette filled with a transparent solution containing an absorbing substance. If 100 photons enter the cuvette and only 50 emerge from the other side, the transmittance is 0.5. If a series of identical cuvettes each containing a portion of the same solution, are placed in the light beam, the result shown is Figure 4.11 is obtained. This illustrates the effect of increasing the path length of an absorbing solution. The first mathematical formulation of this effect is generally credited to Lambert (1760), although it now appears that Bouguer first stated it in 1729.

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100 Photons → → 50 Photons

Transmittance, $T = \frac{50}{100} = 0.50$

Figure 4.10. Light Absorption

BOUGUER'S LAW

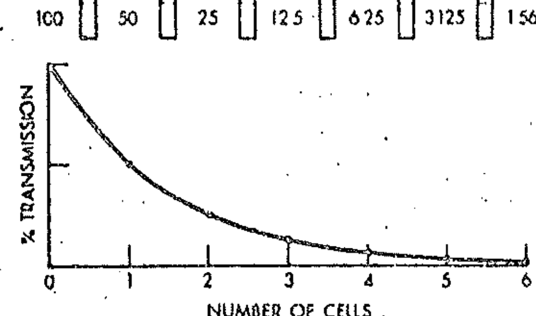


Figure 4.11. Bouguer's Law

The successive values of transmittance from Figure 4.11 are 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625. These correspond to numbers in the following sequence: $(0.5)^1$, $(0.5)^2$, $(0.5)^3$, $(0.5)^4$, $(0.5)^5$, and $(0.5)^6$. Bouguer's Law can be formulated mathematically:

$$T = \frac{P}{P_0} = e^{-\alpha b}$$

or

$$\log_e \frac{1}{T} = \alpha b$$

where:

- $P$ = the radiant power transmitted by the sample
- $P_0$ = the radiant power incident on the sample
- $b$ = path length (in centimeters)
- $\alpha$ = absorption coefficient (characteristic of the sample)
- $e$ = the base of natural logarithms

Bouguer's Law can thus be stated, "The absorbance of a homogeneous sample is directly proportional to the thickness of the sample in the optical path."

### 4.5.2 LAWS OF LIGHT ABSORPTION: BEER'S LAW

Beer's Law is exactly analogous to Bouguer's Law, except that it is stated in terms of both path length and concentration. The amount of light absorbed is proportional to the number of absorbing molecules through which the light passes. This is formulated mathematically, using base 10 logarithms:

$$T = \frac{P}{P_0} = 10^{-abc}$$

or

$$A = -\log T = -\log \frac{P}{P_0} = \log \frac{P}{P_0} = abc$$

where:

- A = absorbance
- a = absorptivity
- b = path length (in centimeters)
- c = concentration (usually in grams/liter)

Beer's Law in the form A = abc is widely used in quantitative analysis, because absorbance is directly proportional to concentration. Most modern spectrophotometers have scales calibrated in absorbance rather than in transmittance because of its convenience. Absorptivity, a, is a characteristic of a given substance only under precisely defined conditions of wavelength, solvent, temperature, and other parameters. If path length is given in terms of centimeters and concentration in grams solute per liter of solution, then the absorptivity unit is liters per gram centimeter. Another useful and well accepted term is molar absorptivity ($\epsilon$), which is the product of the absorptivity, a, and the molecular weight of the substance.

### 4.5.3 A TYPICAL QUANTITATIVE SPECTROPHOTOMETRIC ANALYSIS

A typical analysis is the quantitative analysis of phenobarbital in serum. After extracting the serum as outlined in the procedures section of this manual (Section 2.2.5), dissolve the sample in 0.45N NaOH to a volume of 3.0 ml. Divide this solution evenly between two cuvettes. To one cuvette add 1.5 ml of 0.45N (pH 13) and to the other add 1.5 ml of a boric acid buffer solution (pH 10). Determine the spectrum of each of these solutions with a reagent blank in the reference beam. The resulting spectra will resemble those illustrated in Figure 4.12.

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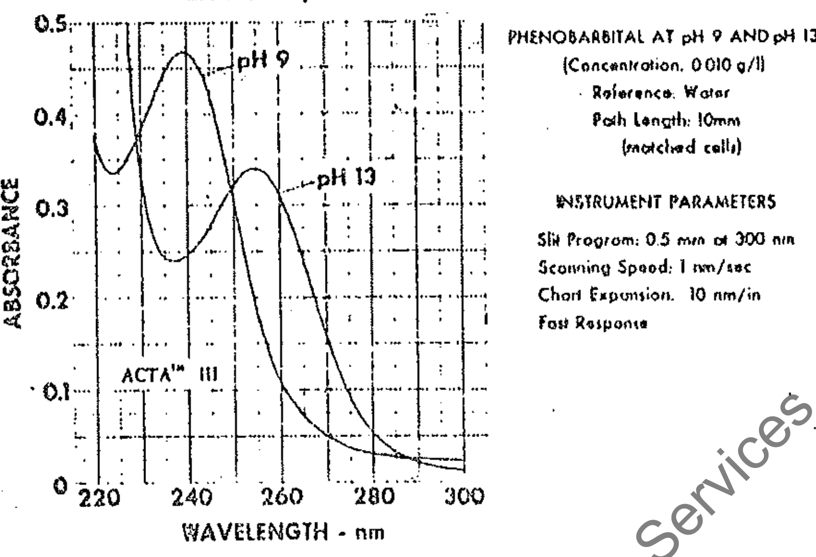


Figure 4.12. Barbiturates—Phenobarbital Spectra

If barbiturates are present, the absorbance of the solution at pH 13 will have a maximum at 260 nm and possibly a minimum at about 240. The maximum absorbance of the solution at pH 10 will shift to 240 nm. If the absorbance curves cross (confirming a barbiturate), the quantity can be determined by measuring the distance between the curves at 260 nm from the equation below:

$$(A_1 - A_2) \times F \times 0.2 \frac{\text{ml } 0.45\underline{N} \text{ NaOH}}{\text{ml Sample}} = \text{mg } 100 \text{ ml (PATIENT)}$$

where:

$A_1$ = absorbance of PATIENT sample—NaOH at 260 nm
$A_2$ = absorbance of PATIENT sample—borate at 260 nm
F = Factor: 42.9—general; 40.6—slow acting; 40.3—intermediate; and 47.8—fast acting barbiturates
0.45N NaOH solution = 6.0 ml
PATIENT sample = 1.0 ml

For further details, especially with respect to differential measurements, refer to Tietz (458).

### 4.5.4 DIFFERENTIAL QUANTITATIVE ANALYSIS

The absorbance at 260 nm for the sample at pH 13 may be subtracted from the absorbance of the sample at pH 10.2 by running the pH 10.2 as the sample and the other as the reference. The difference in the absorbance of these two solutions at 260 nm is proportional to the amount of barbiturate present in the sample. A series of known concentrations of phenobarbital may be carried through the same extraction and dilution process as the samples (as illustrated in Figure 4.13), and the difference in absorbance at 260 nm may be plotted on linear graph paper against concentration.

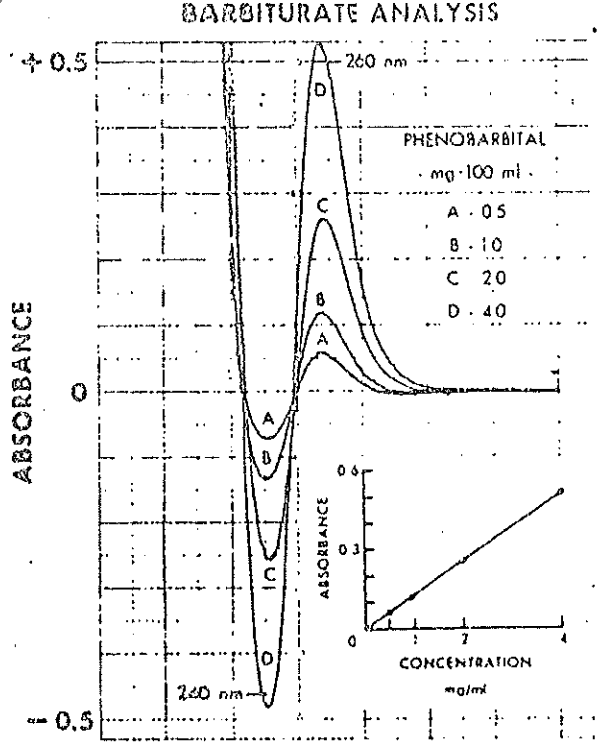


Figure 4.13. Quantitative Differential Barbiturate Analysis

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This would provide a calibration chart for phenobarbital. The concentration of an unknown phenobarbital can then be determined directly from this chart once the difference in absorbance in the sample at pH 13 and at pH 10.2 is determined. This differential reading compensates for contaminants and other absorbing materials that are in both solutions.

Use of a calibration graph as illustrated above is not absolutely necessary. Concentration can be determined directly from Beer's Law once the absorptivity has been evaluated (as defined in Section 4.5.2).

$$c = \frac{A}{ab}$$

To perform the differential quantitation on the DB-GT, place the differential mode switch at the rear of the instrument in the ON position. The instrument will now read out from -0.3 to +0.7A. The sample in 0.45$\underline{N}$ NaOH (pH 13) is placed in the cuvette in the sample beam, (right-hand position), the sample in the 0.45$\underline{N}$ NaOH/boric acid buffer solution (pH 10.2) is placed in the cuvette in the reference position, and the spectrum is scanned from 340 nm to 220 nm. The difference in absorption between the two solutions at 260 nm is proportional to barbiturate in the sample.

### 4.4.5 MULTI-COMPONENT ANALYSES

Sometimes more than one component must be determined in a mixture. As long as there is no overlapping of the absorption bands, each component can be treated exactly as if it were a single component. When there is interference because of overlapping absorption bands, the simple Beer's Law approach described above must be altered to minimize the effect of interference. Absorbances are additive, that is, at a specified wavelength the absorbance of a mixture is equal to the sum of the absorbances of each component. This effect may be noted when more than one drug in a category has been consumed by the patient. The resulting ultraviolet spectrum may not match closely with any of the standard spectra (Appendix II), however, strong UV absorption may indicate the presence of a drug or more than one drug. These results, as always, should be corroborated by one of the other methods – GC or TLC.

The fact that a strong ultraviolet absorption was found in the solution from the UV steps of these procedures is in itself important, for it may aid in the corroboration or confirmation of the TLC or GC results. In itself it must be considered as an INCONCLUSIVE, unless the noted absorption clearly matches a standard spectrum or the combination of two unique standard spectra.

### 4.5.6 SOURCES OF ERROR IN QUANTITATIVE ANALYSIS

Certain instrumental sources of error will be discussed under the appropriate paragraphs on instrument performance tests (Section 4.6). Several other sources of error are discussed in the remainder of this section.

[illegible] scattered by colloidal dispersions, dust, or other particulate matter in the sample appears to the instrument as absorption and can cause appreciable errors. Such samples should be filtered, centrifuged, or allowed to settle before measurement. Where turbid samples must be measured, a correction can sometimes be applied by measuring the scattered light at a wavelength where the sample does not absorb and subtracting that reading from the reading at the center of the absorption band.

Fluorescence – Some samples fluoresce, that is, they emit light of one wavelength when irradiated with light of shorter wavelength. Under ordinary circumstances the instrument cannot distinguish between the desired light and the emitted light. The error caused by fluorescence appears as a non-linearity in the plot of absorbance versus concentration. If the fluorescent substance is the substance being measured, the error can be eliminated by plotting a good calibration curve. Sometimes the effect of fluorescence can be minimized or eliminated by introducing a glass filter in the beam of the instrument between the sample and phototube. A filter is selected which passes the wavelength desired and absorbs the fluorescent light, which is always of a longer wavelength.

Temperature – Two different problems can be created when the temperature of the sample at the time of measurement is different from the temperature at the time of calibration. The volume of the solvent can change significantly, introducing a small error. The other source of error, the temperature coefficient of absorptivity, can, with some samples, be quite significant amounting to as much as 1% to 2% per degree centigrade.

Sample Decomposition – Some samples are quite sensitive to photochemical reactions when exposed to [illegible] light in the instrument or fluorescent light in the [illegible]laboratory.

Solvent Evaporation – Volatile solvents such as ether, acetone, methylene chloride, and many others can evaporate rapidly from an open cuvette and cause a rapid change in sample concentration. Such solvents should always be used in stoppered cuvettes only.

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Dirty Sample Cuvettes—Cuvettes must always be kept scrupulously clean. The optical surfaces should be wiped clean with a soft tissue before each use. Particular care should be taken to avoid fingerprints, because skin oils and perspiration absorb strongly, especially in the ultraviolet regions of the spectrum. Refer to the instructions for care of absorption cuvettes (Section 4.8).

## 4.6 DRUG ANALYSIS BY ULTRAVIOLET SPECTROPHOTOMETRY

### 4.6.1 INSTRUMENTATION: GENERAL DESCRIPTION OF THE BECKMAN DB-GT

This ultraviolet–visible spectrophotometer is a compact, totally solid-state, double-beam instrument. The power supply for the deuterium lamp and all other electronics, including those providing scale expanded modes, are located within the unit. Figure 4.14 shows the DB-GT controls. For a detailed description of the instrument, refer to the instruction manual.

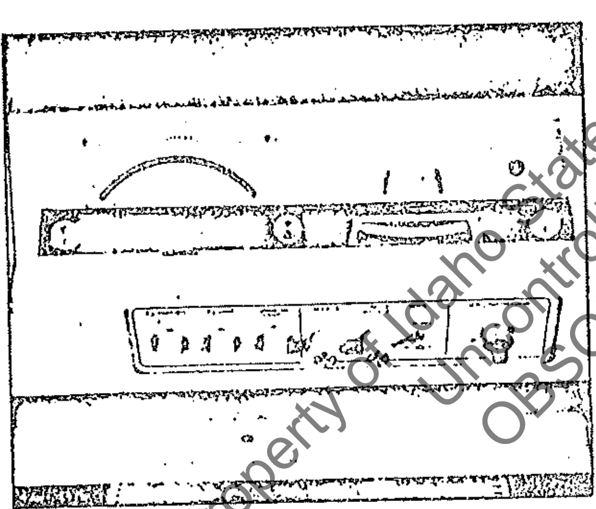

Figure 4.14. DB-GT Controls

### 4.6.2 PERCENT-T OR ABSORBANCE RECORDING IN DOUBLE-BEAM MODE

Although several modes of operation are possible with this instrument, analyses are performed in manual, double beam mode. For the ultraviolet analyses in these procedures, the Double Beam and the Absorbance recording modes will be used.

This operation includes both the spectrophotometer and a recorder. The two instruments should be properly interconnected, particularly connections at the recorder terminals of the spectrophotometer. The remote chart speed cable from the recorder attaches to the two-prong male receptacle on the rear panel of the spectrophotometer. See Figure 4.15.

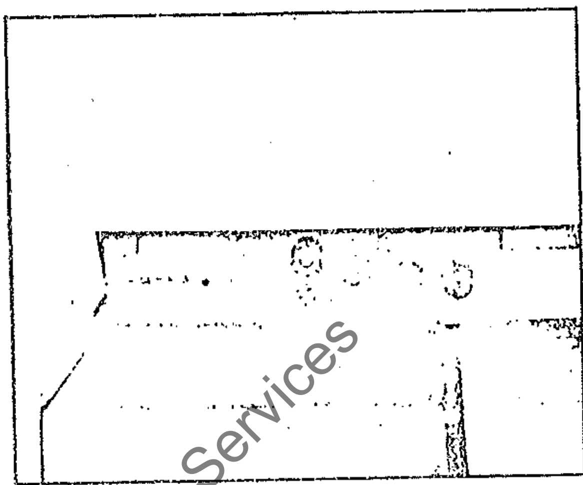

Figure 4.15. Back Panel of DB-GT

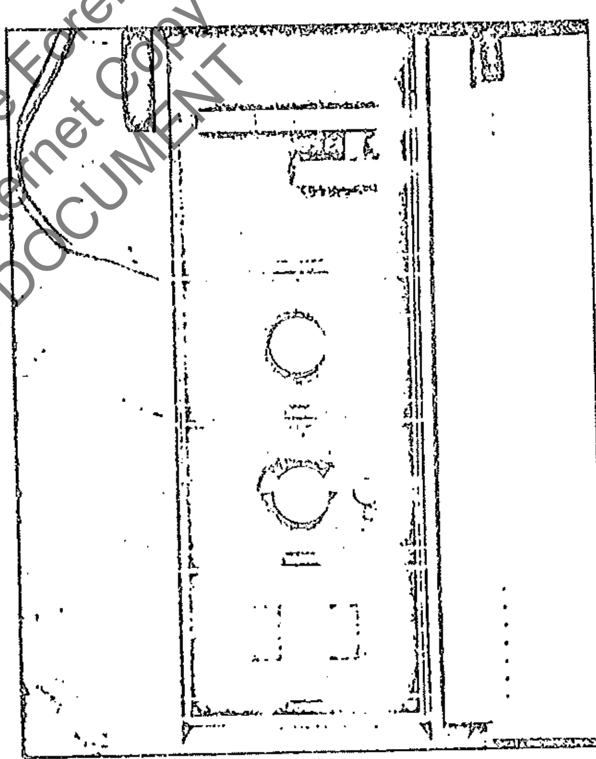

Figure 4.16. Recorder Controls and Top Panel

### 4.6.3 RECORDER FUNCTIONS

The recorder speed selected must be one which offers the best chart presentation at the selected scanning speed. In the SCAN II position of the power switch, the DB-GT monochromator will scan at the rate of 50 nm/minute. Used with a chart speed of five inches per minute on the recorder, this will give a 10 nm per inch

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presentation of the spectrum (Figure 4.16). This presentation will agree with all ultraviolet spectra included in this manual.

4.6.4 INSTRUCTIONS FOR USING THE DB-GT
Sample Preparations – Refer to the steps in the procedures (Section 2.0) detailing the preparation of biological samples for analysis on the DB-GT.

PROCEDURE

1. To start the instrument, turn power switch to ON position. Note that the red pilot light on the control panel glows.
2. For operation in the UV (190-360 nm), the range in which all of our drug analyses will be made:
   a. Place Source Selector in UV position.
   b. Momentarily depress $D_2$ LAMP pushbutton. If the $D_2$ lamp does not fire, depress the $D_2$ IGNITION button for a few seconds. The lamp should now ignite.

NOTE

For operation in the visible region – refer to the instruction manual provided with the DB-GT.

3. Select desired slit program – Program I.
4. Rotate the WAVELENGTH control to 340 nm.

NOTE

Always approach this wavelength from the high side (side of longer wavelength). Motion of the wavelength dial should be slow and even. When using a Beckman Ten-Inch Potentiometer Recorder, turn Power Control Switch to SCAN position. Chart drive will begin when scanning operation starts.

5. Place SPAN Switch in 0–2 A position.
6. Fill sample and reference cuvettes with solvent indicated for the particular determination being made.
7. Seat cuvettes in sample compartment.
8. Close compartment cover.
9. With REF adjust control, adjust meter to read 0 A.
10. Note on the chart paper the sample identification, starting wavelength, slit width setting, chart speed, and wavelength drive speed.
11. Remove sample cuvette, rinse, and fill with drug sample solution. Use the same sample cuvette and reinsert it in the sample compartment in the same orientation in which it was used in Step 3. Close compartment cover.
12. Start scan by placing the Mode Switch in SCAN II.
13. At the completion of this scan, clean and rinse the sample cuvette to prepare for the next sample.

4.7 INSTRUMENT PERFORMANCE CHECKS
Before making any spectrophotometric measurements in the ultraviolet or visible regions, make certain that the instrument is operating properly and that wavelength and absorbance readings are accurate. Until the analyst is very familiar with the operation of his instrument, he should follow the operating procedures outlined in the instrument instruction manual.

4.7.1 WAVELENGTH CALIBRATION

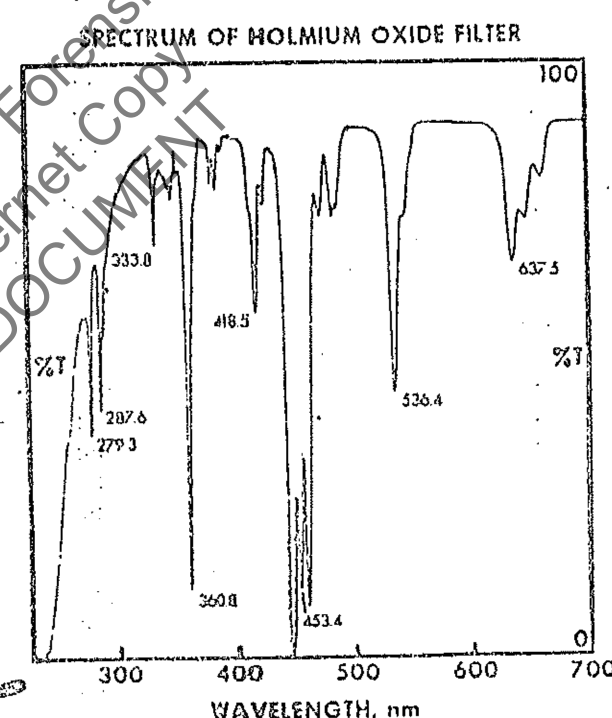


Figure 4.17. Holmium Oxide Filter Spectrum

HOLMIUM OXIDE FILTER TOLERANCES

| WAVELENGTH (nm) | DB (nm) | DB-G, DB-GT (nm) | DU, DU-2 (nm) |
|---|---|---|---|
| 279.3 | ±0.7 | ±0.5 | ±0.12 |
| 287.6 | ±0.7 | ±0.5 | ±0.15 |
| 333.8 | ±0.7 | ±0.5 | ±0.2 |
| 360.8 | ±1.5 | ±0.5 | ±0.2 |
| 418.5 | ±1.5 | ±0.5 | ±0.4 |
| 536.4 | ±2.0 | ±0.5 | ±0.6 |
| 637.5 | ±4.0 | ±0.5 | ±0.6 |

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Wavelength calibration should be checked daily using as a standard the Holmium Oxide Filter Accessory (Beckman Part No. 96157). This is a stable glass filter mounted in a special holder which may be inserted into the sample compartment of Beckman spectrophotometers. Wavelength values are tabulated in Figure 4.17.

WAVELENGTH CALIBRATION PROCEDURE (DB-GT)

DB-GT

| | |
|---|---|
| Mode | Double Beam 0-100%T |
| Slit Program | 1 |
| Source Lamp | < 360 nm, Hydrogen;<br>> 360 nm, Tungsten |

1. Set 100%T with both beams clear, then block the sample beam using the black 0%T block and adjust the 0%T setting.
2. Insert holmium oxide filter in sample beam.
3. Slowly rotate wavelength dial past the selected test point, 360.8 nm, from long to short wavelengths (refer to the wavelengths in Figure 4.17).
4. Observe the wavelength at which the %T is lowest and compare with the values in the table of Figure 4.17.
5. DB-GT's usually need to be checked only at one wavelength; 360.8 nm is the most convenient.
6. If wavelength tolerances are exceeded, do not try to adjust them. This should be done only by qualified service personnel.

### 4.7.2 PHOTOMETRIC CALIBRATION BY NATIONAL BUREAU OF STANDARDS FILTERS

The absorbance accuracy of a spectrophotometer is most rapidly and easily checked by using a set of neutral density glass filters which are calibrated at four wavelengths and certified for transmission values by the National Bureau of Standards. This set of three filters (SRM 930) is available from the Division of Analytical Chemistry, NBS, Washington, D.C., 20234 at a reasonable cost. Transmission values for a typical set of SRM 930 filters are shown in Table 4.2. (Note: each set is provided with its own set of certified values).

### 4.7.3 PHOTOMETRIC CALIBRATION BY CHROMATE SOLUTION

Alkaline potassium chromate, one of the same materials proposed by NBS for testing absorbance accuracy, is also a convenient check for photometric linearity. A solution of 40 mg/l in 0.05N KOH has an absorbance of about 1.0 at approximately 370 nm in a narrow bandpass spectrophotometer such as the DB-GT. Spectra of several solutions of this material are shown in Figure 4.18.

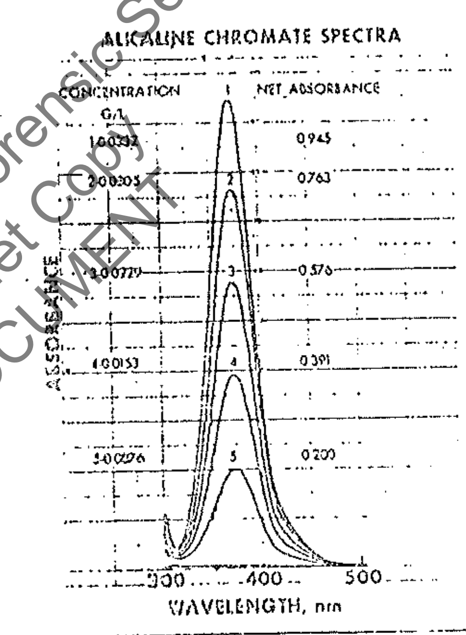


Figure 4.18. Alkaline Chromate Spectra

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TABLE 4.2
TRANSMISSION VALUES-NBS SRM 930 FILTERS

| | FILTER NUMBER | | | | | |
|---|---|---|---|---|---|---|
| | 1-7 | | 2-7 | | 3-7 | |
| WAVELENGTH (nm) | %T (±0.5) | A | %T (±0.5) | A | %T (±0.5) | A |
| 440 | 39.5 | 0.483 | 22.2 | 0.654 | 12.4 | 0.908 |
| 465 | 35.6 | 0.449 | 24.7 | 0.607 | 14.4 | 0.843 |
| 590 | 31.9 | 0.496 | 20.7 | 0.684 | 11.2 | 0.953 |
| 635 | 33.2 | 0.478 | 22.0 | 0.658 | 12.2 | 0.915 |

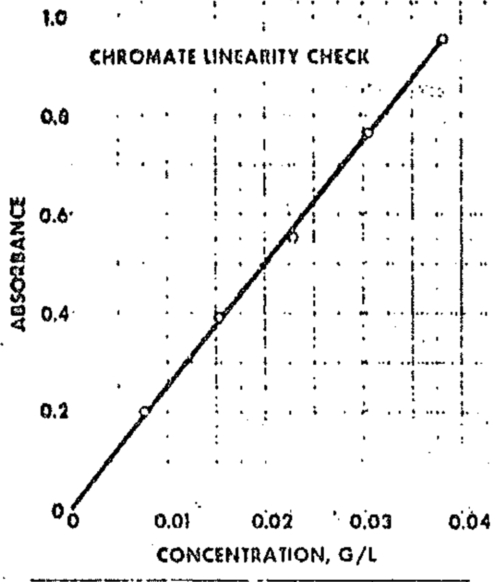


Figure 4.19. Chromate Linearity

SOLUTIONS REQUIRED

1. 0.05N KOH – 3.3 g analytical reagent grade KOH/liter distilled water.
2. Stock potassium chromate ($K_3CrO_4$) – dissolve 20 mg analytical reagent grade $K_2CrO_4$ in 500 ml 0.05N KOH.
3. To four, 50 ml volumetric flasks, add as follows:
   a 40 ml stock $K_2CrO_4$
   b 30 ml stock $K_2CrO_4$
   c 20 ml stock $K_2CrO_4$
   d 10 ml stock $K_2CrO_4$

   Dilute each to 50 ml with 0.05N KOH.

TEST PROCEDURE – FOR THE DB-GT

1. Set the spectrophotometer wavelength dial to 370 nm. Use the hydrogen lamp.
2. Place a cuvette containing 0.05N KOH in sample and reference beam. Set 100% on meter. (On DB-GT, set zero on 0–1 A scale).
3. Remove sample cuvette, rinse twice with $K_2CrO_4$ stock solution, and discard the rinse. Fill cuvette with $K_2CrO_4$ solution and place it in sample beam. Be sure cuvette windows are clean, dry, and free of fingerprints.

NOTE

To clean cuvettes, soak them in a mild solution of liquid detergent. Rinse thoroughly with distilled water and dry by rinsing with ethanol and inverting on tissue paper. Do not use acetone.

4. Read absorbance from meter.
5. Repeat steps 2 through 4 using solutions a, b, c, and d, as prepared above.
6. Plot absorbance vs $K_2CrO_4$ concentration (grams/liter) on linear graph paper. A typical plot is shown in Figure 4.19.

Other solutions sometimes used to check absorbance accuracy are listed in Table 4.3.

4.7.4 PRECAUTIONS FOR PERFORMANCE CHECKS

1. Random errors can be due to day-to-day repeatability of a variety of factors:
   a. The operator(s) setting of wavelength.
   b. Variations in laboratory temperature (some spectrophotometers show a slight temperature effect on wavelength).
   c. Cuvette cleanliness.
   d. Differences in operator interpretation of transmittance dial or meter reading.

TABLE 4.3
ABSORBANCE ACCURACY TEST SOLUTIONS

| SOLUTION | WAVELENGTH (nm) | A | %T |
|---|---|---|---|
| Potassium Chromate 0.0400 g 1 0.05N KOH | [illegible] | [illegible] (±0.01) | 17.3 (±0.5) |
| NBS SRM # 136d[1] | 340 | 0.314 (±0.005) | 48.5 (±0.5) |
| | 375 | 0.991 (±0.02) | 10.2 (±0.5) |
| | 400 | 0.387 (±0.005) | 41.0 (±0.5) |
| Cobalt Ammonium Sulfate 28.96 g 1 1% $H_2SO_4$ | [illegible] | [illegible] (±0.005) | 44.9 (±0.5) |
| Cupric Sulfate ($CuSO_4 \cdot 5H_2O$) 20.00 g 1 1% $H_2SO_4$ | [illegible] | [illegible] (±0.004) | 59.7 (±0.5) |
| | 670 | 0.332 (±0.005) | 48.6 (±0.5) |

[1] May be purchased from National Bureau of Standards.

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2. Maintain an instrument log or notebook, in which the results of each day's wavelength calibration test are recorded. Any other notes on instrument performance or malfunction could also be entered, as well as the results of the weekly absorbance linearity test.
3. Directional errors will appear as either gradual or sudden shifts of readings in one direction or another. A gradual upward trend in absorbance of the test sample can usually be traced to slow changes in sample concentration because of evaporation during handling and storage. This may be verified by using a fresh, accurately prepared test solution—such as the dichromate described above in the performance check on absorbance linearity.

   A downward trend in absorbance readings may arise from several causes. In single beam instruments the most common are gradual decreases in output of source lamps and corrosion or deposits of dust and coatings on mirror surfaces. Errors of this type, which usually take place over rather long periods of time, make it necessary to use gradually wider slits to set 100%T at a given wavelength. Such errors appear slowly in double beam instruments such as the DB-GT because the reference and sample beams are treated the same optically.
4. Serious damage to mirror surfaces because of carelessness in handling solutions in the sample compartment will usually cause stray light levels to increase, sometimes abruptly. Should this occur, attention by qualified service personnel is required.
5. Sudden, very sharp changes in photometric readings can occur if a source lamp or wavelength calibration adjustment is accidentally disturbed, as might occur when moving an instrument from one location to another. Any time an instrument is moved or when a new source lamp or detector is installed, all aspects of its performance should be checked thoroughly.

## 4.8 CARE OF ABSORPTION CELLS (OR CUVETTES)

Instructions for the Care and Cleaning of Beckman Cuvettes

1. CAUSES of Cell deterioration:
   a. Films deposited by solvent evaporation, strong wetting agents, or inadequate cleaning.
   b. Etching caused by continued use of strong alkali or concentrated mineral acids, either in the sample or cleaning solution. Also caused by weak alkaline solutions left in the cuvette for long periods of time.
   c. Contamination caused by solutions being allowed to evaporate in the cuvette, leaving deposits of salts, organic material, etc.
2. EFFECTS
   Reduced transmission and light scattering caused by etching of the cuvette windows. (Etching does not always mean the cuvette is useless because corrections can be made for it.) Films and particulate matter also decrease transmission, and, in addition, may contaminate the sample solvent.
3. DETECTION
   Extent of cell deterioration may be judged by the following specification: at 220, 240, and 270 nm (silica cuvette), or 320 nm (Vycor or Pyrex cuvettes), transmission of a new Beckman cuvette filled with double-distilled water must be at least 70% of transmission with air alone in the cuvette space.
4. PREVENTION
   a. Clean the cuvette with a mild agent as soon as possible after each use.
   b. Always start with distilled water for aqueous solutions, or use any suitable organic solvent for organic materials.
   c. Mild detergents may be used if it is certain that they are true solutions and do not contain particulate matter. Either Beckman 97707 Bio-Degradable Detergent or 97709 Ammoniated Detergent is recommended.
   d. For hard-to-remove deposits, use a mixture of 50% 3N HCl and 50% Ethanol. More convenient, and just as effective, is Beckman 97705 Dual Sonic Solution, which is specially formulated for cleaning the fragile items.
   e. Whenever possible, rinse the cuvette with sample solution before filling.
   f. Remember that if a reagent is not of spectrophotometric grade purity, it may leave a deposit on the cuvette window after evaporation.

   Never blow the cuvette dry with air. It is better to speed evaporation of the solvent with the aid of a vacuum. For example, use the Beckman 188040 Cell Washer. The cell washer includes a stopper and ten gaskets and may be used with a 500 ml Erlenmeyer flask and a 50 ml beaker for cuvette washing or by using a vacuum only, for cuvette drying.

   Never use any brush or instrument

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## CHAPTER 2

# ULTRAVIOLET AND VISIBLE SPECTRA

**2–1.** *Introduction.* 2–2. *The Energy of Electronic Excitation.* 2–3. *The Absorption Laws.* 2–4. *Measurement of the Spectrum.* 2–5. *Vibrational Fine Structure.* 2–6. *Choice of Solvent.* 2–7. *Selection Rules and Intensity.* 2–8. *Chromophores.* 2–9. *Solvent Effects.* 2–10. *Searching for a Chromophore.* 2–11. *Standard Works of Reference.* 2–12. *Definitions.* 2–13. *Conjugated Dienes.* 2–14. *Polyenes.* 2–15. *Polyeneynes and Poly-ynes.* 2–16. *Ketones and Aldehydes; $\pi\rightarrow\pi^*$ Transitions.* 2–17. *Ketones and Aldehydes; $n\rightarrow\pi^*$ Transitions.* 2–18. *$\alpha\beta$-unsaturated Acids, Esters, Nitriles and Amides.* 2–19. *The Benzene Ring.* 2–20. *Substituted Benzene Rings.* 2–21. *Polycyclic Aromatic Hydrocarbons.* 2–22. *Heteroaromatic Compounds.* 2–23. *Quinones.* 2–24. *Porphyrins, Chlorins and Corroles.* 2–25. *Non-conjugated Interacting Chromophores.* 2–26. *The Effect of Steric Hindrance to Coplanarity. Bibliography.*

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### 2–1. Introduction

The visible and ultraviolet spectra of organic compounds are associated with transitions between electronic energy levels. The transitions are generally between a bonding or lone-pair orbital and an unfilled non-bonding or anti-bonding orbital. The wavelength of the absorption is then a measure of the separation of the energy levels of the orbitals concerned. The highest energy separation is found when electrons in $\sigma$-bonds are excited, giving rise to absorption in the 120 to 200 mμ ($1\ \mu = 10^{-4}$ cm.) range. This range, known as the vacuum ultraviolet, since air must be excluded from the instrument, is both difficult to measure and relatively uninformative. Above 200 mμ, however, excitation of electrons from p- and d-orbitals and $\pi$-orbitals, and, particularly, $\pi$-conjugated systems, gives rise to readily measured and informative spectra.

### 2–2. The Energy of Electronic Excitation

The energy is related to wavelength by equation 2–1.

$$E\ (\text{kcals/mole}) = \frac{28{\cdot}6 \times 1000}{\lambda\ (\text{m}\mu)} \qquad (2\text{–}1)$$

That is, for example, 286 mμ is equivalent to 100 kcals—sufficient

energy, incidentally, to initiate many interesting reactions: compounds should not, therefore, be left in the ultraviolet beam any longer than is necessary.

### 2-3. The Absorption Laws

Two empirical laws have been formulated about the absorption intensity. *Lambert's law* states that the fraction of the incident light absorbed is independent of the intensity of the source. *Beer's law* states that the absorption is proportional to the number of absorbing molecules. From these laws, the remaining variables give the equation 2-2.

$$\log_{10} \frac{I_0}{I} = \varepsilon . l . c \qquad (2\text{-}2)$$

$I_0$ and $I$ are the intensities of the incident and transmitted light respectively, $l$ is the path length of the absorbing solution in centimetres, and $c$ is the concentration in moles/litre. $\log_{10} (I_0/I)$ is called the absorbance or optical density; $\varepsilon$ is known as the molar extinction coefficient and has units of 1000 cm.$^2$/mole but the units are, by convention, never expressed.

### 2-4. Measurement of the Spectrum

The ultraviolet or visible spectrum is usually taken on a very dilute solution. An appropriate quantity of the compound (often about 1 mg. when the compound has a molecular weight of 100 to 200) is weighed accurately, dissolved in the solvent of choice (see below) and made up to, for instance, 100 ml. A portion of this is transferred to a silica cell. The cell is so made that the beam of light passes through a 1 cm. thickness (the value $l$ in equation 2-2) of solution. A matched cell containing pure solvent is also prepared, and each cell is placed in the appropriate place in the spectrometer. This is so arranged that two equal beams of ultraviolet or visible light are passed, one through the solution of the sample, one through the pure solvent. The intensities of the transmitted beams are then compared over the whole wavelength range of the instrument. In most spectrometers there are two sources, one of 'white' ultraviolet and one of white visible light, which have to be changed when a complete scan is required. Usually either the visible or ultraviolet alone is sufficient for the purpose in hand. The spectrum is plotted automatically on most machines as a $\log_{10} (I_0/I)$ ordinate and $\lambda$ abscissa. For publication and comparisons these are often

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converted to an $\varepsilon$ versus $\lambda$ or log $\varepsilon$ versus $\lambda$ plot. The unit of $\lambda$ is almost always mμ (1 mμ = 10 Å). Strictly speaking the intensity of a transition is better measured by the area under the absorption peak (when plotted as $\varepsilon$ versus frequency) than by the intensity of the maximum of the peak. For several reasons, most particularly convenience and the difficulty of dealing with overlapping bands, the latter procedure is adopted in everyday use. Spectra are quoted, therefore, in terms of $\lambda_{max}$, the wavelength of the absorption peak, and $\varepsilon_{max}$, the intensity of the absorption peak as defined by equation 2–2.

### 2–5. Vibrational Fine Structure

The excitation of electrons is accompanied by changes in the vibrational and rotational quantum numbers so that what would otherwise be an absorption *line* becomes a broad peak containing vibrational and rotational fine structure. Due to interactions of solute with solvent molecules this is usually blurred out, and a smooth curve is observed. In the vapour phase, in non-polar solvents, and with certain peaks (e.g., benzene with the 260 mμ band), the vibrational fine structure is sometimes observed.

### 2–6. Choice of Solvent

The solvent most commonly used is 95 per cent ethanol (commercial absolute ethanol contains residual benzene which absorbs in the ultraviolet). It is cheap, a good solvent and transparent down

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Table 2–1

Some Solvents used in Ultraviolet Spectroscopy

| Solvent | Minimum wavelength for 1 cm. cell, mμ |
|---|---|
| Acetonitrile | 190 |
| Water | 191 |
| Cyclohexane | 195 |
| Hexane | 201 |
| Methanol | 203 |
| Ethanol | 204 |
| Ether | 215 |
| Methylene dichloride | 220 |
| Chloroform | 237 |
| Carbon tetrachloride | 257 |

to about 210 mμ. Fine structure, if desired, may be revealed by using cyclohexane or other hydrocarbon solvents which, being less polar, have least interaction with the absorbing molecules. Table 2-1 gives a list of common solvents and the minimum wavelength from which they may be used in 1 cm. cells.

The effect of solvent polarity on the position of maxima is discussed in section 2·9.

**2–7. Selection Rules and Intensity**

The irradiation of organic compounds may or may not give rise to excitation of electrons from one orbital (usually a lone-pair or bonding orbital) to another orbital (usually a non-bonding or anti-bonding orbital). It can be shown that:

$$\varepsilon = 0{\cdot}87 \times 10^{20}\ P.a \qquad (2\text{–}3)$$

where $P$ is called the transition probability (with values from 0 to 1) and **a** is the target area of the absorbing system; the absorbing system is usually called a chromophore. With common chromophores of the order of 10 Å long, a transition of unit probability will have an $\varepsilon$ value of $10^5$. This is close to the highest observed values, though—with unusually long chromophores—values in excess of this have been measured. In practice, a chromophore giving rise to absorption by a fully allowed transition will have $\varepsilon$ values greater than about 10,000, while those with low transition probabilities will have $\varepsilon$ values below 1000. An important point is that there is a positive correlation between the *length* of a particular chromophore (more strictly the *area*) and the absorption intensity observed.

There are many factors which affect the transition probability of any particular transition. In the first place there are rules about which transitions are allowed and which are forbidden. These are complicated because they are a function of the symmetry and multiplicity both of the ground state and excited state orbitals concerned. The spectra of chromophores, with $\varepsilon_{max}$ less than about 10,000, are the result of 'forbidden' transitions. Two very important and 'forbidden' transitions are observed: (*a*) the $n \rightarrow \pi^*$ band near 300 mμ of ketones, with $\varepsilon$ values of the order of 10 to 100, and (*b*) the benzene 260 mμ band and its equivalent in more complicated systems, with $\varepsilon$ values from 100 upwards. Both occur because the symmetry which makes absorption strictly forbidden is broken up by molecular vibrations and—in the latter case—by substitution.



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Both types are discussed further under the sections on ketones and aromatic systems.

In this and the following discussions a very simplified theoretical picture is given: there is considerable danger in being satisfied with so little in so well developed a subject. The books by Jaffé and Orchin and by Murrell, listed in the bibliography, give excellent accounts of the state of the art.

### 2–8. Chromophores

The word chromophore is used to describe the system containing the electrons responsible for the absorption in question. Most of the simple unconjugated chromophores described in Table 2·2 below give rise to such high-energy, and therefore such short-wavelength absorption, that they are of little use.

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**Table 2–2**

**The Absorption of Simple Unconjugated Chromophores**

| Chromophore | Transition notation† | $\lambda_{max}$ in mμ |
|---|---|---|
| σ-Bonded electrons | | |
| >C—C< and >C—H | $\sigma \rightarrow \sigma^*$ | ~150 |
| Lone-pair electrons | | |
| —Ö— | $n \rightarrow \sigma^*$ | ~185 |
| —N̈< | $n \rightarrow \sigma^*$ | ~195 |
| —S̈— | $n \rightarrow \sigma^*$ | ~195 |
| >C=Ö | $n \rightarrow \pi^*$ | ~300 |
| >C=Ö | $n \rightarrow \sigma^*$ | ~190 |
| π-bonded electrons | | |
| >C=C< (isolated) | $\pi \rightarrow \pi^*$ | ~190 |

† There are many other notations used

One of the few useful simple unconjugated chromophores is the very weak forbidden $n \rightarrow \pi^*$ transition of ketones mentioned earlier which appears in the 300 mμ region and is of particular importance in connection with optical rotatory dispersion. This band is due to the excitation of one of the lone pair of electrons (designated n) on the oxygen atom to the lowest anti-bonding orbital (designated $\pi^*$)

of the carbonyl group. It is discussed further in the sections on solvent effects and on ketones.

The important chromophores are those in which conjugation is present. An isolated double bond or lone pair of electrons gives rise to a strong absorption maximum at about 190 mμ, corresponding to the transition $x$ in Fig. 2-1, at too short a wavelength for convenient measurement. When the molecular orbitals of two isolated double bonds are brought into conjugation, the energy level of the highest occupied orbital is raised and that of the lowest unoccupied anti-bonding orbital lowered (Fig. 2-1).

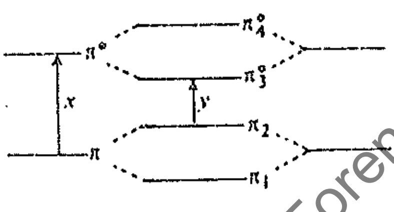


Fig. 2-1.

The $\pi \rightarrow \pi^*$ transition, which is occasioned by absorption, is now associated with the smaller value $y$. This transition appears in the spectrum of butadiene as a strong, easily detected, and easily measured maximum at 217 mμ. The same principle governs the energy levels when unlike chromophores, e.g., those of an $\alpha\beta$-unsaturated ketone, are brought together. For instance, methyl vinyl ketone has an absorption maximum at 225 mμ, while neither a carbonyl group nor an isolated double bond has a strong maximum above 200 mμ.

When more than two $\pi$-bonding orbitals overlap, that is when the chromophore is a longer conjugated system, the separation of the energy levels is further reduced, and absorption occurs at longer wavelength. A long conjugated polyene like carotene absorbs, quite obviously since it is coloured, in the visible. The most important point to be made is that, in general, the longer the conjugated system, the longer the wavelength of the absorption maximum.

The rules and correlations possible with the spectra of conjugated dienes, $\alpha\beta$-unsaturated ketones, and some substituted benzene ring compounds are given in sections 2-13, 2-16 and 2-20. With complicated chromophores, predictions become more difficult. The usual procedure, when one is confronted with the ultra-

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violet spectrum of an unknown substance, is to compare the spectrum, in its general shape and in the intensity and position of its peaks, with the spectra of reasonable model compounds. These models are chosen to possess as nearly as possible the same chromophore as that suspected for the unknown.

**2-9. Solvent Effects**

*(i)* $\pi \rightarrow \pi^*$. The Frank–Condon principle states that during the electronic transition atoms do not move. Electrons, however, including those of the solvent molecules, may reorganize. Most transitions result in an excited state more polar than the ground state;† the dipole-dipole interactions with solvent molecules will, therefore, lower the energy of the excited state more than that of the ground state. Thus it is usually observed that ethanol solutions give longer wavelength maxima than do hexane solutions. In other words, there is a small red-shift of the order of 10 to 20 mμ in going from hexane as solvent to ethanol.

*(ii)* $n \rightarrow \pi^*$. The weak transition of the oxygen lone pair in ketones—the $n \rightarrow \pi^*$ transition—shows a solvent effect in the opposite direction. The solvent effect is now due to the lesser extent to which solvents can hydrogen bond to the carbonyl group in the excited state. In hexane solution, for example, the absorption maximum of acetone is at 279 mμ ($\varepsilon = 15$), whereas in aqueous solution the maximum is at 264·5 mμ. The shift in this direction is known as a blue shift.

**2-10. Searching for a Chromophore**

There is no easy rule or set procedure for identifying a chromophore—too many factors affect the spectrum and the range of structures which can be found is too great. The examination of a spectrum with particular regard for the following points is the first step to be taken.

† This transition is commonly visualized in valence bond terms with the ground state represented without charge separation and the excited state as the dipolar species.

$$\text{CH}_2{=}\text{CH–CO–CH}_3 \xrightarrow{h\nu} {}^{+}\text{CH}_2\text{–CH}{=}\text{C(O}^{-}\text{)–CH}_3$$

Such representations are over-simplified, and misleading: the dipolar structure is not *the* structure of the excited state, rather it is a more important contributor to the excited state than to the ground state. Since the valence bond technique is less exact and less revealing in this field than is the molecular orbital theory, the latter should be used on all occasions.

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(i) *The complexity and the extent to which the spectrum encroaches on the visible region.* A spectrum with many bands stretching into the visible shows the presence of a long conjugated or a polycyclic aromatic chromophore. A compound giving a spectrum with only one band (or only a few bands) below about 300 mμ, probably contains only two or three conjugated units.

(ii) *The intensity of the bands, particularly the principal maximum and the longest wavelength maximum.* This observation can be very informative. Simple conjugated chromophores such as dienes and $\alpha\beta$-unsaturated ketones have $\varepsilon$ values of 10,000 to 20,000. The longer simple conjugated systems have principal maxima (usually also the longest wavelength maxima) with correspondingly higher $\varepsilon$ values. Very low intensity absorption bands in the 270 to 350 mμ region, on the other hand, with $\varepsilon$ values of 10 to 100, are the result of the $n \rightarrow \pi^*$ transition of ketones. In between these extremes, the existence of absorption bands with $\varepsilon$ values of 1000 to 10,000, almost always shows the presence of an aromatic system. Many unsubstituted aromatic systems show bands with intensities of this order of magnitude, the absorption being the result of a transition with a low transition probability, low because of the symmetry of the ground and excited states. When the aromatic nucleus is substituted with groups which can extend the chromophore, strong bands with $\varepsilon$ values above 10,000 appear, but bands with $\varepsilon$ values below 10,000 are often still present.

Having made these observations, one should search for a model system which contains the chromophore and therefore gives a similar spectrum to that which is being examined. This may be difficult in rare cases; but so many spectra are now known, and the changes caused by substitution so well documented, that the task can be a simple one. The first tool which an organic chemist requires is a general knowledge of the simple chromophores and the changes which structural variations make in the absorption pattern. Sections 2.13 to 2.26 give a very brief account of these topics. The remaining task, that of searching through the literature, is greatly facilitated by the existence of the indexes and compilations which are described in section 2.11. The usefulness of these books will be greatly increased by a general knowledge of organic chemistry on which to base a guess as to what chromophores are likely to be known and in what compounds they may be found.

The search for a chromophore is also likely to be assisted by the

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other physical methods described in this book. The range of structures in which a search must be made can be narrowed, for example, to aromatic compounds on the strength of infrared or NMR aromatic C–H absorptions. Similarly the presence of an $\alpha\beta$-unsaturated ketone may be inferred from the C=O stretching vibration observed in the infrared spectrum and confirmed from the ultraviolet spectrum, and the extent of alkylation deduced by a consideration of Woodward's rules (section 2–16) and by reference to the NMR spectrum. A very important stage in determining the structure of a natural product is the positive identification of the chromophore, by comparison of the spectrum with that of some known model compound.

**2–11. Standard Works of Reference**

In the search for a model chromophore, a number of source books are available. In addition several textbooks, larger than the single chapter of this book, are devoted to the subject and are mentioned in the bibliography at the end of this chapter.

The major collections of *data* are the following.

(*i*) H. M. Hershenson, *Ultraviolet and Visible Absorption Spectra*, Index for 1930–1954, Academic Press, New York, 1956; Index for 1955–1959 (1961).

The index gives the names of compounds which have been examined together with literature references.

(*ii*) R. A. Friedel and M. Orchin, *Ultraviolet Spectra of Aromatic Compounds*, Wiley, New York, 1951.

This is a catalogue showing the actual spectra of 579 aromatic compounds.

(*iii*) A.P.I. Research Project 44, *Ultraviolet Spectral Data*, Carnegie Institute and U.S. Bureau of Standards.

This compilation shows the actual spectra of 917 compounds (up to October 1962), mostly aromatic hydrocarbons.

(*iv*) H. E. Ungnade and M. J. Kamlet (Eds.), *Organic Electronic Spectral Data*, Interscience, New York, Vol. I, 1946–1952 (1960); Vol. II, 1953–1955 (1960); Vol. III, 1956–1957 (1965); Vol. IV, 1958–1959 (1963).

This most valuable collection has been prepared by a complete search of the major journals since 1945. The compounds are indexed by their empirical formulae, and absorption maxima are quoted together with literature references.

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(e) *UV Atlas of Organic Compounds*, Butterworths, London, 1965.

A collection of the spectra of nearly 1000 compounds elaborately cross indexed by chromophoric groups.

**2-12. Definitions**

The following words and symbols are commonly used.

*Red shift* or *bathochromic effect*. A shift of an absorption maximum towards longer wavelength. It may be produced by a change of medium, or by the presence of an auxochrome.

*Auxochrome*. A substituent on a chromophore which leads to a red shift. For example, the conjugation of the lone pair on the nitrogen atom of an enamine has shifted the absorption maximum from the isolated double bond value of 190 mμ to about 230 mμ. The nitrogen substituent is the auxochrome. An auxochrome, then, extends a chromophore to give a new chromophore.

*Blue shift* or *hypsochromic effect*. A shift towards shorter wavelength. This may be caused by a change of medium and also by such phenomena as the removal of conjugation. For example, the conjugation of the lone pair of electrons on the nitrogen atom of aniline with the π-bond system of the benzene ring is removed on protonation. Aniline absorbs at 230 mμ (ε 8600), but in acid solution the main peak is almost identical with that of benzene, being now at 203 mμ (ε 7500). A blue shift has occurred.

*Hyperchromic effect*. An effect leading to increased absorption intensity.

*Hypochromic effect*. An effect leading to decreased absorption intensity.

$\lambda_{max}$ The wavelength of an absorption maximum.

$\varepsilon$ The extinction coefficient defined by equation 2-2.

$E^{1\%}_{1\,cm.}$ Absorption ($\log_{10}(I_0/I)$) of a 1 per cent solution in a cell with a 1 cm. path length. This is used in place of ε when the molecular weight of a compound is not known, or when a mixture is being examined.

*Isosbestic point*. A point common to all curves produced in the spectra of a compound taken at several pH values.

**2-13. Conjugated Dienes**

The energy levels of butadiene have been illustrated in Fig. 2-1. The transition y gives rise to strong absorption at 217 mμ (ε 21,000).

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Alkyl substitution extends the chromophore, in the sense that there is a small interaction between the $\sigma$-bonded electrons of the alkyl group and the $\pi$-bond system. The result is a small red shift with alkyl substitution, just as there is a red shift (though a relatively large one) in going from an isolated double bond to a conjugated diene.

Fortunately the effect of alkyl substitution, in dienes at least, is additive; and a few rules suffice to predict the position of absorption in open chain dienes and dienes in six-membered rings. Open chain dienes exist normally in the s-*trans* conformation, while homoannular dienes must be in the s-*cis* conformation. These conformations are illustrated in the part structures I (heteroannular diene) and II (homoannular diene). It is not entirely clear why, but the s-*cis* conformation leads to longer wavelength absorption than does the s-*trans* conformation. Also, due to the shorter distance between the ends of the chromophore, s-*cis* dienes give maxima of lower intensity ($\varepsilon \sim 10{,}000$) than the maxima of s-*trans* dienes ($\varepsilon \sim 20{,}000$).

I

II

The actual rules for predicting the absorption of open chain and six-membered ring dienes were first made by Woodward in 1941. Since that time they have been modified by Fieser and by Scott as a result of experience with a very large number of dienes and trienes. The modified rules are given in Table 2-3.

For example the diene I would be calculated to have a maximum at 234 mμ by the following addition:

| | | |
|---|---|---|
| Parent value | | 214 mμ |
| Three ring residues (marked *a*) | 3 × 5 = | 15 mμ |
| One exocyclic double bond (the $\Delta^4$ bond is exocyclic to ring B) | | 5 mμ |
| Total | | 234 mμ |

An observed value is 235 mμ ($\varepsilon$ = 19,000).

By a similar calculation, the diene II would be expected to have a maximum at 273 mμ, and does actually have one at 275 mμ.

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**Table 2–3**

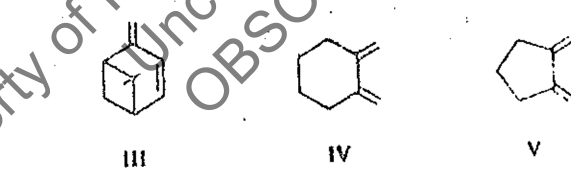
**Rules for Diene and Triene Absorption**

| | |
|---|---|
| Value assigned to parent heteroannular or open chain diene | 214 mμ |
| Value assigned to parent homoannular diene | 253 mμ |
| Increment for | |
| (a) each alkyl substituent or ring residue | 5 mμ |
| (b) the exocyclic nature of any double bond | 5 mμ |
| (c) a double bond extension | 30 mμ |
| (d) auxochrome OAcyl | 0 mμ |
| OAlkyl | 6 mμ |
| SAlkyl | 30 mμ |
| Cl, Br | 5 mμ |
| NAlkyl$_2$ | 60 mμ |
| Total | |
| $\lambda_{calc}$ | |

(Reprinted with permission from A. I. Scott, *Interpretation of the Ultraviolet Spectra of Natural Products*, Pergamon Press, Oxford, 1964.)

Though ethanol is the usual solvent, change of solvent has little effect. The actual appearance of the spectrum of a simple conjugated diene with the chromophore of I is illustrated in Fig. 2–2. The discussion above has referred to the highest intensity band and, indeed, the weaker bands are not always apparent.

There are a large number of exceptions to the rules, where special factors can operate. Distortion of the chromophore may lead to red or blue shifts, depending on the nature of the distortion.




The strained molecule verbenene (III) has a maximum at 245·5 mμ, whereas the usual calculation gives a value of 229 mμ. The diene IV might be expected to have a maximum at 273 mμ; but distortion of the chromophore, presumably out of planarity with consequent loss of conjugation, causes the maximum to be as low as 220 mμ with a similar loss in intensity (ε 5500). The diene V, in which coplanarity of the diene is more likely, gives a maximum at 248 mμ (ε 15,800) showing that this is so although it still does not agree with the expected value. Change of ring size in the case of

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simple homoannular dienes also leads to departures from the predicted value of 263 mμ as follows: cyclopentadiene, 238·5 mμ (ε 3400); cycloheptadiene, 248 mμ (ε 7500); while cyclohexadiene is close at 256 mμ (ε 8000). The lesson, an important one, is that when the ultraviolet spectrum of an unknown compound is to be compared with that of a model compound, then the choice of model must be a careful one. Allowance must be made for the likely shapes of the molecule and for any unusual strain. Some general comments on the effect of steric hindrance to coplanarity are given in section 2–26.

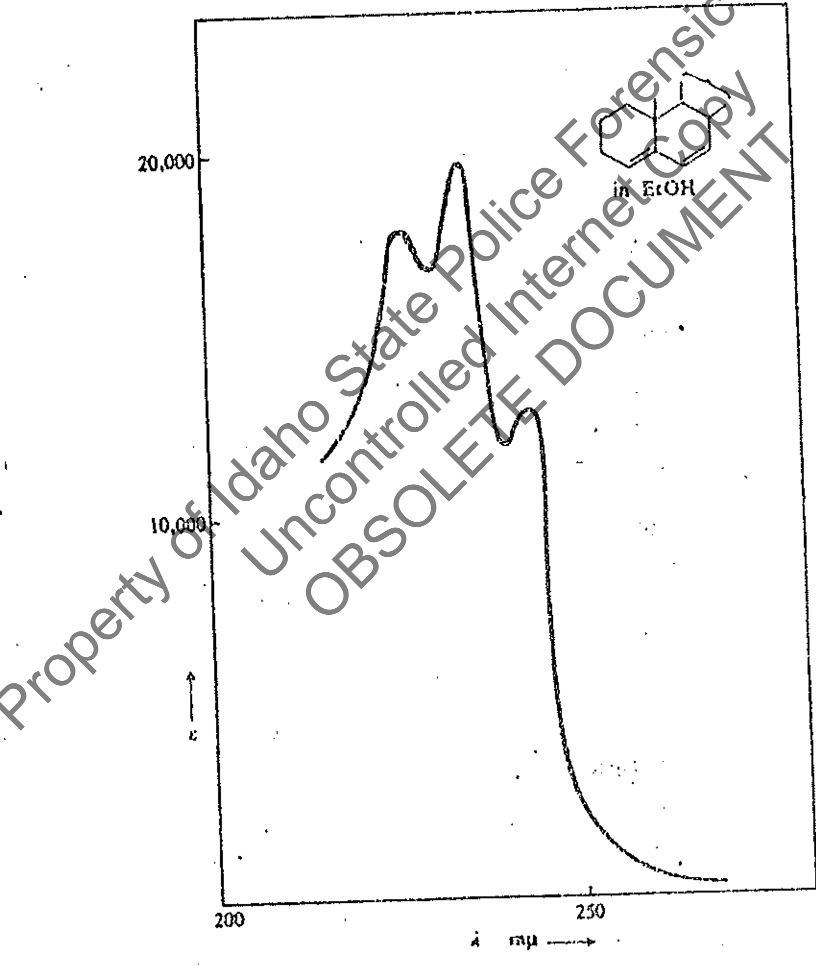


Fig. 2–2

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## 2-14. Polyenes

As the number of double bonds in conjugation increases, the wavelength of maximum absorption encroaches on the visible region. A number of subsidiary bands also appear and the intensity increases. Table 2-4 gives examples of the longest wavelength maxima of some simple conjugated polyenes, showing these trends.

**Table 2-4**

**Longest Wavelength Maxima of Some Simple Polyenes**

| | $Me(CH{=}CH)_nMe$ | | $Ph(CH{=}CH)_nPh$ | |
|---|---|---|---|---|
| $n$ | $\lambda_{max}$ mμ | $\varepsilon$ | $\lambda_{max}$ mμ | $\varepsilon$ |
| 3 | 274·5 | 30,000 | 358 | 75,000 |
| 4 | 310 | 76,500 | 384 | 86,000 |
| 5 | 342 | 122,000 | 403 | 94,000 |
| 6 | 380 | 146,500 | 420 | 113,000 |
| 7 | 401 | — | 435 | 135,000 |
| 8 | 411 | - | — | — |

The appearance of the spectra of some simple polyenes is illustrated in Fig. 2-3, which should be compared with the simpler spectrum of the diene in Fig. 2-2.

Several attempts both empirical and theoretical have been made to relate the principal or longest wavelength maximum with chain length. Some of the theoretical treatments have been based on the classical 'electron in the box' wave equation, in which the walls of the box are usually considered to be one average bond length beyond each end of the chromophore. This leads to the correct conclusion that increasing values of $\lambda_{max}$ are found for increasing length in a conjugated polyene, quantitative predictions are, however, less satisfactory. The simple theory might indicate that as the chain length increases, the value of $\lambda_{max}$ for long chains would increase proportionately; whereas in practice there is a convergence, which can be seen in Table 2-4. More sophisticated treatments, allowing for the variation in bond lengths between the double and single bonds, have been made and are described in Murrell's book. An interesting simplification is provided by the cyanine dye analogues VI in which resonance leads to uniform bond lengths and bond orders along the polyene chain.

$$Me_2\overset{+}{N}{=}CH{-}(CH{=}CH)_n{-}NMe_2 \longleftrightarrow Me_2N{-}(CH{=}CH)_n{-}CH{=}\overset{+}{N}Me_2$$

VI

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Calculations based on the 'electron in the box' model lead to values very close to those observed: $\lambda_{max}$ 309 ($n$ = 1), 409 ($n$ = 2) and 511 ($n$ = 3) mμ.

In a long chain polyene, change from *trans*- to *cis*- configuration at one or more double bonds lowers both the wavelength and the intensity of the absorption maximum.

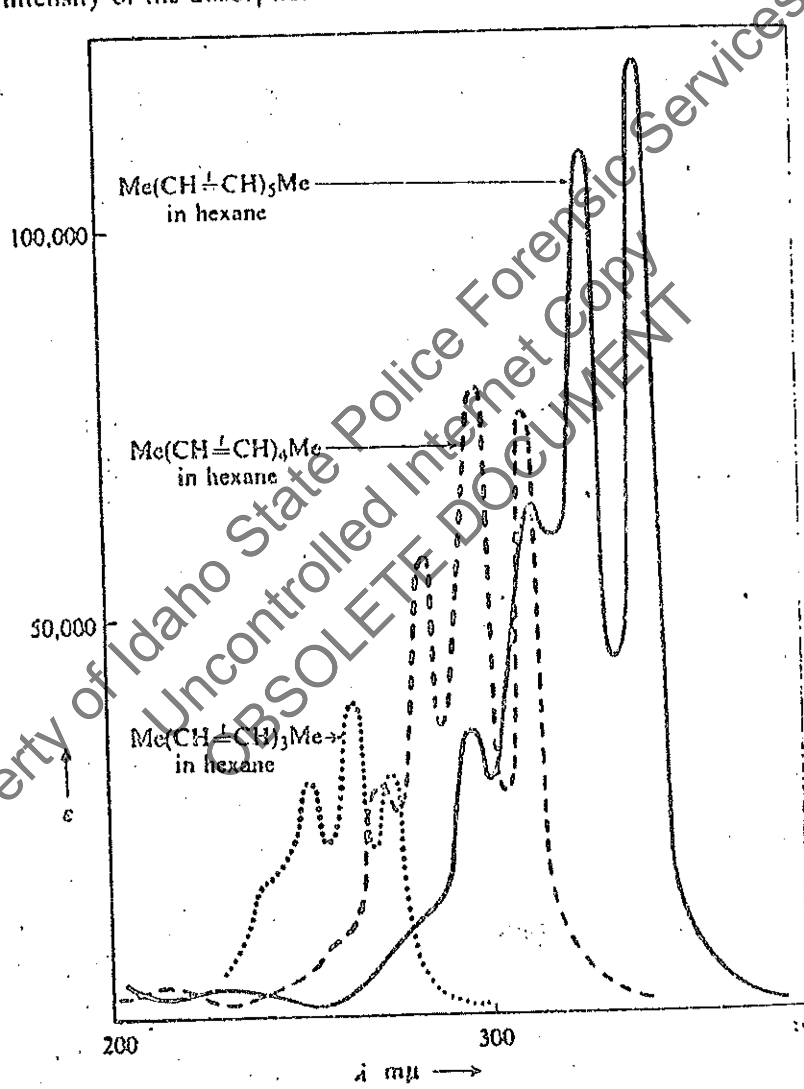


Fig. 2–3

(Replotted from Nayler and Whiting, *J. Chem. Soc.*, 1955, 3042.)

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**2-15. Polyeneynes and Poly-ynes**

As a result of interest in natural polyeneynes and poly-ynes, the ultraviolet spectra of many such compounds are known and have been of considerable use in the elucidation of structure. The characteristic spiky appearance of the spectra has been very helpful during the screening of crude plant extracts for acetylenic compounds. When more than two triple bonds are conjugated, the spectrum shows a characteristic series of low intensity bands ($\varepsilon \sim 200$) at intervals of 2300 $cm^{-1}$ (note the *frequency* units, frequency being directly proportional to energy whereas wavelength is not) and high intensity bands ($\varepsilon \sim 10^5$) at intervals of 2600 $cm^{-1}$. The principal maxima in each group are shown in Table 2-5.

**Table 2-5**

**Principal Maxima of Conjugated Poly-ynes $Me(C{\equiv}C)_nMe$**

| $n$ | $\lambda_{max}$ mμ | $\varepsilon$ | $\lambda_{max}$ mμ | $\varepsilon$ |
|---|---|---|---|---|
| 2 | — | — | 250 | 160 |
| 3 | 207 | 135,000 | 306 | 120 |
| 4 | 234 | 281,000 | 354 | 105 |
| 5 | 260·5 | 352,000 | 394 | 120 |
| 6 | 284 | 445,000 | — | — |

Fig. 2-4 shows the spectrum which is, like a fingerprint, diagnostic of the triyne-ene chromophore present in the dehydromatricaria ester (VII).

$$Me{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CO_2Me$$

**VII**

A similar compound VIII, which has, effectively, an alkyl group in place of the carbomethoxyl group of the ester VII, shows a similar pattern shifted to the blue by about 15 mμ, as shown on Fig. 2-4.

$$Me{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH_2CH_2COEt$$

**VIII**

This is an example of the way in which an organic chemist deals

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with the comparison of ultraviolet spectra: most of the chromophore of VII is present in VIII, and the latter will therefore continue to show the characteristic features of the former, with a small blue shift due to the relatively small loss of conjugation.

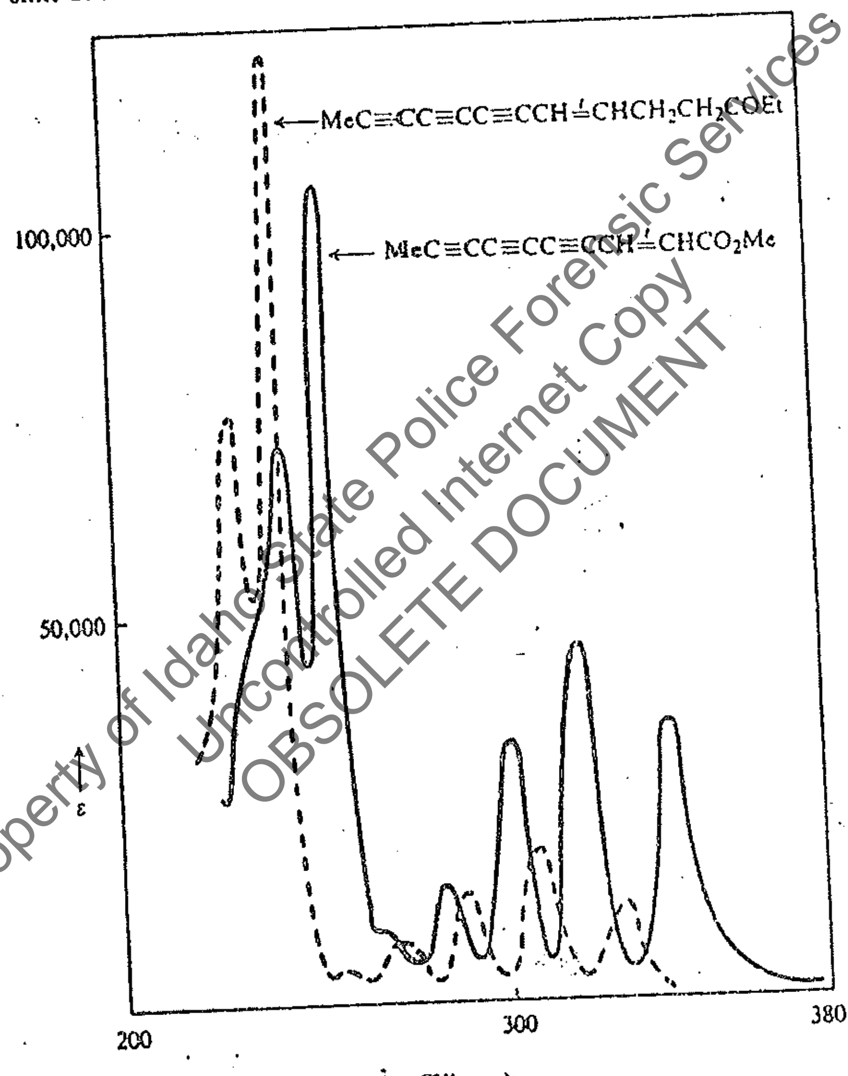

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Fig. 2–4

(Replotted from Sörensen, Bruun, Holme and Sörensen, *Acta Chem. Scand.*, 1954, 8, 28 and Bohlmann, Mannhardt and Viehe, *Chem. Ber.*, 1955, 88, 365.)

### 2-14. Ketones and Aldehydes; $\pi \rightarrow \pi^*$ Transitions

Like the dienes considered in section 2–13, $\alpha\beta$-unsaturated ketones and aldehydes have been the subject of much study and their absorption, too, is susceptible to prediction by a set of rules first formulated by Woodward and modified by Fieser and by Scott. The modified rules for calculating the expected position of the absorption maximum are given in Table 2-6.

**Table 2-6**

**Rules for $\alpha\beta$-unsaturated Ketone and Aldehyde Absorption**

$\overset{\delta}{C}=\overset{\gamma}{C}-\overset{\beta}{C}=\overset{\alpha}{C}-C=O$

$\varepsilon$ values are usually above 10,000 and increase with the length of the conjugated system.

| | | |
|---|---|---|
| Value assigned to parent $\alpha\beta$-unsaturated six-ring or acyclic ketone | | 215 mμ |
| Value assigned to parent $\alpha\beta$-unsaturated five-ring ketone | | 202 mμ |
| Value assigned to parent $\alpha\beta$-unsaturated aldehyde | | 207 mμ |
| Increments for | | |
| (a) a double bond extending the conjugation | | 30 mμ |
| (b) each alkyl group or ring residue | $\alpha$ | 10 mμ |
| | $\beta$ | 12 mμ |
| | $\gamma$ and higher | 18 mμ |
| (c) auxochromes | | |
| (*i*) —OH | $\alpha$ | 35 mμ |
| | $\beta$ | 30 mμ |
| | $\delta$ | 50 mμ |
| (*ii*) —OAc | $\alpha, \beta, \delta$ | 6 mμ |
| (*iii*) —OMe | $\alpha$ | 35 mμ |
| | $\beta$ | 30 mμ |
| | $\gamma$ | 17 mμ |
| | $\delta$ | 31 mμ |
| (*iv*) —SAlk | $\beta$ | 85 mμ |
| (*v*) —Cl | $\alpha$ | 15 mμ |
| | $\beta$ | 12 mμ |
| (*vi*) —Br | $\alpha$ | 25 mμ |
| | $\beta$ | 30 mμ |
| (*vii*) —$NR_2$ | $\beta$ | 95 mμ |
| (d) the exocyclic nature of any double bond | | 5 mμ |
| (e) homodiene component | | 39 mμ |
| $\lambda_{calc}^{EtOH}$ | Total | |

For $\lambda_{max}^{calc}$ in other solvents a solvent correction (Table 2-7) must be subtracted from the above value.

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In this case, spectra are affected significantly by the solvent as a result of the change in polarity on excitation. A solvent correction (from Table 2–7) is subtracted from the calculated value (Table 2–6) to obtain the value expected for a solvent other than the standard solvent ethanol.

Table 2–7

Solvent Corrections for $\alpha\beta$-unsaturated Ketones

| Solvent | Correction mμ |
|---|---|
| Ethanol | 0 |
| Methanol | 0 |
| Dioxan | +5 |
| Chloroform | +1 |
| Ether | +7 |
| Water | −8 |
| Hexane | +11 |
| Cyclohexane | +11 |

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For example, mesityl oxide ($Me_2C{=}CHCOMe$) may be calculated to have $\lambda_{max}$ at $(215+2\times12) = 239$ mμ. The observed value is 237 mμ (ε 12,600). A more complicated example, the trienone chromophore IX, would be calculated to have a maximum at 349 mμ by the following addition.

IX

| | |
|---|---|
| Parent value | 215 mμ |
| β-substituent (marked *a*) | 12 mμ |
| ω-substituent (marked *b*) | 18 mμ |
| 2 × extended conjugation | 60 mμ |
| Homoannular diene component (a special addition for this component when it is a linear part of the chromophore) | 39 mμ |
| Exocyclic double bond (the αβ-double bond is exocyclic to ring A) | 5 mμ |
| Total | 349 mμ |

The observed values of $\lambda_{max}$ are 230 mμ ($\varepsilon$ 18,000), 278 mμ ($\varepsilon$ 3720) and 348 mμ ($\varepsilon$ 11,000). As was the case with simple polyenes, the long chromophore present in this example gives rise to several peaks, with the longest wavelength peak in excellent agreement with prediction.

An important general principle is illustrated by the calculation for the cross-conjugated trieneone X. In this case the main chromophore is the linear dieneone portion, since the $\Delta^5$-double bond is not in the longest conjugated system. The calculation, along the lines above, gives a value of 324 mμ. The observed values are 256 mμ and 327 mμ. The former might be due to the $\Delta^5$-7-one system ($\lambda_{calc}$ 244 mμ), but a positive identification of this sort in a complicated system is largely unjustified.

X XI

Certain special changes in structure, as noted in the case of dienes in section 2-13, also lead to departures from the rules given above. The effect of the five-membered ring in cyclopentenones is accommodated in the rules; but when the carbonyl group is in a five-membered ring and the double bond is exocyclic to the five-membered ring, a parent value of about 215 mμ holds. Another special case, verbenone, XI, would be calculated to have a maximum at 239 mμ but actually has a maximum at 253 mμ, an increment for strain of 14 mμ, close to the increment for the corresponding diene III.

### 2-17. Ketones and Aldehydes: n→π* Transitions

Saturated ketones and aldehydes show a weak symmetry forbidden band, in the 275–295 mμ range ($\varepsilon \sim 20$), due to excitation of an oxygen lone-pair electron to the anti-bonding π-orbital of the carbonyl group. Aldehydes and the more heavily substituted ketones absorb at the upper end of this range. Polar substituents on the α-carbon atoms raise (when axial) or lower (when equatorial) the extremes of this range. When the carbonyl group is substituted by an auxochrome as in an ester, an acid, or an amide,



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the $\pi^*$ orbital is raised but the n level of the lone-pair left largely unaltered. The result is that the n→$\pi^*$ transition of these compounds is shifted to the relatively inaccessible 200–215 mμ range. The presence, therefore, of a weak band in the 275–295 mμ region is positive identification of a ketone or aldehyde carbonyl group (nitro groups show a similar band and, of course, impurities must be absent). The low intensity of this transition is responsible for the ease with which the Cotton effect may be measured in studies of the optical rotatory dispersion of ketones.

$\alpha\beta$-unsaturated ketones show a slightly stronger n→$\pi^*$ band or series of bands ($\varepsilon \sim 100$) in the 300–350 mμ range. The precise position of these bands is not predictable from the extent of alkylation, but is a regular function of the conformation of $\gamma$-substituents, axially substituted isomers absorbing at longer wavelengths than equatorially substituted isomers.

The position and intensity of n→$\pi^*$ bands are also influenced by transannular interactions (see section 2–25) and by solvent (see section 2–9 (*ii*)).

The n→$\pi^*$ transitions of $\alpha$-diketones in the diketo form give rise to two bands, one in the usual region near 290 mμ ($\varepsilon \sim 30$) and a second ($\varepsilon$ 10 to 30), which stretches into the visible in the 340–440 mμ region and gives rise to the yellow colour of some of these compounds. (See also quinones in section 2–23, quinones being $\alpha$-, or vinylogous $\alpha$-, diketones.)

### 2–10. $\alpha\beta$-unsaturated Acids, Esters, Nitriles and Amides

$\alpha\beta$-unsaturated acids and esters follow a trend similar to that of the ketones but at slightly shorter wavelength. The rules for alkyl

**Table 2–8**

**Rules for $\alpha\beta$-unsaturated Acids' and Esters' Absorption**

$\varepsilon$ values are usually above 10,000

| | |
|---|---|
| $\beta$-monosubstituted | 208 mμ |
| $\alpha\beta$- or $\beta\beta$-disubstituted | 217 mμ |
| $\alpha\beta\beta$-trisubstituted | 225 mμ |
| Increment for | |
| (a) a double bond extending the conjugation | 30 mμ |
| (b) the exocyclic nature of any double bond | 5 mμ |
| (c) when the double bond is endocyclic in a five- or seven-membered ring | 5 mμ |
| $\lambda_{calc}$ Total | |

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substitution, summarized by Nielsen, are given in Table 2-8. The change in going from acid to ester is usually not more than 2 mμ.

αβ-unsaturated nitriles have been little studied but usually come slightly below the corresponding acids.

αβ-unsaturated amides have maxima lower than the corresponding acids, usually near 200 mμ (ε ~ 8000).

αβ-unsaturated lactams have an additional band at 240–250 mμ (ε ~ 1000).

## 2–19. The Benzene Ring

Benzene absorbs at 184 (ε 60,000), 203·5 (ε 7400) and 254 (ε 204) mμ in hexane solution. The latter band, illustrated in Fig. 2–5, shows vibrational fine structure. Although a 'forbidden' band, it owes its appearance to the loss of symmetry caused by molecular vibrations; indeed, the 0→0 transition (the transition between the ground state vibrational energy level of the electronic ground state to the ground state vibrational energy level of the electronic excited state) is not observed.

When the aromatic ring is substituted by alkyl groups, for example, or is an aza analogue such as pyridine, the symmetry is lowered; the 0→0 transition is then observed, although the spectrum is little changed otherwise. The presence of fine structure resembling that in Fig. 2–5 is characteristic of the simpler aromatic molecules.

When, however, the benzene ring is substituted by lone pair donating or by π-bonded systems, the chromophore is extended more usefully; in these cases the fine structure tends to be lost and the intensity and wavelength of the absorption maximum increases. Unfortunately, prediction of the effects of various substituents is not always possible in the manner useful with dienes and unsaturated ketones. Section 2–20 gives an account of some of the trends observed and the predictions possible in compounds containing a substituted benzene ring. Sections 2–21 and 2–22 give very brief accounts of polycyclic aromatic hydrocarbons and heteroaromatic compounds.

## 2–20. Substituted Benzene Rings

Table 2-9, giving the wavelength of absorption maxima in the spectra of a range of monosubstituted benzenes, shows how the wavelength and intensity of the absorption peaks increase with an increase in the extent of the chromophore. The more extensive the

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in 95% EtOH

Fig. 2–3

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conjugation, the less obvious is the vibrational fine structure. The list given in Table 2–9 gives the main peaks in the accessible range above 200 mμ. Other strong bands occur below this and are also shifted to longer wavelength by substitution, giving rise to what is known as end absorption, since in simple cases it is not at high enough a wavelength to be resolved as a maximum.

**Table 2–9**

**Absorption Maxima of the Substituted Benzene Rings Ph R**

| R | $\lambda_{max}$ mμ (ε) (solvent $H_2O$ or MeOH) | | | | | |
|---|---|---|---|---|---|---|
| H | 203·5 | (7400) | 254 | (204) | | |
| $NH_3^+$ | 203 | (7500) | 254 | (160) | | |
| Me | 206·5 | (7000) | 261 | (225) | | |
| —I | 207 | (7000) | 257 | (700) | | |
| —Cl | 209·5 | (7400) | 263·5 | (190) | | |
| —Br | 210 | (7900) | 261 | (192) | | |
| —OH | 210·5 | (6200) | 270 | (1450) | | |
| —OMe | 217 | (6400) | 269 | (1480) | | |
| —$SO_2NH_2$ | 217·5 | (9700) | 264·5 | (740) | | |
| —CN | 224 | (13,000) | 271 | (1000) | | |
| —$CO_2^-$ | 224 | (8700) | 268 | (560) | | |
| —$CO_2H$ | 230 | (11,600) | 273 | (970) | | |
| —$NH_2$ | 230 | (8600) | 280 | (1430) | | |
| —$O^-$ | 235 | (9400) | 287 | (2600) | | |
| —NHAc | 238 | (10,500) | | | | |
| —COMe | 245·5 | (9800) | | | | |
| —CH=$CH_2$ | 248 | (14,000) | 282 | (750) | 291 | (500) |
| —CHO | 249·5 | (11,400) | | | | |
| —Ph | 251·5 | (18,300) | | | | |
| —OPh | 255 | (11,000) | 272 | (2000) | 278 | (1800) |
| —$NO_2$ | 268·5 | (7800) | | | | |
| —CH$\stackrel{t}{=}$CHCO$_2$H | 273 | (21,000) | | | | |
| —CH$\stackrel{t}{=}$CHPh | 295·5 | (29,000) | | | | |

(Most values taken with permission from H. H. Jaffé and M. Orchin, *Theory and Applications of Ultraviolet Spectroscopy*, Wiley, New York, 1962.)

In disubstituted benzenes, two situations are important. (a) When electronically complementary groups, such as amino and nitro, are situated *para* to each other as in XII, there is a pronounced red shift in the main absorption band, compared to the effect of either substituent separately, due to the extension of the chromophore from the electron donating group to the electron withdrawing group through the benzene ring. (b) Alternatively, when two groups are situated *ortho* or *meta* to each other or when the *para* disposed groups are not complementary, as in XIII, then the observed spectrum is usually closer to that of the separate, non-interacting, chromophores.

These principles are illustrated by the examples in Table 2–10.

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The values in this table should be compared with each other and with the values for the single substituents separately given in Table 2·9.

$H_2\ddot{N}-C_6H_4-\overset{+}{N}(=O)O^-$ $\lambda_{max}$ 375 mμ (ε 16,000) XII

$O_2N-C_6H_4-NO_2$ $\lambda_{max}$ 260 mμ (ε 13,000) XIII

In particular it should be noted that those compounds with non-complementary substituents, or with an *ortho* or *meta* substitution pattern, actually have a band (though a much weaker one) at longer wavelength than the compounds with interacting *para* disubstituted substituents. This fact is not in accord with the simple resonance picture; neither is the similarity of the *ortho* to the *meta* disubstituted cases. This is another case in which the molecular orbital theory (too complicated to be introduced here but dealt with in Murrell's book) gives a better picture.

Table 2-10

Absorption Maxima of the Disubstituted Benzene Rings $R-C_6H_4-R'$

| R | R' | Orientation | $\lambda_{max}^{EtOH}$ in mμ (ε) | | | | | |
|---|---|---|---|---|---|---|---|---|
| —OH | —OH | *o*- | 214 | (6000) | 278 | (2630) | | |
| —OMe | —CHO | *o*- | 253 | (11,000) | 319 | (4000) | | |
| —$NH_2$ | —$NO_2$ | *o*- | 229 | (16,000) | 275 | (5000) | 405 | (6000) |
| —OH | —OH | *m*- | 277 | (2200) | | | | |
| —OMe | —CHO | *m*- | 252 | (8300) | 314 | (2800) | | |
| —$NH_2$ | —$NO_2$ | *m*- | 235 | (16,000) | 373 | (1500) | | |
| —Ph | —Ph | *m*- | 251 | (44,000) | | | | |
| —OH | —OH | *p*- | 225 | (5100) | 293 | (2700) | | |
| —OMe | —CHO | *p*- | 277 | (14,800) | | | | |
| —$NH_2$ | —$NO_2$ | *p*- | 229 | (5000) | 375 | (16,000) | | |
| —Ph | —Ph | *p*- | 280 | (25,000) | | | | |

In the case of disubstituted benzene rings in which the electron donating (*o*,*p*-directing) group is complemented by an electron withdrawing carbonyl group, some quantitative assessments may be made. These apply to the compounds $R-C_6H_4-COX$ in

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which X is alkyl, H, OH, or OAlkyl, and refer to the strongest band in the accessible region; this is often the only measured band in the highly conjugated *para* disubstituted systems. The calculation is based on a parent value with increments for each substituent. Poly-substituted benzene rings should be treated with caution, particularly when the substitution might lead to steric hindrance preventing coplanarity of the carbonyl group and the ring. Table 2-11 gives the rules for this calculation. In the absence of steric hindrance to coplanarity, the calculated values are usually within 5 mμ of the observed values.

**Table 2-11**

**Rules for the Principal Band of Substituted Benzene Derivatives** $R—C_6H_4—COX$

| | Orientation | $\lambda_{max}^{EtOH}$ mμ |
|---|---|---|
| Parent Chromophore: | | |
| X = alkyl or ring residue | | 246 |
| X = H | | 250 |
| X = OH or OAlkyl | | 230 |
| Increment for each substituent: | | |
| R = alkyl or ring residue | *o-*, *m-* | 3 |
| | *p-* | 10 |
| R = OH, OMe, OAlkyl | *o-*, *m-* | 7 |
| | *p-* | 25 |
| $R = O^-$ | *o-* | 11 |
| | *m-* | 20 |
| | *p-* | 78 |
| R = Cl | *o-*, *m-* | 0 |
| | *p-* | 10 |
| R = Br | *o-*, *m-* | 2 |
| | *p-* | 15 |
| $R = NH_2$ | *o-*, *m-* | 13 |
| | *p-* | 58 |
| R = NHAc | *o-*, *m-* | 20 |
| | *p-* | 45 |
| R = NHMe | *p-* | 73 |
| $R = NMe_2$ | *o-*, *m-* | 20 |
| | *p-* | 85 |

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A single example, that of 6-methoxytetralone (XIV), will show the method.

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MeO

XIV

| | |
|---|---|
| Parent value | 246 mμ |
| *Ortho* alkyl | 3 mμ |
| *Para* methoxyl | 25 mμ |
| $\lambda_{calc}$ | 274 mμ |

The maximum actually occurs at 276 mμ (ε 16,500).

Other electron withdrawing groups, e.g., in nitriles and nitro compounds, show similar trends but with different and less well documented substituent effects.

### 2–21. Polycyclic Aromatic Hydrocarbons

The range of polycyclic aromatic hydrocarbons is too great for detailed consideration in this book. Their spectra are usually complicated, and for that reason are useful as fingerprints. This is

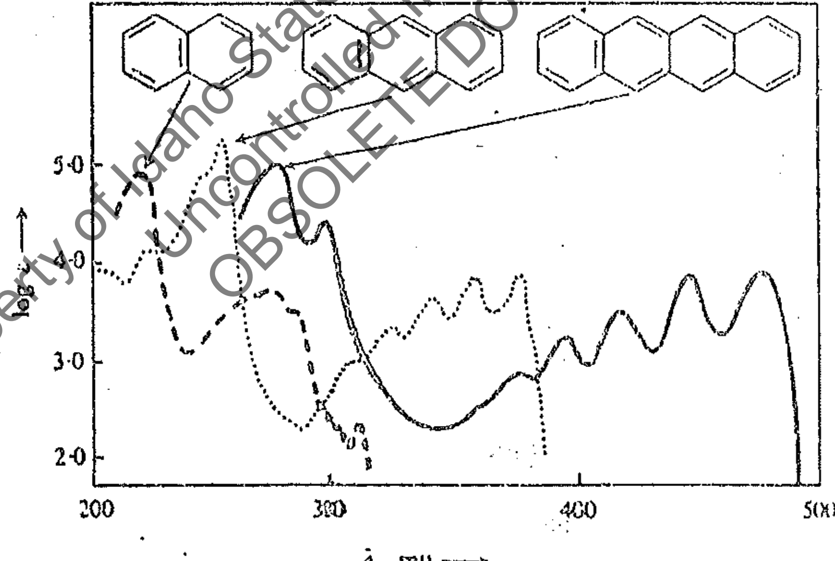


Fig. 2–6

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particularly so in that the relatively non-polar substituents, such as alkyl and acetoxyl groups, have only a small effect on the shape and position of the absorption peaks of the parent hydrocarbon. The degradation products of natural materials often contain polycyclic nuclei which can be identified in this way as, for example, a phenanthrene or a perylene. The spectra of a typical series, naphthalene, anthracene and naphthacene, are illustrated in Fig. 2-6; the logarithmic ordinate should be noted.

Fortunately, the collections of spectra mentioned in section 2-11 show the actual spectra of a great many of the known aromatic systems and make the identification of such systems a relatively simple matter.

### 2-22. Heteroaromatic Compounds

The range of heteroaromatic compounds is too great for detailed consideration in this book. In general they resemble the spectra of their corresponding hydrocarbons, but only in the crudest way. The heteroatom, whether like that in a pyrrole or that in a pyridine, leads to pronounced substituent effects which depend on the electron donating or withdrawing effect of the substituent and the heteroatom and on their orientation. The effects of these factors are predictable, in a qualitative way, using the same sorts of criteria as were used in section 2-20 when considering the effects of more than one substituent on a benzene ring. For example, a simple pyrrole XV and a pyrrole with an electron withdrawing substituent XVI have strikingly different absorption maxima. The conjugation present from the nitrogen lone-pair through the pyrrole ring to the carbonyl group increases the length of the chromophore and leads to longer wavelength absorption. The following illustrations of heterocyclic systems give some indication of the spectra observed.

| XV | XVI | XVII |
|---|---|---|
| $\lambda_{max}^{EtOH}$ 203 mμ ($\varepsilon$ 5670) | $\lambda_{max}^{EtOH}$ 262 mμ ($\varepsilon$ 12,000) | $\lambda_{max}^{EtOH}$ 245 mμ ($\varepsilon$ 4800) |

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XVIII

$\lambda_{max}^{EtOH}$ 300 mμ (ε 5000)

XIX

$\lambda_{max}^{MeOH}$ 520 mμ

XX

$\lambda_{max}^{CHCl_3}$ 245 mμ (ε 12,000)
275 mμ (ε 2800)
282 mμ (ε 3020)

XXI

$\lambda_{max}^{cyclohexane}$ 220 mμ (ε 26,000)
262 mμ (ε 6310)
280 mμ (ε 5620)
288 mμ (ε 4170)

XXII

$\lambda_{max}^{CHCl_3}$ 218 mμ (ε 79,000)
266 mμ (ε 3900)
305 mμ (ε 2000)
318 mμ (ε 3000)

Compare these values with the spectrum of naphthalene in Fig. 2-6.

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XXIII

$\lambda_{max}$ pH 4 259·5 mμ
pH 7 260 mμ (ε 11,000)
pH 9·5 261 mμ

XXIV

$\lambda_{max}$ pH 1 210 mμ (ε 9700)
276 mμ (ε 10,000)
pH 5 269 mμ (ε 6650)
pH 7 267 mμ (ε 6130)
pH 12 272 mμ (ε 5630)

XXV

$\lambda_{max}$ pH 2 262 mμ
pH 7 260 mμ (ε 13,500)
pH 12 267 mμ

XXVI

$\lambda_{max}$ pH 1 248 mμ
271 mμ
pH 6 246 mμ (ε 10,000)
275 mμ (ε 7800)
pH 11 245 mμ
273 mμ

In the case of potentially tautomeric molecules the change in the absorption maxima with the change of pH is due sometimes to a change in the chromophore as a result of the tautomerism and sometimes to simple protonation or deprotonation. This point is mentioned here in order to stress the importance of careful control of the medium in which spectra are taken. The changes in absorption maxima with change of pH are very useful diagnostically since they serve in some systems to identify the pattern of substitution. The stable tautomeric species have been identified, using ultraviolet spectroscopy. For example, the 2-hydroxypyridine (XXVII, R = H): pyrid-2-one (XXVIII, R = H) equilibrium has been shown to lie far to the right; the ultraviolet spectrum of the solution resembles that of a solution of N-methylpyrid-2-one (XXVIII, R = Me) and is different from that of 2-methoxypyridine (XXVII, R = Me).

XXVII
R = Me
$\lambda_{max}$ < 205 mμ (ε > 5300)
269 mμ (ε 3230)

XXVIII
R = Me
$\lambda_{max}$ 226 mμ (ε 6100)
297 mμ (ε 5700)

R = H
$\lambda_{max}$ 224 mμ (ε 7230)
293 mμ (ε 5900)

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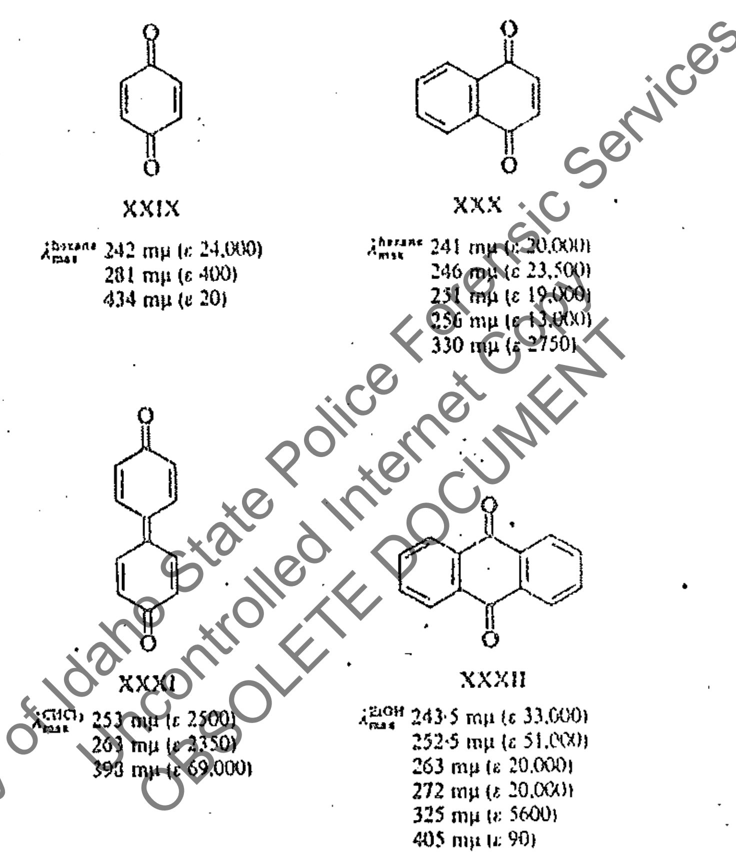
**2–23. Quinones**

A few representative quinones are illustrated below. The colour of the simpler members is due to the weak n→π* transition, similar to that of α-diketones.

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**2–24. Porphyrins, Chlorins and Corroles**

Our knowledge of the chemistry of these important groups of macrocyclic compounds has benefited considerably from the ease with which each class, in many of its various oxidation levels and with varying substitution patterns, can be recognized by the relative intensity of the four bands found in the visible region between 400 mμ and 700 mμ. In addition to these, a very strong sharp band (the Soret band) occurs near 400 mμ (ε 100,000). It is interesting that another conjugated macrocyclic aromatic system, [18]-annulene, shows a similar intense band at 369 mμ (ε 303,000).

These compounds are mentioned here to stress the importance and usefulness of ultraviolet and visible spectroscopy in the study of groups of compounds possessing a long, complicated chromophore. Although little can be accomplished in such systems from a theoretical point of view, the very large number of model systems available makes an empirical approach quite straightforward and very rewarding. These remarks apply to a large number of systems which, for one reason or another, have been studied, but which cannot be dealt with in this chapter.

### 2–25. Non-conjugated Interacting Chromophores

Non-conjugated systems usually have little effect on each other; diphenyl methane has a spectrum similar to that of toluene; the cross conjugation of the trieneone X was successfully ignored when calculating the expected absorption maximum; and even diphenyl ether is not very different from anisole. However, several special cases of non-conjugated interaction are known, two examples of which are given below. The unsaturated ketones XXXIII show the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions shifted in opposite directions when X becomes more electronegative. Presumably the $\pi^*$ orbital is raised by transannular interaction with the $>\overset{+}{N}Me_2$ group, but since the n electron is closer to the $>\overset{+}{N}Me_2$ group in the excited state than in the ground state, the $n \rightarrow \pi^*$ transition is of lower energy. The diene XXXIV has absorption in the accessible ultraviolet whereas the isolated ethylenic double bond has no maximum above 190 mμ.

XXXIII

| X | $\lambda_{max}$ | |
|---|---|---|
| $>CH_2$ | 238 mμ | 308·5 mμ |
| $>\overset{+}{N}Me_2$ | 229 mμ | 318·5 mμ |

XXXIV

$\lambda_{max}$ 205 mμ (ε 2100)
214 mμ (ε 1480)
220 mμ (ε 870)
230 mμ shoulder (ε 200)

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**2–26. The Effect of Steric Hindrance to Coplanarity**

(*i*) *Steric hindrance to coplanarity about a double bond*, as in the hydrocarbon XXXV, raises the ground state energy level but leaves the excited state relatively unchanged (the latter is probably of lowest energy in the conformation in which the biphenyl systems are at right angles). The result (in this case a series of bands culminating at 458 mμ [ε 23,000]) is a shift toward the red from what might have been expected.

$NO_2$ R R $NH_2$ O O O O

XXXV XXXVI XXXVII

(*ii*) *Mild steric hindrance to coplanarity about a single bond* has only a small effect on the position and intensity of absorption maxima.

(*iii*) *Medium steric hindrance to coplanarity about a single bond* gives rise to a marked decrease in intensity but may also lead to either a blue shift or a red shift. For example, the absorption maximum of the nitroaniline XXXVI (R = Me) is at 385 mμ (ε 4840), showing a red shift and marked reduction in intensity from that of the parent compound XXXVI (R = H) at 375 mμ (ε 16,000). Another example, in the opposite direction, is that of 2,4,6-trimethylacetophenone absorbing at 242 mμ (ε 3200), which is to be compared with the calculated value (Table 2–11) of 262 mμ and with *p*-methylacetophenone which has a maximum at 252 mμ (ε 15,000).

(*iv*) *Extreme steric hindrance to coplanarity about a single bond* leads to a situation with no overlap between the separated chromophores. The dilactone XXXVII produced from shellolic acid showed no maximum in the accessible ultraviolet region but on hydrolysis of the αβ-unsaturated lactone grouping an acid with $\lambda_{max}$ 227 mμ (ε 5500) was obtained. This shows that the steric constraint of the lactone ring prevents conjugation and that release of this constraint then allowed the overlap of the double bond and carbonyl orbitals.

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## Bibliography


TEXTBOOKS

A. E. Gillam and E. S. Stern, *Electronic Absorption Spectroscopy*, Arnold, London, 2nd Ed., 1957.

C. N. R. Rao, *Ultraviolet and Visible Spectroscopy*, Butterworths, London, 1961.

A. I. Scott, *Interpretation of the Ultraviolet Spectra of Natural Products*, Pergamon Press, Oxford, 1964.

W. West (Ed.), *Technique of Organic Chemistry, Vol. IX, Chemical Applications of Spectroscopy*, Interscience, New York, 1956.

S. F. Mason, Chapter 7, The Electronic Absorption Spectra of Heterocyclic Compounds, *Physical Methods in Heterocyclic Chemistry, Vol. II*, Academic Press, New York, 1963.

THEORETICAL TREATMENTS

H. H. Jaffé and M. Orchin, *Theory and Applications of Ultraviolet Spectroscopy*, Wiley, New York, 1962.

J. N. Murrell, *The Theory of the Electronic Spectra of Organic Molecules*, Methuen, London, 1963.

G. R. Barrow, *Introduction to Molecular Spectroscopy*, McGraw-Hill, New York, 1962.

R. E. Dodd, *Chemical Spectroscopy*, Elsevier, Amsterdam, 1962.


See also p. 14 for catalogues of ultraviolet spectra.

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methods as a result of fatigue, poor ability to establish intensities, and other characteristics of the average eye.

## 16.2 BEER'S LAW

Whenever a beam of broadly polychromatic radiation passes through a medium, e.g., a liquid or gas, some loss of intensity occurs. First, reflection takes place at the phase boundaries as a result of refractive index differences between the medium and its surroundings (Section 10.8). Second, scattering caused by inhomogeneities (in mixtures) or by thermal fluctuations in the bulk of the medium produces an additional small loss of power from the main beam (Section 10.10). Neither of these is as significant in accounting for the intensity diminution,* however, as the fact that the medium itself is not perfectly transparent but will absorb the radiant frequencies that promote energy changes within its molecules and ions. A schematic representation of the effect of reflection and scattering is given in Fig. 16.1.

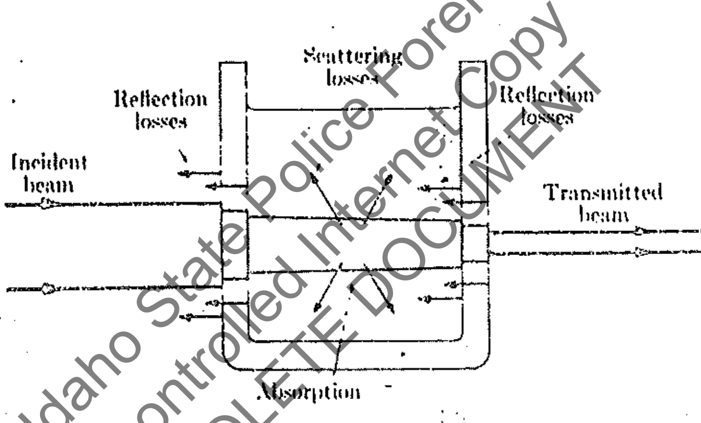


Fig. 16.1 The effect of a cell and sample on the intensity of a transmitted beam. Intensity is represented by the breadth of the beam. The cell walls are not shown as absorbing but may absorb in other cases.

A distinction should be made between the process by which the power level of the radiation is changed (e.g., absorption) and the quantitative measure of the effect. For easy identification let the suffix *-ion* refer to the process, and *-ance* to the measured value. For example, *transmission, reflection*, and *absorption* are occurring in Fig. 16.1 and lead to a measurable *transmittance, reflectance*, and *absorbance*. These terms are still to be defined. Since the absorption will not be directly measurable, it must be derived from a determinable quantity, the *radiant power P* of the beam. $P$ is simply the energy of the radiation reaching a given area per second.†

* It should be noted that scattering becomes significant for large ions or molecules such as polyelectrolytes or polymers. In these the intensity of the scattered light serves as a measure of molecular weight and shape (see Section 20.4).

† It is closely related to, but not identical with, the intensity $I$ of radiation, which is the power per unit solid angle.

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The absorbance depends on (a) the nature of the medium, i.e., its composition, and (b) the length of optical path in the medium. This dependence is expressed by Beer's law.* The assumptions that are made in obtaining the law are important. They are:

1. The incident radiation is monochromatic,
2. The absorbing centers (molecules and ions) act independently of one another regardless of number and kind, and
3. The absorption is limited to a volume of uniform cross-section.

We will consider later the effect of these assumptions for practical work. Beer's law may be expressed as: *the absorption of a medium is directly proportional to the number of absorption centers.* In other words, each centimeter thickness of a solution obeying Beer's law will absorb an equal fraction of the power incident upon it.

From the differential equation [28] expressing Beer's law, there is obtained

$$\ln \frac{P}{P_0} = -kn, \tag{16.1}$$

where $P_0$ is the power incident on *the sample*, $P$ is the power leaving *the sample* (see Fig. 16.1), ln indicates a natural logarithm, $k$ is a constant, and $n$ is the number of absorbing centers of *one kind* in a volume of unit cross section. This equation predicts that the power of the emergent beam will drop off logarithmically (exponentially) as the number of centers in the beam increases.

Equation (16.1) is not operationally useful as it stands. The number of absorbing sites in the beam must be expressed in terms of a path length for the beam and of the concentration of the absorbent. Assuming for the moment a rectangular cell, the total number $N$ of absorbers in the beam will be the product

$$N = c \times 6.02 \times 10^{23} \times b \times S,$$

where $c$ is the concentration of absorbent substance in mol ml$^{-1}$, $6.02 \times 10^{23}$ is the number of molecules in a mole, $b$ is the thickness of the vessel in cm, and $S$ is the cross-sectional area perpendicular to the radiation in cm$^2$. The number $n$ of absorbers in a unit cross-sectional area of the path will be $N/S$ or $cb$. Finally, as a matter of convenience, the logarithmic term of Eq. (16.1) may be shifted from the natural to the Naperian base, 10 (designated by "log"), the concentration changed to units of molarity, and the constant $k$ modified accordingly. Let the new constant be $\epsilon$. With these substitutions, and the additional relation $T = P/P_0$, Eq. (16.1) may now be written

$$\log \frac{P}{P_0} = -\epsilon bc = \log T. \tag{16.2}$$

* Several scientists, Beer, Bouger, Lambert, Bunsen, and Roscoe, have contributed by their investigations and theorizing to the development of this relation. Often it is called the Beer Lambert or the Bouger Beer law. However, it has been shown by Liebhafsky and Pfeiffer [28] that Beer's original conception was sufficiently broad to include both concentration and length dependence. For that reason and for simplicity, the formal statement will be termed Beer's law.

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Here $\epsilon$ is the molar absorptivity and $T$ is the transmittance, the fraction of the incident power transmitted. Taking the reciprocal of the ratio $P/P_0$ removes the negative sign and gives

$$\log \frac{P_0}{P} = A = \epsilon bc. \tag{16.3}$$

This expression defines the absorbance $A$ and is the simplest mathematical statement of Beer's law.*

In absorption photometry, both the transmittance and the absorbance figure prominently. The latter is the more useful, however, because of its linear dependence on concentration and path length. The difference in behavior of $T$ and $A$ may be seen clearly in Fig. 16.2. For purposes of compound identification, the logarithm of $A$ may be preferable to either $T$ or $A$. (See Fig. 16.15 and accompanying discussion in the text.)

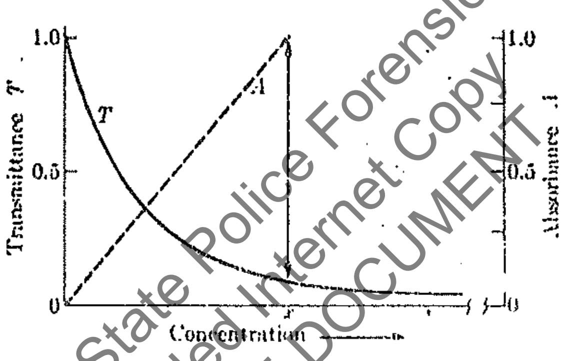


Fig. 16.2 The absorbance and transmittance of a solution at a given wavelength as a function of concentration. For example, at concentration $x$ an arrow links the transmittance (0.10) and the absorbance (1.00). The path length $b$ and other variables are constant.

The important definitions and concepts of absorption photometry are summarized in Table 16.1. Other names and symbols that have been given these variables are noted in parentheses. Two typical calculations will provide illustrations of the interrelationship of these variables by use of Eqs. (16.2) and (16.3).

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**Example 1** The absorbance of a solution of a colored inorganic material at a particular frequency is found to be 0.90, using a 1.00 cm cell and a concentration of 0.0020 M. For a more accurate measurement, an absorbance of 0.43 is desired. What concentration must be made up?

*Solution.* Knowing that $A = 0.90$, find $\epsilon$:

$$A = \epsilon bc = 0.90 = \epsilon \cdot 1 \cdot 0.002; \qquad \epsilon = 450.$$

The new conditions call for $A = 0.43 = 450 \times 1 \times c_2$, and the desired concentration is

$$c_2 = 9.5 \times 10^{-4}\ \text{M}.$$

* Often other concentration units are used in Eq. (16.3). The concentration may be given in g l$^{-1}$, for example. If that is done, $\epsilon$ is replaced by $a$, the (specific) absorptivity.

**Example 2** The incident power of a beam of a particular frequency is reduced 20% in passing through 1.00 cm of a colored solution. What will be the reduction after going through 5.00 cm of the same solution?

*Solution.* The transmittance at a distance of 1 cm is 0.80/1.00 = 0.80. The product $\epsilon c$ of Eq. (16.2) is constant and must be evaluated.

$$\epsilon c = \frac{1}{1} \log \frac{1.00}{0.80} = 0.096.$$

When $b = 5$ cm,

$$\log \frac{P_0}{P} = 0.096 \times 5 = 0.48$$

and

$$\frac{P_0}{P} = \text{antilog } 0.48 = 3.02,$$

so that

$$P = \frac{P_0}{3.02} = 0.331 P_0.$$

The power is reduced to 33% of its original value.

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**Table 16.1** Concepts and Symbolism of Absorption Photometry

| *Term* | *Symbol and Definition* |
|---|---|
| Absorbance (optical density, extinction) | $A(D, E) = \log \frac{P_0}{P}$* |
| Transmittance (transmission) | $T = \frac{P}{P_0}$ |
| Path length | $b(l, d)$ |
| Absorptivity (extinction coefficient, absorbancy index) | $a(k) = \frac{A}{bc}$ ($c$, in g l$^{-1}$) |
| Molar absorptivity (molecular or molar extinction coefficient, molar absorbing index) | $\epsilon = \frac{A}{bc}$ ($c$, in mol l$^{-1}$) |

* Absorbance can also be stated in terms of the intensity ratio $I_0/I$.

Note that for substances following Beer's law the molar absorptivity $\epsilon$ for a substance is independent of concentration and optical path length. It is truly a characteristic constant determined by the nature of the absorbing substance, the solvent, and by the wavelength of the incident radiation. Its occasional apparent dependence on temperature and other variables will be taken up in Section 16.4. Beer's law also extends to mixtures of absorbers of different types. So long as they

act independently, the law holds. Each species has a different molar or specific absorptivity, and the absorbances may be added. Equation (16.3) in the form applicable to homogeneous mixtures is

$$\log \frac{P_0}{P} = A_t = \epsilon_1 bc_1 + \epsilon_2 bc_2 + \cdots = b \sum_i \epsilon_i c_i, \tag{16.4}$$

where solvent, path length $b$, and wavelength are presumed constant.

*Concentration limit on validity.* The restriction that absorption centers do not interact with themselves or other species causes Beer's law to be a limiting law applicable mainly in dilute solutions (concentrations $< 10^{-2}$ M). The interference alters the charge distribution either in the absorbing or excited species or both and thus changes the energy needed for excitation. As a result, the position, shape, and height of the absorption region may be altered. Not all interactions are between neighboring molecules; through electrostatic forces, influences may be exerted at a relatively great distance. Many complex organic molecules, such as eosin and methylene blue, are particularly susceptible to interference and may follow Beer's law only below $10^{-5}$ M if certain simple salts are present.

A second limit on the validity of the law is that the index of refraction of the solution alters as the concentration changes. It may be shown that it is $\epsilon \cdot n/(n^2 + 2)^2$ rather than $\epsilon$ itself that is constant with concentration. [26] Whenever the variation of $n/(n^2 + 2)^2$ exceeds the experimental error over a concentration region of interest, again Beer's law does not hold. In this instance a correction can be used at high concentrations; however, deviations larger than 0.01% from this source occur only at concentrations above $10^{-2}$ M.

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## 16.3 ABSORPTION PROCESSES AND INTENSITIES

Beer's law relates absorption to concentration, but provides no clue as to the dependence of absorption on molecular structure. The connection is established through quantum-mechanical study of the characteristic "motions" that permit a molecule to interact with radiation. Since the energy of such motions, especially vibrations, is characteristic of particular molecules, absorption spectra are a sensitive structural tool. The manifold processes related to the absorption (and emission) of EM radiation are classified in Table 16.2. This section deals with the simpler aspects of molecular absorption; transition probabilities and intensities, line widths, and lifetimes of excited states were treated earlier in Section 13.3.

The energy of a molecule is usually characterizable as being distributed among motions of sufficiently different energy that each can be treated separately,* that is,

$$E_{\text{molecule}} = E_{\text{translation}} + E_{\text{rotation}} + E_{\text{vibration}} + E_{\text{electronic}} + E_{\text{nuclear orientation}}.$$

The absorption of a quantum of incident light may, depending upon its size and the particular molecule, simultaneously promote transitions in one or several categories of motion. Quantum-mechanical *selection rules* describe the combinations allowed.

* At high levels of excitation, interactions among such "motions" are no longer negligible and correction terms must be added.

Table 16.2 Processes of Absorption and Emission of Radiation

| Wavelength, $\mu m$ | → 1000 | 100 | 10 | 1 | 0.1 | 0.01 | 0.001 |
|---|---|---|---|---|---|---|---|
| 1 | 10 | 100 | 1000 | 10 000 | $10^5$ | $10^6$ | ← Wave number, $cm^{-1}$ |

| Spectral regions: | Microwave | Infrared (Far, Middle, Near) | Visible | Ultraviolet (Near, Far) | X-Ray |
|---|---|---|---|---|---|
| Characteristic molecular process excited in region: | Rotation of molecules in gases | Rotations of molecules in gases and intermolecular vibrations in crystals, liquids; Vibrations in molecules | | Electronic transitions of outer shell electrons in atoms and $n$, $\pi$ electrons in molecules | Electronic transitions of inner shell electrons in atoms |
| Characteristic transition: | Pure rotation | Rotation-vibration | | Electronic band spectra (rotation-vibration-electronic) | |

| Energy of transition: | | | | |
|---|---|---|---|---|
| ($kJ\ mol^{-1}$) | 0.1196 | 11.96 | 1196 | 119,600 |
| ($eV\ mol^{-1}$) | $1.24 \times 10^{-3}$ | 0.124 | 12.4 | 1240 |

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Neither translational nor nuclear orientation changes are treated in this chapter;* the latter are reserved for Chapter 19.

*Rotational transitions.* For gaseous, i.e., isolated molecules, energy changes associated with pure rotational shifts are observable, but only in the microwave and far IR range. In the easily accessible 4000 to 650 $cm^{-1}$ IR range, only those rotational changes that accompany vibrational transitions are observed. Molecular rotational levels are spaced on the order of $10^{-2}$ J $mol^{-1}$, corresponding to radiation of a frequency of about 10 $cm^{-1}$.

It can be shown that the rotational energy of a molecule is generally expressible as a sum of terms, each proportional to the reciprocal of one of the molecular moments of inertia. These moments are taken around a set of internal, mutually orthogonal axes. For example, rotation of a rigid linear molecule lying along the $z$-axis can occur around both $x$- and $y$-axes. Its two moments of inertia are equal, and the rotational energy is given by the equation,

$$E_{rotation} = J(J + 1)(h^2/8\pi^2 I) \qquad (16.5)$$

where $J$ is the rotational quantum number ($J = 0, 1, 2, 3, \ldots$), $h$ is Planck's constant, and $I$ is the single moment of inertia. Exactly $2J + 1$ different orientations of a particular rotational axis in space are allowed. In the absence of an external field, these are of equal energy, giving each rotational energy level a $(2J + 1)$-fold degeneracy. The quantum-mechanical selection rule that applies to pure rotational transitions is $\Delta J = \pm 1$.

---

**Example** The microwave rotational spectrum of $^{12}C^{16}O$ consists of evenly spaced lines at 3.84 $cm^{-1}$ ($J = 0$ to $J = 1$), 7.68 $cm^{-1}$ ($J = 1$ to $J = 2$), 11.52 $cm^{-1}$ ($J = 3$ to $J = 4$), 15.36 $cm^{-1}$ ($J = 4$ to $J = 5$), etc. The wavenumber spacing is $2B$ $hc$ where $B = h^2/8\pi^2 I$.

---

A further source of complexity in treating rotation may be noted. A molecule in a high rotational state is stretched, causing each moment of inertia to be larger. If a molecule is in a high vibrational state, anharmonicity (see below) and an average lengthening of bonds lead to greater moments of inertia.

In liquids and solids rotational motions need not be treated as quantized. The reason is that molecular collisions or cooperative vibrations are frequent. Any rotation that occurs is satisfactorily treated by classical models. Actually, in crystalline solids free rotation is usually not possible.

*Vibrational transitions.* In contrast to rotational changes, vibrational transitions persist through all the states of matter. Since chemical bonds are stretched or bent in molecular vibrations, much larger energies are involved. These are of the order of 10 kJ $mol^{-1}$, corresponding to frequencies of the order of 2000 $cm^{-1}$. Thus quanta in the middle IR range are sufficiently large to promote vibrational changes.

Each vibrational degree of freedom of a molecule or *normal mode* can be treated in first approximation as a separate harmonic oscillator. Its potential energy curve

* An essentially continuous range of translational energies is available. Collisions are more important in bringing about such changes than interaction with radiation.

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is parabolic, and during vibration atoms move equal distances on either side of equilibrium positions. The vibrational energy in each mode is given by the expression

$$E_{vibrational} = (V + \tfrac{1}{2})h\nu_0 \tag{16.6}$$

where $V$ is the vibrational quantum number ($V = 0, 1, 2, 3 \cdots$) and $\nu_0$ the frequency of vibration. There are no degeneracies, except as symmetry causes two or more modes to be identical in energy. At room temperature, most molecules are in the ground state ($V = 0$) and possess the so-called *zero point energy* $\frac{1}{2}h\nu_0$ predicted by Eq. (16.6). Thus, the vibrational transition commonly observed in molecular absorption spectra is of the $V = 0$ to $V = 1$ type.

Actually, molecular vibrations are slightly anharmonic, the degree of anharmonicity increasing with vibrational amplitude. The potential function is a distorted parabola. Although Eq. (16.6) predicts an even spacing of vibrational levels, the separation between them actually diminishes as $V$ increases. Because of anharmonicity no selection rule really holds; any transition is allowed to a degree. For example, overtones of *diminished* intensity such as a $V = 0$ to $V = 2$ transition are observed and appear at a frequency somewhat less than twice the fundamental ($V = 0$ to $V = 1$). Correction terms can be added to Eq. (16.6) to compensate for anharmonicity.

In the quantum-mechanical treatment of molecular vibrations the formula for the frequency $\nu_0$ is identical to that derived from a classical Hooke's law treatment. If we treat a molecule as a simple harmonic oscillator we obtain

$$\nu_0 = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \tag{16.7}$$

where $k$ is the bond force constant and $\mu$ the reduced mass. Of course the amplitudes of vibration are quantized as required by Eq. (16.6).

---

**Example** Equation (16.7) can be used to find the force constant of the bond in a diatomic molecule like CO. For the $V = 0$ to $V = 1$ transition of CO, absorption occurs at 2140 $cm^{-1}$. Its reduced mass, $[\mu = m_1m_2/(m_1 + m_2)]$, is just

$$\mu = \left(\frac{12.0 \times 16.0}{12.0 + 16.0}\right) g\ mol^{-1} \times \frac{10^{-3}\ kg\ g^{-1}}{6.02 \times 10^{23}\ atom\ mol^{-1}} = 1.14 \times 10^{-26}\ kg.$$

Since $\nu_0 = c\sigma$, where $\sigma$ is the wave number of the absorption,

$$\nu_0 = 2140\ cm^{-1} \times 3.0 \times 10^{10}\ cm\ s^{-1}.$$

Substitution in Eq. (16.7) gives

$$\nu_0 = 6.42 \times 10^{13}\ s^{-1} = \frac{1}{6.28}\sqrt{\frac{k}{1.14 \times 10^{-26}\ kg}}$$

and

$$k = 18.4 \times 10^2\ N\ m^{-1}.$$

The selection rule that defines allowed transitions in the gas phase states that only vibrational transitions for which $\Delta V = \pm 1$ are allowed. Nearly always rotational

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changes accompany vibrational transitions for a gas molecule, and the selection rule $\Delta J = \pm 1$ simultaneously applies. Accordingly, each vibrational absorption gives rise to a collection of lines or a band. Figure 16.3 shows an example under both high and low resolution.

The so-called "P-branch" of such a spectrum includes all vibrational transitions for which the accompanying rotational shifts are $\Delta J = -1$ and is the "wing" at lowest frequencies. An absorption peak for which $\Delta J = 0$, the "Q-branch," appears for most molecules.* Note its presence as the strong absorption in the middle of the pattern in Fig. 16.3. Finally, transitions for which $\Delta J = +1$ comprise the highest frequency set, the "R-branch."

Infrared spectra become more complex the greater the number of atoms $N$ in a molecule. Each of the $3N - 6$ degrees of vibrational freedom ($3N - 5$ for a linear molecule) gives rise to a fundamental frequency and an absorption pattern like that in Fig. 16.3. We approach the situation mathematically by establishing a set of internal coordinates that reduce complex vibrations to so-called *normal modes* of vibration that are mathematically more straightforward. While it is difficult to relate such modes to IR absorption peaks, assignments have been made for most simple molecules.

Exact motions of atoms during a vibration are hard to describe. All atoms participate but fortunately there is often a great deal of localization in one chemical bond or a set of bonds. For example, if a molecule has an "unassociated" N—H bond, a vibration that stretches the bond almost always absorbs IR in the 3550 to 3340 cm$^{-1}$ range. The motion of other atoms in the molecule exercises a small perturbing force so that a wave number range rather than a specific value has to be given. Thus, vibrational spectra provide a very important basis for identification of chemical bonds in molecules (see Section 16.10).

*Electronic transitions.* No other characteristic molecular "motion" has as great a charge displacement as an electronic transition. The resulting large dipole moment leads to high intensities for such transitions; even forbidden electronic transitions are commonly observed. For this reason and also because there is no general expression covering these transitions in terms of properties that are readily observable, electronic selection rules are seldom useful in the study of absorption electronic transitions. Characteristically, quanta of order of magnitude 20 000 cm$^{-1}$ are absorbed, and most electronic transitions occur in the visible and UV from about 750 to 110 nm (15,400 to 91,000 cm$^{-1}$).

Typically an electronic transition in a molecule is accompanied by a change in vibrational and, in the gaseous state, rotational motion as well. In Fig. 16.4 a possible transition is shown for a diatomic molecule. The chart also provides a comparison with other characteristic transitions.

What vibrational change occurs during an electronic transition? We may partly resolve the question by applying the Franck-Condon principle, which states that nuclei in a molecule do not move during the short interval required for such excitation. The vibrational change also depends on (a) the relative positions of ground and

* For diatomic molecules, as predicted by quantum mechanics, the Q-branch is absent. It is also worth recalling that for homonuclear diatomics like $N_2$ and $O_2$ even the basic vibrational transitions cannot be brought about by IR radiation since there is no transition dipole moment.

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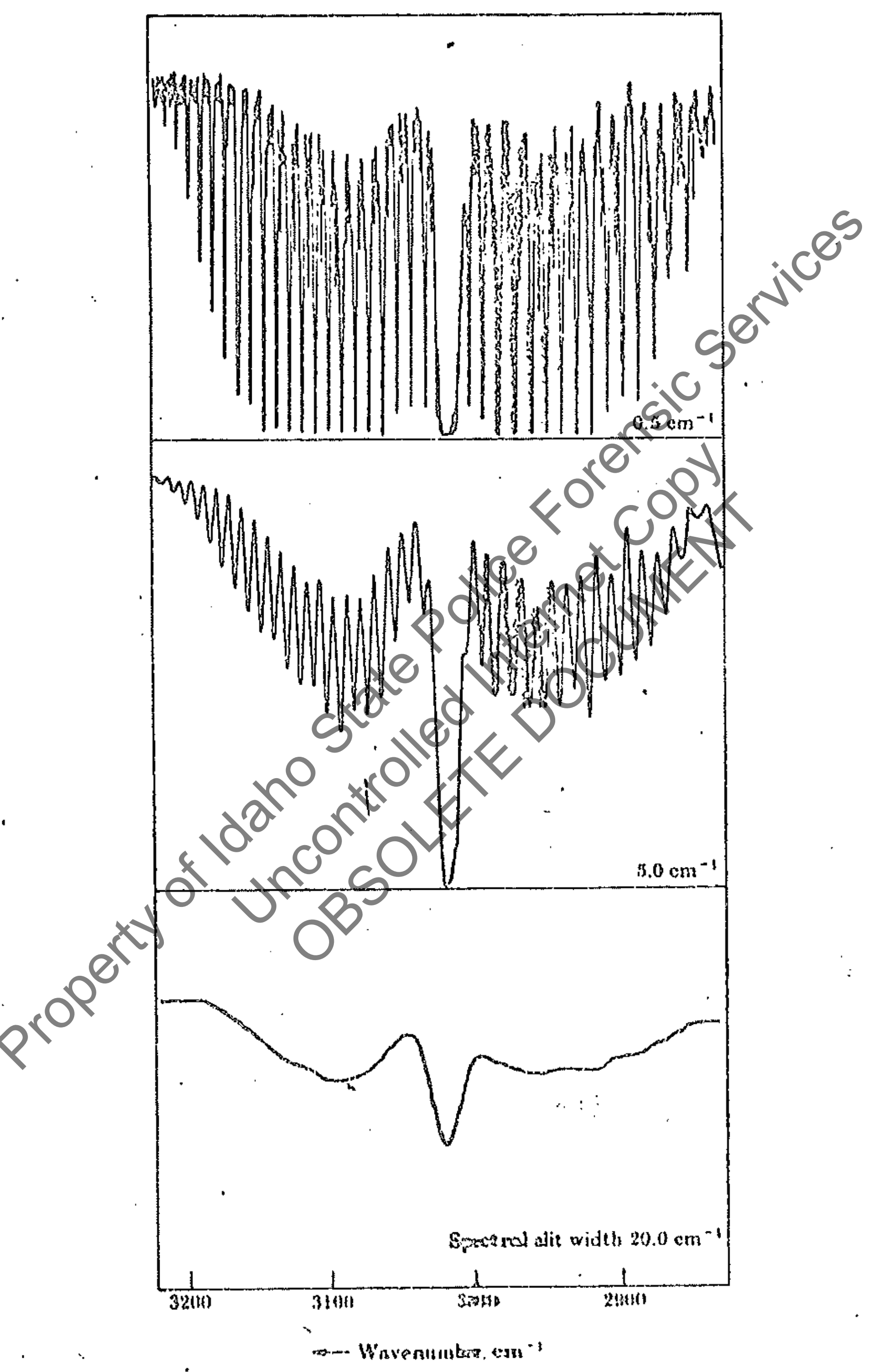

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Fig. 16.3 The C-H stretch band of methane as observed with widely different resolutions. The instrument spectral slit width is noted underneath each trace. (Courtesy of Beckman Instruments.)

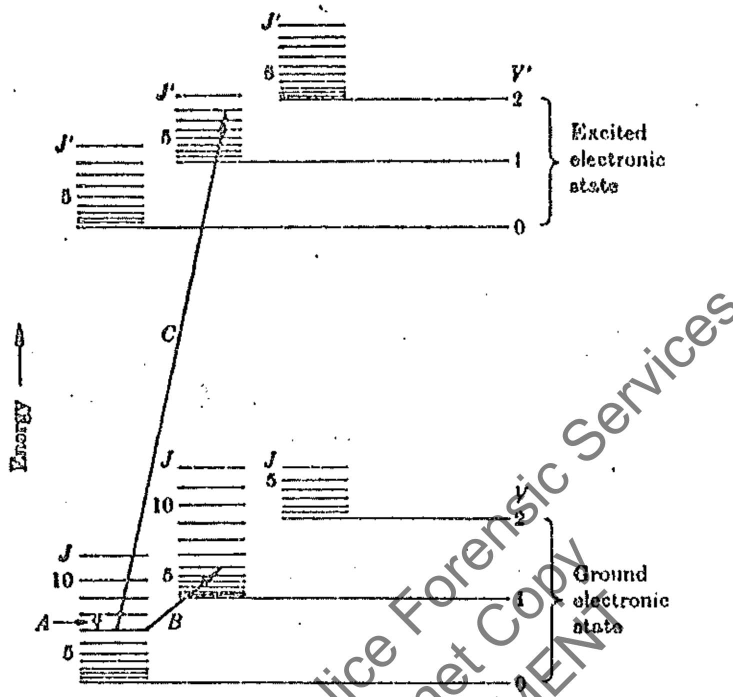


Fig. 16.4 Schematic illustration of energy levels in a diatomic molecule. Representative types of transitions and the region in which they are observed are as follows: *A*, pure rotational (far IR), *B*, vibrational-rotational (middle and near IR), *C*, electronic (VIS and UV).

excited state potential energy curves (or surfaces for a polyatomic molecule) and (b) the most probable position of atoms in the initial and final states. In the ground state the equilibrium separation of atoms is likely; in higher vibrational states either the minimum or the maximum separation of atoms is more probable.

---

**Example** To predict a probable vibrational transition accompanying an electronic excitation, potential energy curves for the ground and excited states are sketched first. With the information at hand, vibrational states are added next. A line is then drawn vertically (Franck-Condon principle) from the center of the ground vibrational state, a position that corresponds to the equilibrium separation of atoms, upward with the first vibrational level in the excited electronic state until it intersects. That transition is the most probable one.

---

The localization of one or more electronic levels is common. For instance, aldehydes and ketones are typified by a UV absorption band between 280 and 320 nm (2800 to 3200 Å). This has been ascribed to a transition from an (nonbonding) orbital on the carbonyl oxygen to an antibonding $\pi^*$ orbital associated with the carbonyl bond. The phenomenon of localization is important analytically; it provides a foundation for use of UV spectra in qualitative analysis and for rather precise structural analyses of molecules (Section 16.10).

*Classification of electronic states.* Molecular electronic states are classified mainly according to total electron spin or symmetry. The total spin or intrinsic angular

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momentum of a molecule is well described by the vector sum of the spins of its electrons provided there is only weak coupling between spin and orbital angular momentum of electrons. In general this condition holds for simple molecules with light atoms. Given that the spin per electron is $+1/2$ or $-1/2$, for a molecule the possible value of total spin $S$ for two electrons is 0 or $\pm 1$. For a total of three electrons, $S = \pm 1/2$ or $\pm 3/2$; for four, $S = 0, \pm 1$, or $\pm 2$.

Since many states of the same energy have the same total spin, the multiplicity, $2|S| + 1$, is frequently cited instead of the total spin. For example, any state in which all electrons are paired has $S = 0$ and a multiplicity of unity. Such a state is a *singlet*. If there is one unpaired electron the multiplicity is two, and the state is a *doublet*. Similarly, with two unpaired electrons with parallel spins, the state is a *triplet* since $S = \pm 1$ and $2|S| + 1 = 3$.

Though the electronic states of linear molecules can be further detailed in terms of angular momentum, for most polyatomic molecules states are classified principally in terms of symmetry. This is possible because the potential field in which electrons move has the same symmetry as the molecule.

*Integrated intensities.* An absorption band may be characterized by its intensity and band half-width as well as its frequency. For quantitative work it is usually sufficient to know $\epsilon_{max}$, the molar absorptivity at the absorption peak. For molecular studies, however, the integrated intensity is usually of greater interest. In particular it is closely related to the polar properties of molecules.

The absolute intensity of an absorption is defined by abs. intensity $= \int_{\nu_1}^{\nu_2} \alpha_\nu \, d\nu$, where $\alpha_\nu$, the absorption coefficient, is integrated over the entire band. In most spectrophotometric work an *integrated* intensity is defined by

$$\int_{\nu_1}^{\nu_2} \epsilon_\nu \, d\nu = \frac{1}{cb} \int_{\nu_1}^{\nu_2} A_\nu \, d\nu, \qquad (16.8)$$

where the molar absorptivity (or the absorbance) is integrated. The reliability of the integration depends greatly on whether the spectral slit width (Section 12.2) is appropriately narrow for the absorption peak. It should be noted that an integrated intensity can often be approximated as $\epsilon_{max} \cdot \Delta\nu_{1/2}$ where $\Delta\nu_{1/2}$ is the bandwidth at half-intensity. Representative integrated intensities in the IR are of the order of 0.1 l mol$^{-1}$ cm$^{-2}$ with the largest values appearing for OH and C=O vibrations.

In certain cases the integrated intensities of IR vibrational bands can be related more effectively to molecular properties than can molar absorptivities. [32] In any event better functional group identification can be made if both intensity and frequency data are used.

The absolute intensity of an electronic transition is often described in terms of its "$f$" number or *oscillator strength*. This term arises from the classical treatment of dispersion and is just the "$f$" given in Eq. (10.11). It can be regarded as the effective number of electrons set into oscillation in a given absorption. Further, $f$ can be related to both the quantum-mechanical dipole strength $D$ of an oscillator and the Einstein transition probability of absorption $B_{mn}$ (Section 13.3). It can be shown that

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$$f = \left(\frac{8\pi^2 m_e c g_n}{3he^2}\right) 2\pi\nu D$$

where $m_e$ is the mass of an electron, $g_n$ is the degeneracy of the excited state, and $\nu$ is the frequency of absorption. The second expression is

$$f = \left(\frac{m_e h c^2 \, 2\pi\nu}{\pi e^2}\right) B_{abs}.$$

Finally, one has

$$f = \frac{2303 m_e c^2}{\pi N_0 e^2} F \int_{\nu_1}^{\nu_2} \epsilon \, d\nu$$

where $F$ is a factor near unity that corrects for the refractive index of the solution medium. This expression relates $f$ to the usual integrated intensity of a band. Typical values of $f$ for electronic transitions are $10^{-4}$ to $10^{-3}$.

## 16.4 SOURCES OF ERROR

The uncertainty in photometric measurements can conveniently be treated as arising both from "static" errors, i.e., errors inherent in determinations at set wavelengths, and "dynamic" errors that arise only in spectral scanning. In this section the first type is examined; they are characteristic of all chemical photometric work. Since those photometric errors peculiar to scanning relate particularly to instrument parameters such as scanning speed, they are discussed below in Section 17.7.

In *quantitative* photometric work, probably the most generally useful indicator of error is Beer's law. True failures of the law in homogeneous systems, e.g., solutions, are unknown, [2] so long as there is no interference between absorbing centers and no refractive index correction. Both factors as sources of true failure of the law were discussed in Section 16.2. The upper limit of concentration for validity of the law ranges from $10^{-5}$ to $10^{-2}$ M. It follows then, in dilute solution, that in most cases of apparent failure one or more determinant errors must have occurred. The evidence for failure will be the production of a nonlinear curve when a series of absorbances at a given wavelength is plotted against concentration. Whether the error should then be traced to its source will depend on the circumstances. New analytical procedures, new reagents, and new instruments should always be scrutinized for error. If, after investigation, the source of deviation proves to be something that cannot easily be controlled, subsequent photometry can probably still be performed reliably on a relative basis, i.e., by the comparison of unknown absorbances with those obtained under the same conditions with standards. Actually, most quantitative photometric analyses are performed in this way to minimize the influence of undetected errors. The calibration curves are usually obtained as $A$ vs. $c$ or $\%$ $T$ vs. $c$ plots and can conveniently be called *working curves*.

In *qualitative* photometric studies, error is detected by the use of line spectra or the spectra of known compounds.

*Chemical.* Several chemical sources of error can be listed: uncontrolled pH, temperature variations, the presence of impurities, and the changing of solvents. They may

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give rise either to positive or to negative Beer's law deviations. These variables influence absorption mainly through their effect on equilibria involving the dissolved species. Particular conditions for a given analysis should be understood and followed.

**Example 1** Beer's law will not hold for sodium chromate in water unless a small amount of strong base is added, for the chromate tends to condense somewhat depending on concentration, and the dichromate formed absorbs at different wavelengths. The equilibrium involved is

$$2CrO_4^{2-} + 2H_3O^+ \rightleftharpoons Cr_2O_7^{2-} + 3H_2O.$$

Added base will ensure that condensation is suppressed.

**Example 2** If a particular solvent and solute interact, the absorption spectrum of the solute may be markedly altered when another solvent is used. Thus, acetic acid gives an absorption characteristic of the molecule in hexane, but in water the spectrum has many features attributable to the ionic species that result from dissociation.

$$CH_3COOH + H_2O \rightleftharpoons CH_3COO^- + H_3O^+$$

**Example 3** When the analytical species has been formed by complexing, a suitable excess of complexing agent must be present. The stability of the complex will determine the concentration needed. For example, copper(II) can be determined in aqueous solution by adding ethylenediamine (EDA):

$$Cu(H_2O)_4^{2+} + 2EDA \rightarrow Cu(EDA)_2^{2+} + 4H_2O.$$

An insufficient excess of EDA will mean the presence of a little of the aquo complex.

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*Instrumental.* Instrumental and procedural errors are less easily traced. The finite spectral width of radiation beams, the presence of stray radiation, photocell fatigue, source fluctuations, the loss of wavelength calibration, and many other factors are properly classified as instrumental errors. The paper of Goldring *et al.* is an interesting study of such errors. [22] Initial checks and routine calibrations, such as the two procedures described below, are highly desirable to keep such errors under control. In addition, the most favorable general conditions for measurement can often be deduced theoretically, as will be discussed.

*Wavelength calibration.* The wavelength control of a monochromator can be standardized by using known absorption or emission spectra. For example, in the visible, one can employ the transmission of a piece of Corning 5120 didymium glass. The didymium spectrum is shown in Fig. 16.5. In the visible and UV the emission spectrum of mercury, as produced by a mercury vapor lamp, or of various lasers are often used. Tables of reference wavelengths are readily available. [38, 40]

*Photometer calibration.* In similar fashion solutions of known absorbance may be used to standardize the photometric response. [1, 2, 27A]

*Bandwidth.* The proper choice of spectral bandwidth is of major importance in photometric investigations. In the accurate analysis of a substance that has relatively

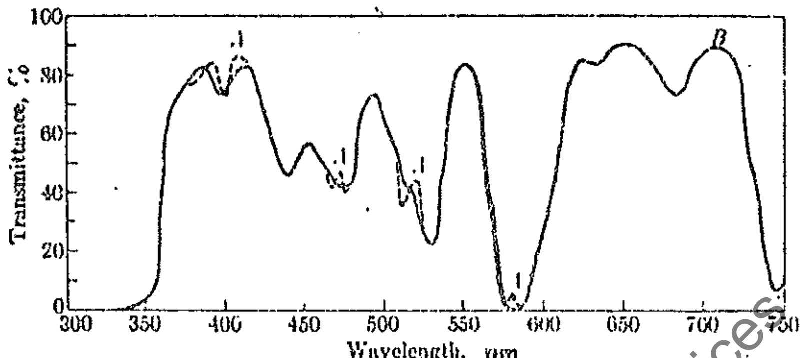


Fig. 16.5 The absorption spectrum of Corning 5120 didymium glass. Spectral slit width for $A$, 0.3 nm; for $B$, 30 nm.

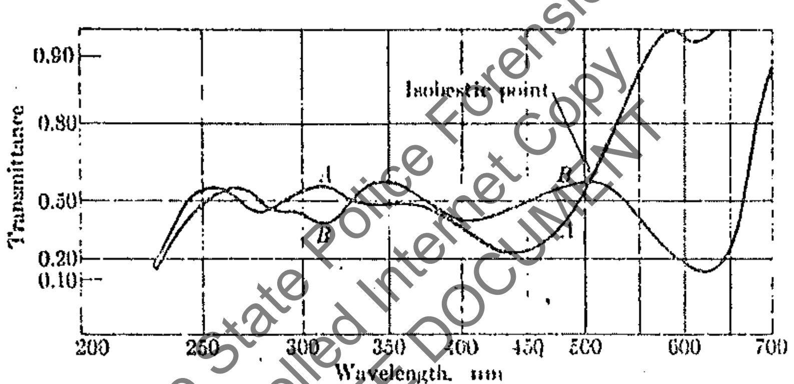


Fig. 16.6 Absorption spectrum of bromthymol blue in water. Curve $A$, pH 5.45. Curve $B$, pH 7.50. An isobestic point is evident at about 500 nm.

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sharply defined regions of absorption, such as gaseous methane (Fig. 16.3), a beam with a very narrow spread of wavelengths is needed. By contrast, bromthymol blue can be determined with accuracy even when the radiation beam is perhaps 30 nm wide judging by Fig. 16.6. The degree of resolution at two different bandwidths is illustrated by Figs. 16.3 and 16.5. Clearly the wide-band absorption study produces a somewhat inaccurate absorption curve for didymium glass.

Many negative deviations from Beer's law may be traced to the use of instruments with band passes that are too wide. The reason may be explained in terms of Fig. 16.7. Suppose the bandwidth isolated by the monochromator or filter is $ab$. At each concentration the instrument will report an effective absorbance that is between the extreme values at wavelengths $a$ and $b$, but it will not be the mean.

This is because the detector response varies directly with the transmitted power. Since the absorbance varies logarithmically (and inversely) with the power, the absorbance calculated from the average of power received across the band will be different from the absorbance $d$ or $d'$ at the mean $\lambda$. For the case illustrated by curve $B$ in Fig. 16.7, the high level of power at wavelengths near $a$ will contribute more to the

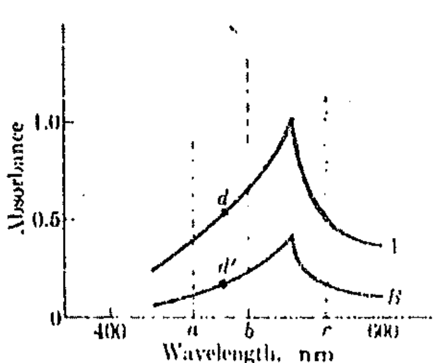


Fig. 16.7 Absorbance of a substance obeying Beer's law. Curve A, 3 concentration units; curve B, 1 concentration unit.

instrument response than will the much lower levels at wavelengths near *b*. The absorbance determined using radiation of bandwidth *ab* will thus be *smaller than the absorbance at the mean wavelength*. At the higher concentration (curve A) the difference in the values of the transmitted power at *a* and at *b* is greater than for curve *B*, and the difference between the measured and "mean" absorbances is larger. In general, the higher the concentration, the greater the variation. Since the absorbances at the mean wavelength are not obtained, an apparent negative deviation from Beer's law is observed. A similar deviation will occur for wavelength band *bc* even though it is centered on a maximum, since the curve is asymmetrical. Clearly, the extent of the deviation will be determined by the exact dependence on wavelength of the power in the beam, the transmitted power, and the sensitivity of the detector.

Since a working curve may be readily prepared, are such negative deviations from Beer's law of any consequence? Since the working curve has a lessened slope at high concentration, it should be evident that they are. Any error or uncertainty in absorbance will yield a greater uncertainty in concentration than if the law held. If the bandwidth error is large, it is desirable to work at low concentrations. In quantitative IR analyses, where bandwidths are often comparable to the half-width of an absorption band, this approach is often used.

*Pressure broadening in gases.* In the spectrophotometric analysis of gaseous mixtures some special sources of error enter. It is found, for example, that gaseous absorption curves vary not only as a function of concentration but also with the total pressure. At a fixed concentration of a constituent, increasing pressure usually produces broadening of its absorption bands (Section 13.3). An effectively larger absorbance will thus be detected instrumentally. At higher pressures the absorption is fortunately much less sensitive to further changes. If the total pressure can be maintained in that range and a narrow band of radiation used, Beer's law may be expected to hold.

*Stray radiation.* In all photometric apparatus some stray radiation adds to the regularly transmitted beam registered by the detector (Section 12.8). The magnitude of the error so introduced will depend both on the power level of stray radiation *detected* and on the absorbance of the sample under study. At high absorbances the error will be greatest, and vice versa. In this situation also, Beer's law will appear to fail negatively.

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**Example** Assume the stray radiation $S$, registered by a detector, is 1.5% of the power level $P_0$ transmitted by the reference cell. Consider a given sample with a transmittance of 0.1. If there were no stray radiation, the true absorbance $A^0$ would be measured as

$$A^0 = \log_{10}\frac{P_0}{P} = \log_{10}\frac{1.00}{0.1} = 1.$$

With stray light $S$ also detected, $P_0$ and $P$ increase. The measured absorbance is

$$A = \log_{10}\frac{P_0 + S}{P + S} = \log_{10}\frac{100 + 1.5}{10 + 1.5} = \log_{10} 8.83 = 0.95.$$

An error of 5% in the absorbance is incurred.

---

*Fluorescence.* If the mixture under photometric examination fluoresces under the incident illumination, the fluorescent radiation will produce an error exactly like that induced by stray radiation. It must be blocked somehow.

Since the fluorescence will be of longer wavelength than the incident radiation (Section 15.1), it can often be absorbed by a suitable filter placed just before the detector. The filter must, of course, pass the primary beam.

*Optimum concentration or cell thickness.* It is possible to determine a range of optimum transmittance values for each type of photometric device and procedure. This information in turn will allow the most favorable concentration or cell thickness for an analysis to be calculated.

Most photometers produce a response directly proportional to the power falling on the detector. In addition, the conventional operational procedure calls for adjusting slits or other beam intensity controls so that the transmittance of the reference is 1.00. In this situation Eq. (16.2) applies.

The methods of Section 1.7 may be used to calculate the error in the concentration resulting from error in the photometric response. The range of transmittance values in which the propagation of error is smallest may be ascertained in a second operation.

The relative error in the concentration $c$ is $dc/c$, where $dc$ represents a very small error in $c$. Rearranging Eq (16.2) results in

$$c = -\frac{1}{\epsilon b}\log T. \tag{16.9}$$

For a given absorbing species and path length, $\epsilon$ and $b$ are constant and all the error in the determined concentration can be attributed to the uncertainty $dT$ in the measured transmittance $T$. By taking the total differential of Eq. (16.9) and dividing through both sides by $c$ one obtains

$$\frac{dc}{c} = -\frac{1}{c\epsilon b}\frac{\log_{10} e}{T}dT. \tag{16.10}$$

To find the transmittance at which the propagation of error is smallest, $dc/c$ must be minimized. This may be done by differentiating Eq. (16.10) and setting the derivative equal to zero. First, a simplification may be made. By using Eq. (16.2),

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$$\frac{1}{\epsilon b}\frac{\log_{10} e}{T} = \frac{\log_{10} e}{T \log T} = \frac{\log_{10} e}{A}\log^{-1} A. \tag{16.11}$$

Differentiating Eq. (16.11) and equating it to zero yields

$$\frac{d\{[\log_{10} e/(T \log T)]\, dT\}}{dT} = -dT(\log T + \log_{10} e) = 0. \tag{16.12}$$

This yields the nontrivial solution $\log T = -0.4343$ or $T_{optimum} = 0.368$ and $A_{optimum} = 0.4343$. The relative error $dc/c$ as expressed by Eq. (16.10) is graphed in Fig. 16.8 as a function of absorbance.

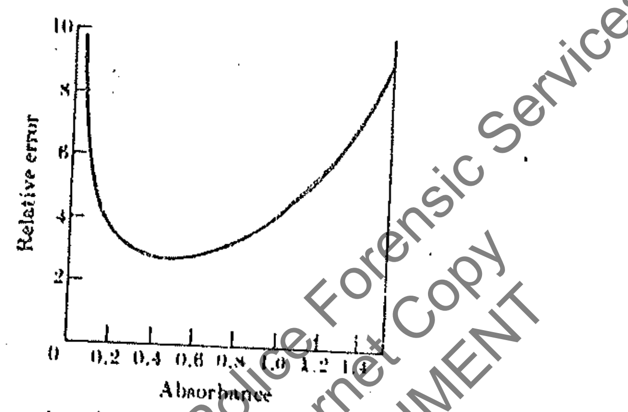


Fig. 16.8 Relative error, $[\log_{10} e \cdot \log^{-1} A]/A$, in the concentration traceable to the instrumental uncertainty in the transmittance. ($dT$ is assumed constant.)

From Fig. 16.8 it is seen that the best analytical results will be secured in an ordinary photometric procedure if the absorbance is maintained in the range from 0.2 to 0.9. The concentration of the sample or the thickness of the cell should be adjusted accordingly. As would be expected, the range for optimum transmittance holds whether the data are obtained under single- or double-beam operation.

If a differential spectrophotometric procedure is used instead, better precision can be secured as will be considered in Section 16.7.

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## GUIDELINES FOR ANALYSIS

If UV is used for qualitative analysis, a standard sample must be run under similar conditions on the instrument. Standard spectra must be kept in a file for the instrument. Quantitative analysis requires that a known standard solution be run at the time of case analysis. Since standards may be used for relatively long periods of time, they should be maintained in such a way as to prevent solution change.

Spectra used to develop conclusions for casework must be kept with case files. Periodically a holmium crystal standard should be run on the instrument.

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# REFERENCES

A Textbook of Pharmaceutical Analysis, 3rd Ed., K.A. Connors, John Wiley & Sons, New York, 1982.

Handbook of Spectrophotometric Data of Drugs, I. Sunshine, Ed., CRC Press, Boca Raton, Fla., 1981.

Handbook of Modern Analytical Instruments, R.S. Khandpur, TAB Books, Blue Ridge Summit, Penn., 1981.

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