Infrared Spectroscopy

The energy associated with electromagnetic radiation is given by Planck's Equation as :
$E = h.c/\lambda$
where E is the energy, h is Planck's constant, c is the velocity of light and λ is the wavelength of light. Electromagnetic radiation (light) is divided into spectral regions based on energy and defined in terms of wavelength regions. Different spectral regions are characterized by the ability to excite different molecular motions.

High energy Short wavelengths						Low energy Long wavelengths	
X-ray	UV	Visible	Near IR	mid IR	far IR	Microwave	Radiowave
0.1-50Å (1Å=10^{-10}m)	100(160)- 400nm	400-720nm VIBGYOR (rainbow)	720-2500nm 12500-4000cm^{-1}	2500-25000nm 4000-400cm^{-1}	25000-1000000nm 400-10cm^{-1}	0.01-100cm	0.1-10m
Inner electron excitation or ionization. Scattering and diffraction	Excitation of electrons		Excitation of molecular vibrations			Molecular rotations and translations or Unalignment of electrons aligned in magnetic field	Unalignment of protons aligned in magnetic field
X-ray Fluorescence (XRF) Crystallography	Electronic Spectroscopy		Vibrational Spectroscopy			Microwave spectroscopy or Electron Spin Resonance (ESR)	Nuclear Magnetic Resonance (NMR)
	Broad intense absorption High ε Quantification 0.01mg/ml Strong scattering Solutions are better			Many, weaker sharp bands Low ε Identification 1mg/ml Less scattering Solids are possible			

Fig. 1 : The spectral regions of the electromagnetic spectrum

Infrared spectroscopy is the study of the scattering, reflection, absorption, or transmission of infrared radiation in the spectral range 800nm to 1000,000nm (0.8 to 1000 μm). In older literature (pre-1970), IR radiation was referred to in terms of wavelengths as microns (μm). Nowadays, the wavenumber ($\tilde{\nu}$) unit is used almost exclusively. The relationship between wavenumber in cm^{-1} and wavelength (λ) in μm is given by :

$$\tilde{\nu} = \frac{10^4}{\lambda}$$

The infrared spectrum is usually divided into three regions, 12,500 to 4000cm^{-1} (0.8 to 2.5μm) (*near infrared*), 4000 to 400cm^{-1} (2.5 to 25μm) (*mid infrared*), and 400 to 10cm^{-1} (25 to 1000μm) (*far infrared*). Only the mid infrared region (often referred to simply as *infrared*) is considered here because it is the region which is widely used in the analysis of drugs and pesticides. However, some instruments scan from 5000 to about 200cm^{-1} and the extension to the far infrared is useful for halogenated compounds and for inorganic substances.

Infra red radiation has sufficient energy to excite molecular vibrations (and associated molecular rotations). At room temperature, a molecule is generally in its ground electronic state where it sits in its ground vibrational state. Providing the incoming IR has the appropriate energy (wavelength, wavenumber), resonance will occur. The molecule will be excited to a higher vibrational state and the IR radiation will be absorbed. These transitions give rise to an absorption spectrum characteristic of the compound. There are several factors that characterise this absorption spectrum : the number of the absorption features and their associated wavenumbers, the strength (intensity) of the absorption features and the sharpness of these features.

Energy/Wavenumber of an IR Absorption by a Molecular Vibration

Molecules can undergo two types of vibrations *viz.* stretching vibrations involving changes in bond length and bending vibrations involving changes in bond angles. The vibrational modes associated with the methylene CH_2 group are illustrated in Fig. 1.

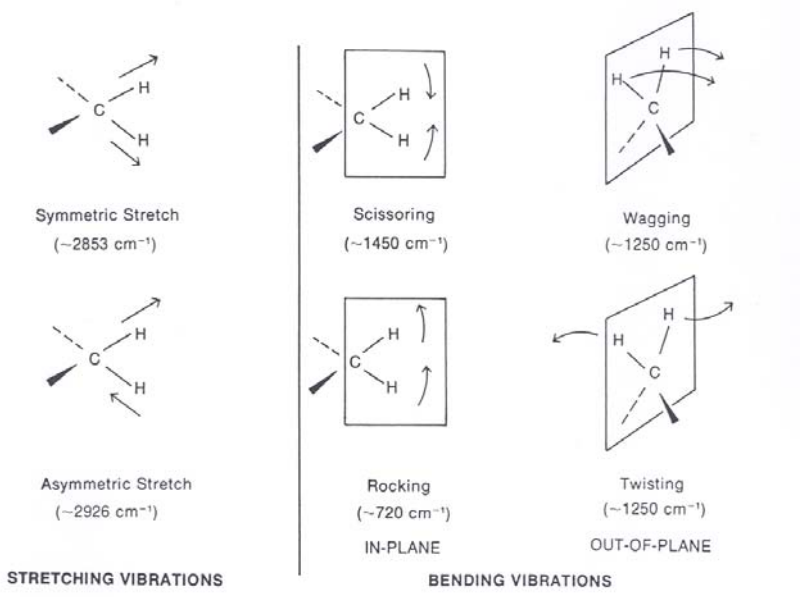


Fig. 1. The molecular vibrations of the methylene CH_2 group (Courtesy of D.L. Pavia, G.M. Lampman and G.S. Kriz, Jr. Introduction to Spectrosocpy : Guide for Students of Organic Chemistry, Saunders College Publishing / Holt, Rinehart and Winston, USA, 1979).

Theoretically a drug molecule has (3N-5) such modes of vibration, where N is the number of atoms in the molecule. These are called fundamental modes and require IR energy in the range 4000-400cm^{-1} (mid-IR) to become excited. Not all vibrations in a molecule will be assignable and generally only the most prominent are readily assigned to a given vibrational mode. These characteristic vibrations are a good way of detecting the existence of functional groups in a chemical compound. The precise wavenumber where a particular vibration absorbs light is associated with bond strength and the atomic masses of the atoms in the bond. The wavenumbers required for the excitation of typical vibrations is given in Table 2.

$\tilde{\nu}$ (cm^{-1})	Vibration
3400	N-H stretch (sharp)
3300 to 2600	O-H stretch (broad)
3050	Aromatic C-H stretch
2900	Aliphatic C-H stretch
1800-1600	C=O stretch (strong – prominent) ester (1740), acid (1700), amide (1650), ketone (1800 – 1700)
1300-1000	C-O stretch
1800-400	Forest of vibrations – fingerprint region

Table 2 : List of typical infrared vibrations

In addition there will be overtones (the excitation of a vibration to a double or higher frequency) and combinations which are the sum or difference of two or more fundamental bands. There are no fundamental vibrations that require energy greater than 4000cm^{-1} to become excited. All vibrations in the near IR are therefore overtones or combination

bands. The reader is referred to spectroscopic texts for a more detailed explanation of the origin of IR bands (eg Spectroscopic Methods in Organic Chemistry, D. Williams & I. Fleming, McGraw-Hill Education Europe, ISDN 0077091477).

Changes in the wavenumber of a band can be related to changes in either the structural environment or the physical state of the molecule. However, many bands in the complex region from 1800 to 400cm^{-1}, which is usually referred to as the `fingerprint region', remain of unconfirmed origin. Many of the bands are characteristic of the molecule as a whole and cannot be directly assigned to particular bonds. Nevertheless, inspection of IR spectra can form the basis of qualitative analytical work in IR spectroscopy to confirm the identity of a sample. A complete molecular structure cannot be deduced directly from an IR spectrum. Rather, functional groups are identified and total molecular identity is confirmed by comparison with IR spectra in a compendium.

The Strength of Absorption by a Molecular Vibration Absorption

Traditionally an IR spectrum is reported as a plot of percent transmittance (T%) against wavenumber ($\tilde{\nu}$). The IR transmission spectrum of a polystyrene film used to calibrate the wavenumber scale is given in Fig. 2.

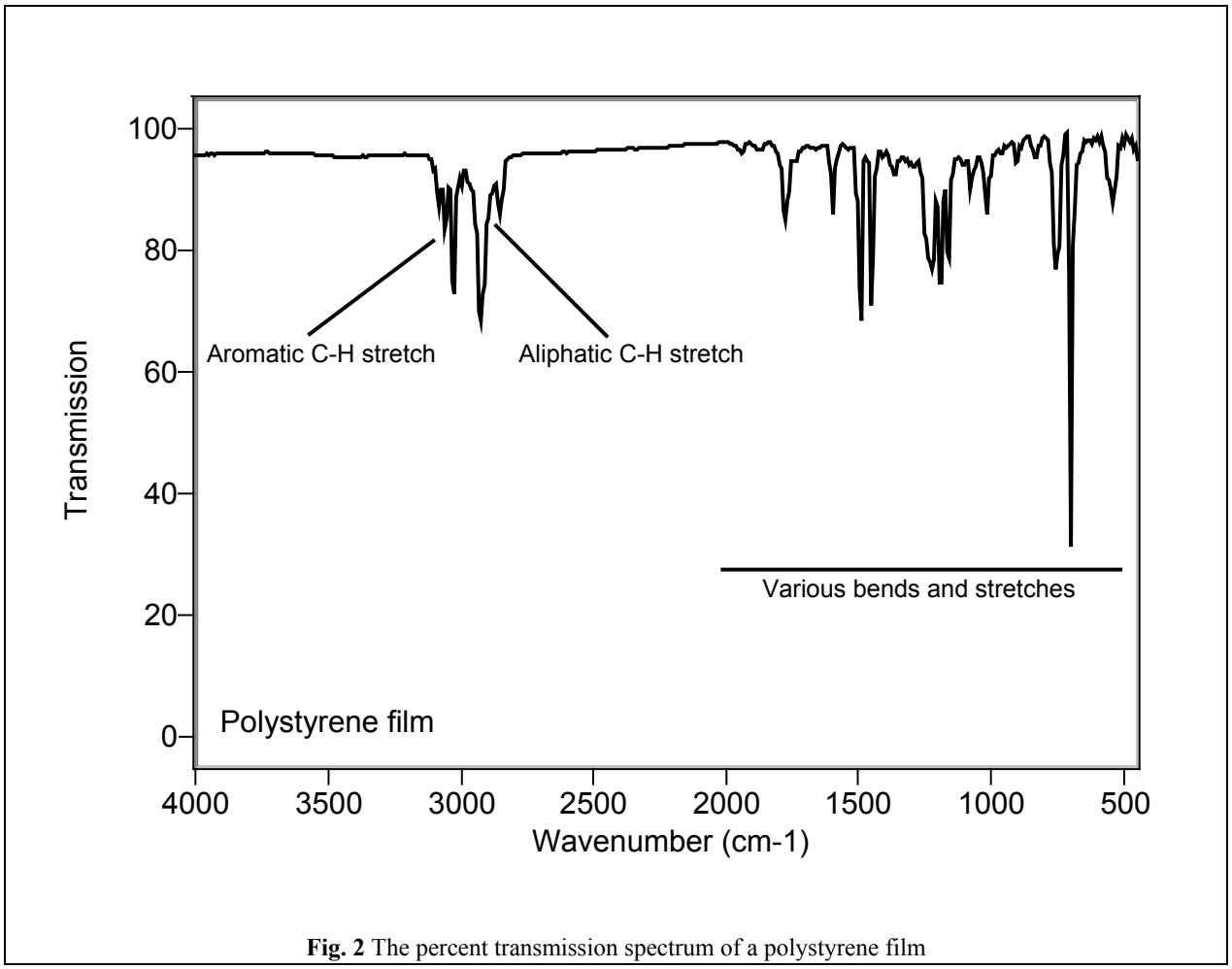
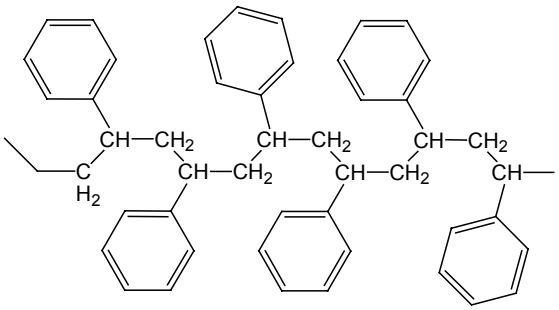


Fig. 2 The percent transmission spectrum of a polystyrene film

Polystyrene



The absorption of light in Fig. 2 is registered as the transmission of light. The simple ratio of the transmitted intensity to the incident intensity is known as the Transmittance. The percentage transmittance is 100 times the transmittance (see Fig. 3).

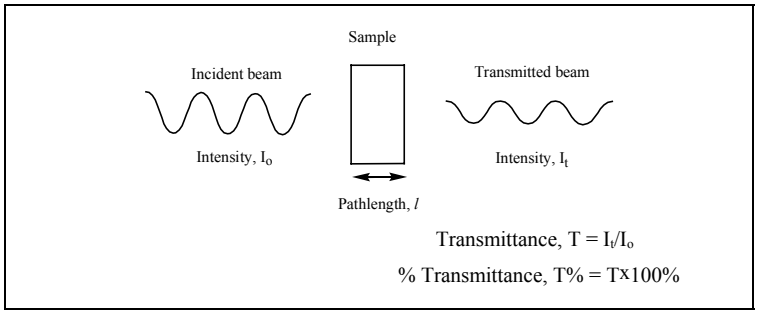


Fig. 3 The transmission of light by a sample.

The absorption of light is quantified through Beer's law as :

$A = \log(I_o/I_t) = \varepsilon.c.l$

where A is the absorbance, c is the molar concentration, l is the pathlength of the sample and ε is the molar extinction coefficient. Absorbance is the log of the inverse of transmittance.

$A = \log(I_o/I_t) = \log(1/T) = 1-\log T = 2-\log(T\%/100)$

The transmission spectrum of the polystyrene film illustrated in Fig.2 is reproduced in Fig. 4 as the absorption spectrum.

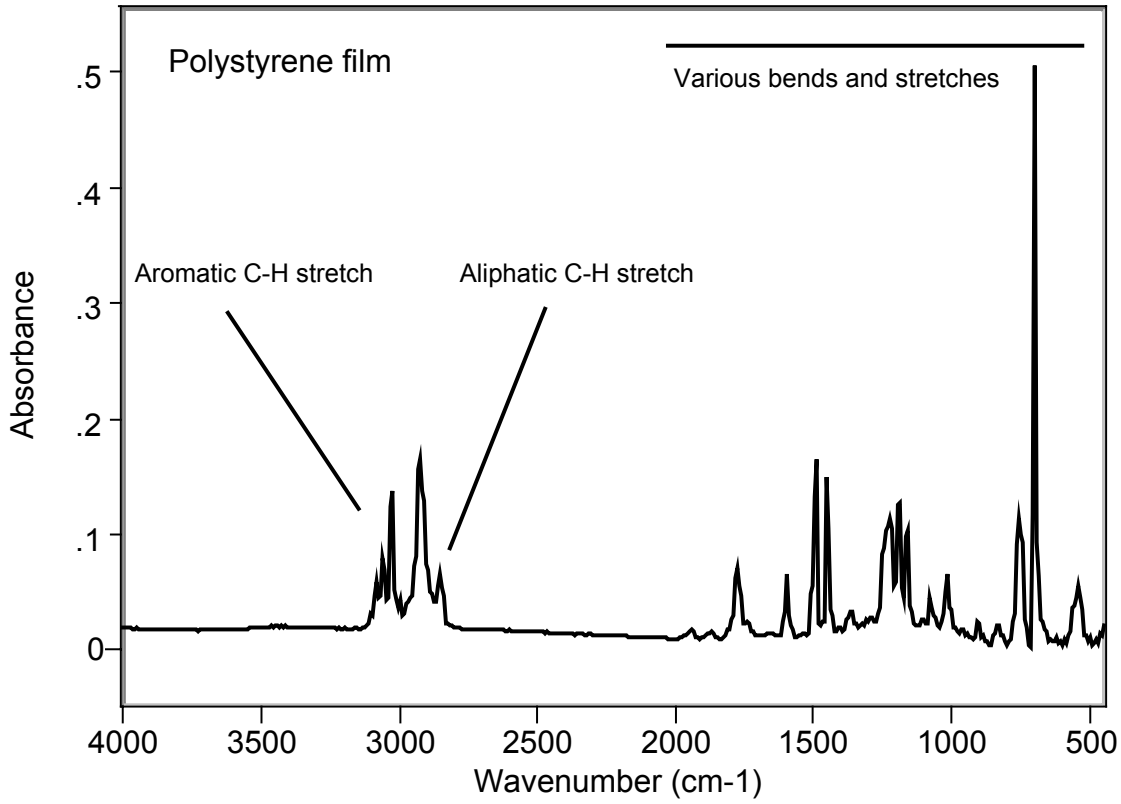


Fig. 4 The Absorbance spectrum of a polystyrene film

Whether an IR spectrum should be reported in terms of transmittance or absorbance is open to debate. Transmittance is traditional from the days when electronics were less sophisticated and log amplification was less reliable. This argument no longer holds. The only advantage that transmittance offers is that weaker peaks are more prominent in the transmittance mode than in absorbance and so are more readily recognised by eye. The absorbance scale is preferred for the following reasons :

i. The criterion that the best signal-to-noise ratio (performance) is best produced by an A~0.4 can be more easily adhered to.
ii. Samples with very strong absorbance can be difficult to distinguish in the transmittance mode. Transmittance <1% give flattened peaks heights difficult to define precisely. (A=1 corresponds to T%=1, A=2 corresponds to T%=0.1, A=3 corresponds to %T=0.01).
iii. Light scattering distorts the baseline particularly for solid sample. With an absorbance scale the varying baseline is less apparent giving an aesthetically more pleasing presentation.
iv. Light scattering can be corrected for using log(A) versus log(λ) plots.
v. Absorbance is proportional to concentration. This is important for quantitative work involving normalisation, relative peak heights or Beer's law.
vi. For the reason given in (iv.) absorbance plots will be a better presentation for database matching.
vii. Modern computation methods, such as curve fitting and derivative plots, operate better in the absorbance mode.
viii. The absorbance plot provides a better representation of the relative magnitudes of peak intensities (ε values).

Nevertheless, in this book, as with many other compilations, IR spectra are presented as transmission plots. This was the way they were originally measured.

Just because light incident on a molecule has the appropriate energy ($E=hc/\lambda$) to produce resonant excitation does not mean that excitation and absorption will necessarily occur. The charge movement during an excitation must travel in a straight line and correlate with the oscillating electric field of the light beam. In turn, this means that the vibration must behave like a translation of charge with an associated transition electric dipole moment. Thus a stretching vibration has a higher probability of being excited than an out-of-plane twisting mode (Fig. 5).

The various vibrational modes have different tendencies to absorb, different molar extinction coefficients and therefore they will have different intensities in the spectrum (see Fig. 5). The carbonyl (C=O) stretch at around 1650cm^{-1} has a particularly strong transition electric dipole moment therefore ε is large for a vibration (~100) and the carbonyl absorption is a very prominent feature. The C=O stretch is said to be spectroscopically allowed. The C-H stretching mode generates a lower transition electric dipole moment and the ε value is smaller at ~10. However, there are usually many CH's in a molecule and the additive absorption of these will make the C-H vibration a prominent feature.

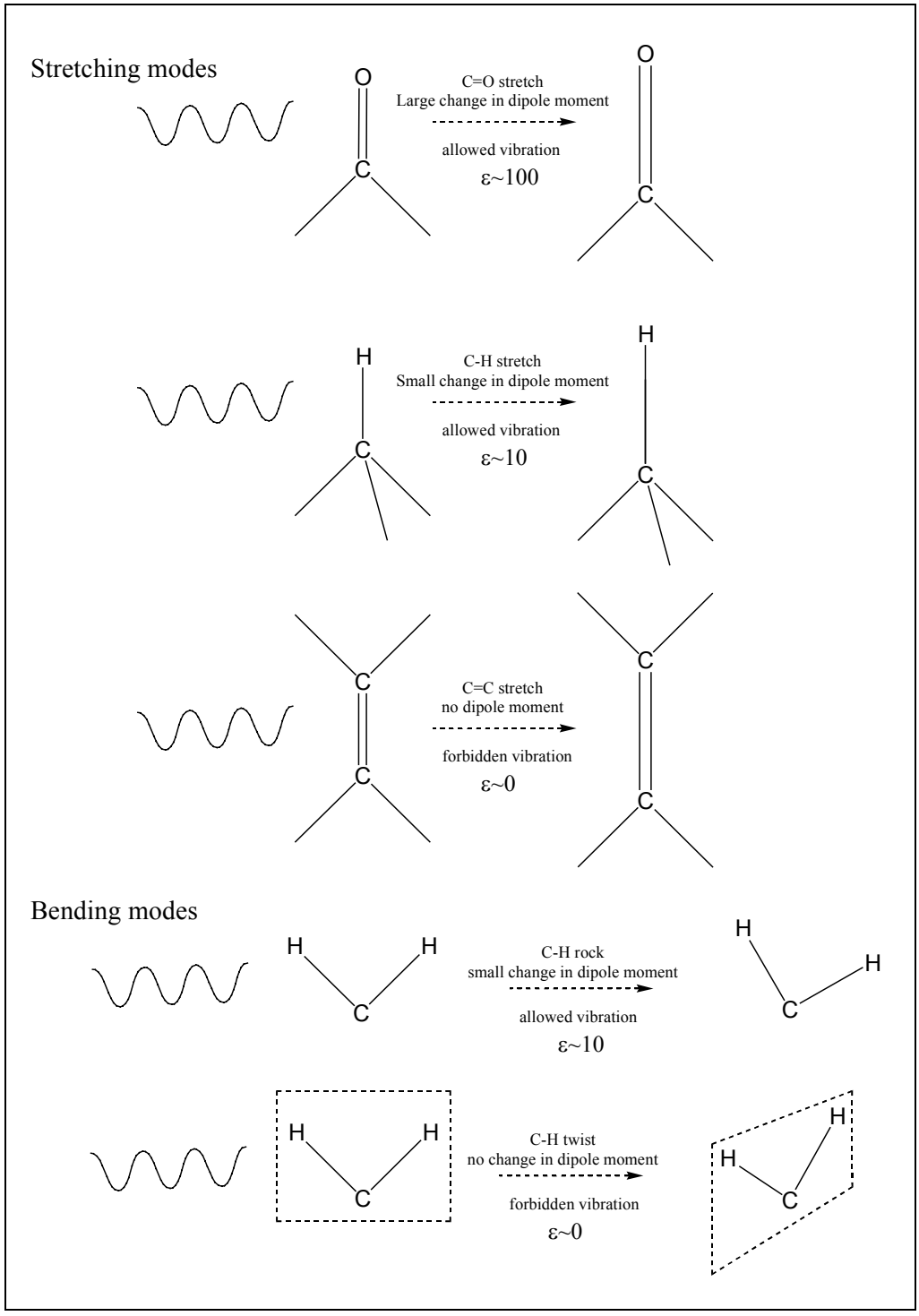


Fig. 6 The interaction of IR light with a vibration. Bond movements have been exaggerated to illustrate the point that a stretching vibration correlates with the oscillating electric field, twisting modes do not.

The stretches of symmetrical bonds such as H-H (hydrogen gas), -C-C- (ethane), O-O (oxygen), N-N (nitrogen) do not have a transition dipole moment and therefore $\varepsilon \rightarrow 0$ and they are not observed and said to be forbidden. Similar vibrations in complex molecules do absorb but extremely weakly. On the other hand gases such as NO, NO_2, CO, CO_2, SO_2, CH_4 and H_2O (water vapour) do have IR active vibrations that can be measured to monitor environmental levels. The low value of ε and low concentrations of these gases mean that gas cells for the IR are very long (up to 1meter or more). Water vapour and CO_2 present in the normal atmosphere will produce absorption effects in an IR spectrometer in the absence or presence of a sample (Fig. 7). High precision work will therefore benefit from flushing with dry air or nitrogen. The water vibrations centred at $3782cm^{-1}$ and $1587cm^{-1}$ show fine structure associated with the rotations of the water molecule.

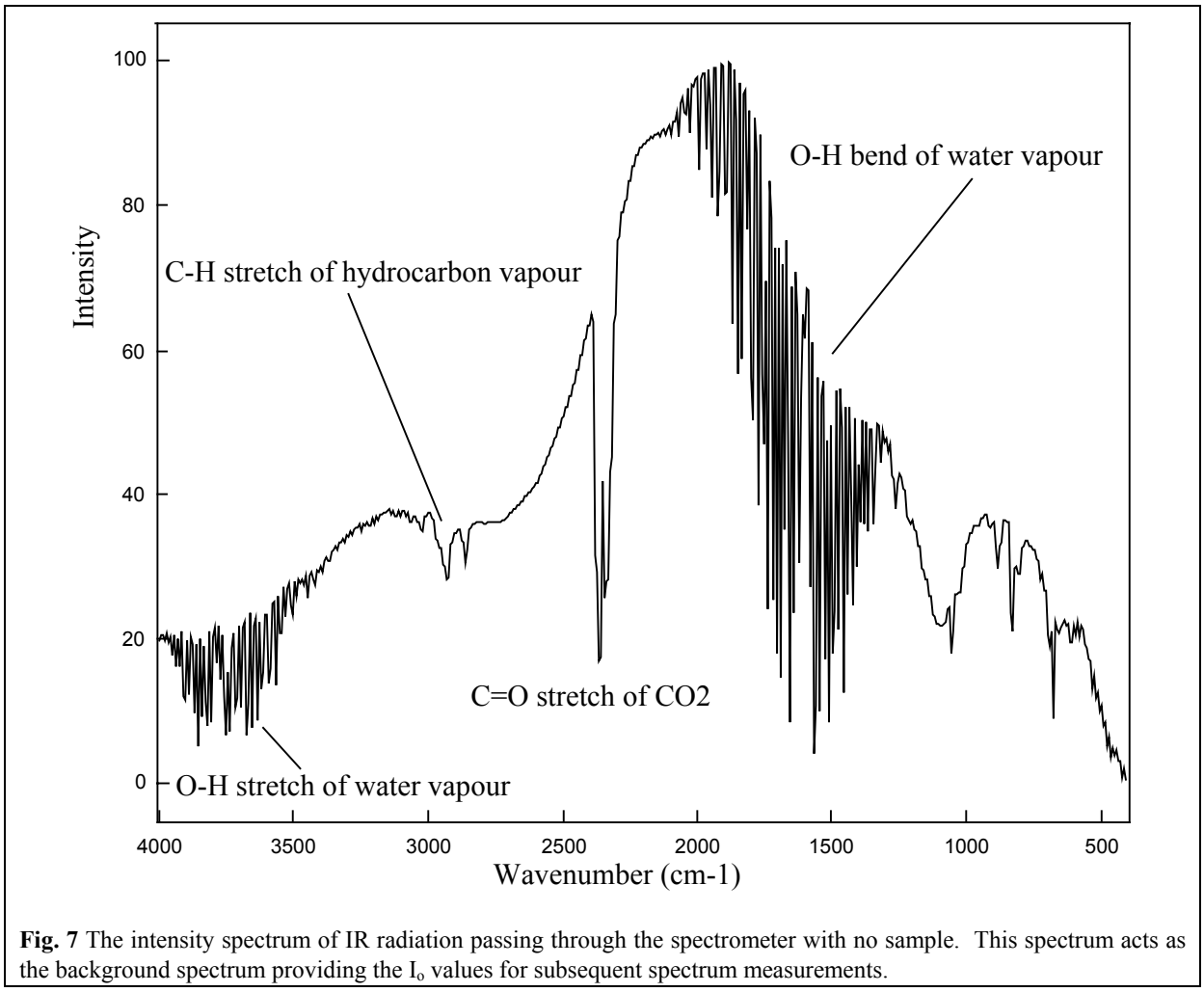


Fig. 7 The intensity spectrum of IR radiation passing through the spectrometer with no sample. This spectrum acts as the background spectrum providing the I_o values for subsequent spectrum measurements.

The actual movement of charge during a vibration is in practice very small and ε~200 is a practical upper limit. In tables, the intensity of a vibrational band is designated as vs (very strong), s (strong), m (medium) and w (weak) to reflect the variation in extinction coefficient (intensity of spectral feature) – see Table. For older designs of instrument the measurement of IR intensity was unreliable making quantitative work in the IR unreliable. This is less true for modern instruments.

The spectroscopic character of overtones and combinations is less well defined. Accordingly, ε in the near IR is low and absorption will only be detected for concentrated solutions whose absorption may be too strong for spectral regions where ε is larger.

The molar extinction coefficient, ε, is largely responsible for setting the sensitivity of an analysis

The Width of an IR Absorption Band

In the UV/Vis electronic absorption spectroscopy, absorption is limited to the excitation of a single electron in a chromophore, albeit to one of several excited states. An electronic spectrum is rarely composed of more than three prominent spectral features. However, the energy required to excite an electron is enough to also excite associated vibrational and rotational states and the absorption profile is broad. The UV/Vis spectrum is characterised by only a few broad features. A typical half-height width of a UV/Vis absorption is between 2000 and 5000cm^{-1}.
The IR sees the excitation of bond vibrations and associated rotational motions. The IR spectrum is composed of many relatively sharp features that provide an excellent fingerprint for identification. A typical half-height width of an IR absorption band is 10 to 20cm^{-1}.

INSTRUMENTATION
Dispersive Spectrometers

Conventional spectrometers start with an appropriate light source giving light that is focussed onto the entrance slit of a monochromator that splits the light up into its wavelength components. The monochromator exit slit selects a particular emerging wavelength. The monochromatic light passes through a sample where it may or may not be absorbed before being detected by a light detector (photomultiplier or photodiode). This type of spectrometer is known as a *dispersive instrument*. To measure transmittance or absorbance, both the incident intensity I_o and the transmitted intensity I_t need to be measured at every wavelength.

Single beam dispersive spectrometers

With a single beam instrument, the I_o spectrum is measured first with air (or solvent) as a reference in the light beam. In a separate measurement the I_t spectrum of the sample is recorded. In pre-computer days, the I_o and I_t values were listed and arithmetically converted manually into $T=(I_t/I_o)$ values at every wavelength. Absorbance values could also be calculated from $A = \log(I_o/I_t) = \log(I_o)-\log(I_t)$. Computers allow data to be stored and processed automatically. To ensure accuracy and precision, all components in the instrument (light source, detector and electronics) need to be very stable to ensure that I_o does not drift.
Nowadays, single beam dispersive IR spectrometers are likely to be found only in monitoring processes (eg environmental pollution). The Fourier transform IR (FTIR) spectrometer described below normally operates as a single beam instrument.

Double beam spectrometer

Double beam spectrometers are designed to compensate for instrument drifts and the need to determine I_o and I_t in separate measurements. The layout of a typical double beam spectrometer is illustrated in Fig. 8. Note that dispersive instruments in the IR have the sample located next to the light source before the monochromator (see Fig. 8) unlike their UV/Vis counterparts.

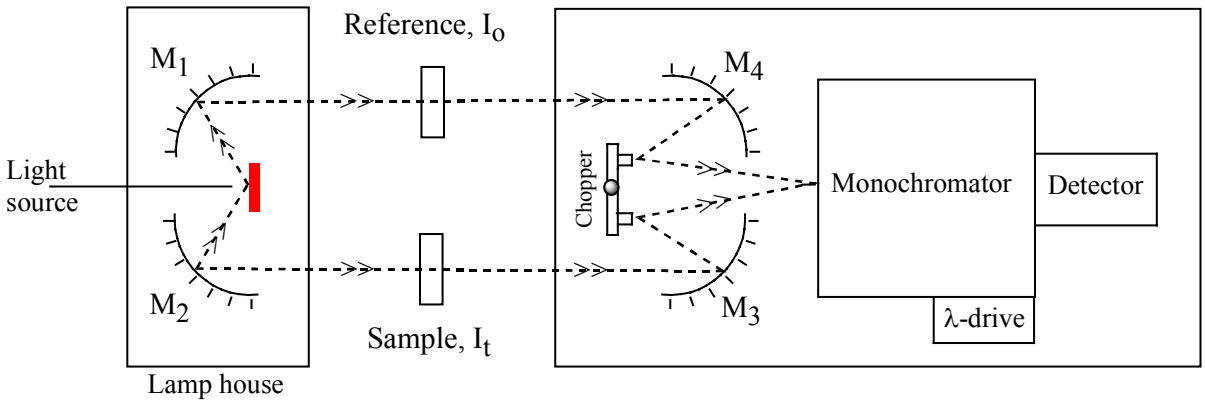


Fig. 8 The double beam dispersive IR spectrometer

IR light from the source, typically an electrically conducting element such as a Globar maintained at about 1000K, illuminates equally two mirrors M_1 and M_2. The light from mirror M_1 acts as the reference beam; the light from M_2 is the sample beam. Mirrors M_3 and M_4 send the light beams to mirrors on a mechanical chopper. The mechanical chopper is a rotating disc carrying mirrors that alternately reflect the reference and sample beams into the monochromator. After passage through the monochromator, monochromatic light from the sample and reference beams is detected alternately by the single detector as the wavelength drive changes the wavelength passing through the system. In the reference-beam chopper period the detector registers I_o and in the sample-beam chopper period I_t is measured. The alternating signals are amplified and ratioed to give $I_t/I_o = T$ or $\log(I_o/I_t) = A$. The ratiometry was achieved mechanically in very old instruments but electronically by the late 1970's. The detector preferred certainly by the 1980's was a TGS (deuterated triglycine sulphate). This is a pyroelectric detector whose electrical resistance is very sensitive to heat (IR intensity).

All measurements can be made simply with air in the reference beam. Placing a cell with solvent in the reference beam enables compensation for unwanted absorption (eg from solvents). The measurement cell pathlengths in the reference and sample beams must be identical. A computer interfaced with the spectrometer will allow post-measurement subtraction of a reference spectrum from the sample spectrum.

The amount of polychromatic IR light hitting the detector coming from the natural, black-body, room-temperature radiation from the cell compartment walls can be 10 fold or greater than the desired monochromatic IR radiation coming from the reference or the sample. This background radiation is stray light which severely affects the accuracy of the (I_o/I_t) value. Locating the sample before the monochromator ensures that the "chopped" signal selected by the detector system is related almost exclusively to light derived from either the sample or reference beams. Artificial reduction of absorbance values is thereby greatly reduced. However, locating a sample close to an IR source can cause deleterious heating effects.

Important comments in the article produced by Prof D. Chapman for the previous edition of this book are reproduced here as they are of historical relevance

In optical null instruments, radiation is passed through the sample and reference paths simultaneously or the instrument may be designed to pass the radiation through each path alternately (pre-sample chopping). A wedge or comb attenuator is moved in or out of the reference beam until absorption in both beams is equal. The movement of the attenuator is linked to the recorder pen, which records the spectrum directly. These instruments lack sensitivity when the sample absorption is high, because of the lack of energy to activate the system. Thus, measurements made at below about 15% transmittance are imprecise. This problem is overcome with ratio-recording instruments in which the ratio of the intensities of the sample and reference beams is measured; the pen response remains constant, so enabling precise and reproducible values of the ordinate to be obtained, even at very small transmission values.
Infrared spectrophotometers may not always record the correct frequency of the absorption bands because of various errors. Such errors may be inherent in the instrument, errors of adjustment, errors in fitting the paper to the recorder, or in the printing of the paper. The error in the frequency is greater at higher wavenumbers than at lower ones. For older dispersive instruments, the error at 5000cm^{-1} inherent in the instrument alone can be as large as ±50cm^{-1}, and it is essential to calibrate each spectrum before it is removed from the recorder. With modern dispersive instruments, the error should be ±5cm^{-1} or less at 5000cm^{-1}, and within ±1cm^{-1} below 2000cm^{-1}. However, occasional calibration ensures that the instrument is up to specification.

Interferometric Spectrophotometers

Fourier transform IR spectrometers have the sample next to the detector after wavelength selection. This reduces heat effects due to having a sample in proximity to an IR source. Locking into the mirror oscillation frequency coupled to signal filtering associated with the Fourier transform and the improvements in optics and detectors makes the preferred sample position viable.

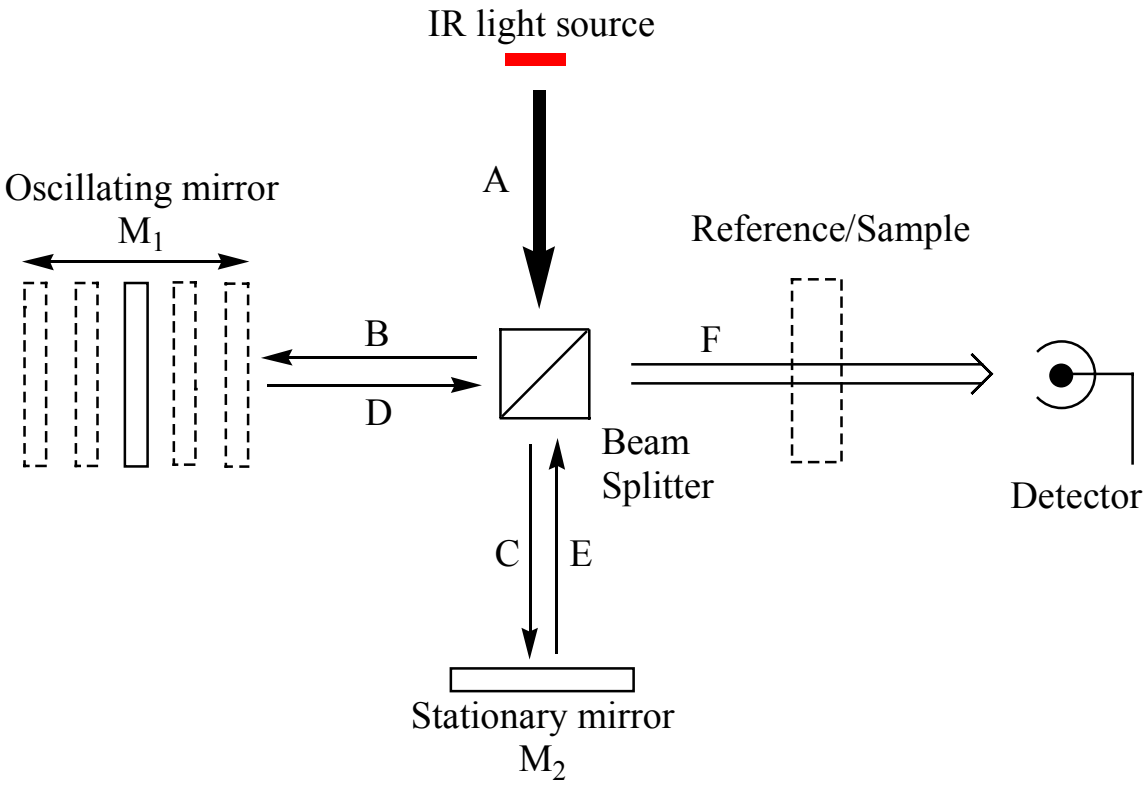


Fig. 9 The layout of an IR interferometer (FTIR spectrometer)

The Fourier transform IR spectrometer incorporates an interferometer in place of a monochromator (Fig 9). The way a FT IR spectrometer operates is presented here as a series of points with reference to Fig 9 :

i) The IR light beam A, containing all IR wavelengths coming from the IR source, is directed onto the beam splitter. Two equal intensity beams are produced, B and C.

ii) Beams B and C are reflected back by mirrors M_1 and M_2 as beams D and E respectively.

iii) At the beam splitter, beams D and E recombine to give beam F.

iv) Beam F passes through the reference or the sample to the detector.

v) If the distances travelled by beams (B + D) and (C + E) are identical, the two beams D and E will recombine constructively to give beam F (see Fig 10A)

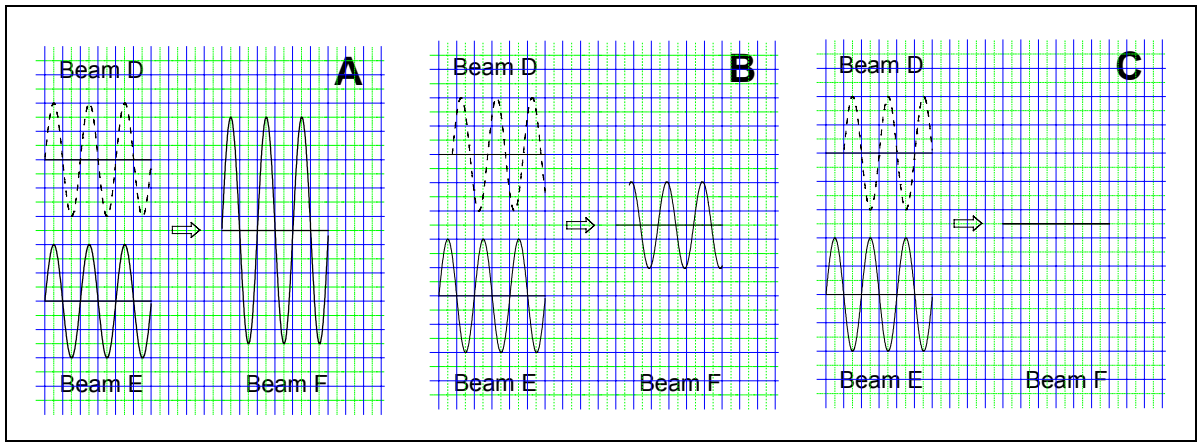


Fig 10 : The variation in the relative phases of beams D and E. 10A illustrates the beams D and E in-phase (beams fully constructive); 10B illustrates the beam D ¼ wave in advance of beam E; 10C illustrates beam D being ½ wave in advance of beam E to give complete destructive interference.

vi) If the mirror M_1 is set to oscillate along the optic axis, the distance travelled by beam (B + D) will vary whilst the distance travelled by (C + E) will remain unchanged.

vii) Identical (B + D) and (C + E) distances will mean that D and E are in-phase and recombination is fully constructive. As mirror M_1 moves towards the beam splitter, the beam D will arrive "ahead "of beam E; recombination will not be fully constructive and the intensity of beam F will be reduced and its phase will change (Fig 10B). Eventually the mirror movement of M_1 will lead to beam D being a half wave ahead of beam E. The recombination is now fully destructive and the intensity of beam F will be zero (see Fig 10C). At this point, the movement of M_1 is reversed passed the oscillation mid-point to eventually make D retarded compared to E to give zero intensity at half wave retardation before returning to the mid-point of the oscillation.

viii) As the mirror moves back and forth through a single mirror oscillation period, the intensity of a single wavelength of IR light will vary considerably. In practice, all wavelengths are passing through the system simultaneously. Therefore, the total IR light intensity registered falling on the detector during a single mirror oscillation period is very complicated and takes the form illustrated in Fig 11

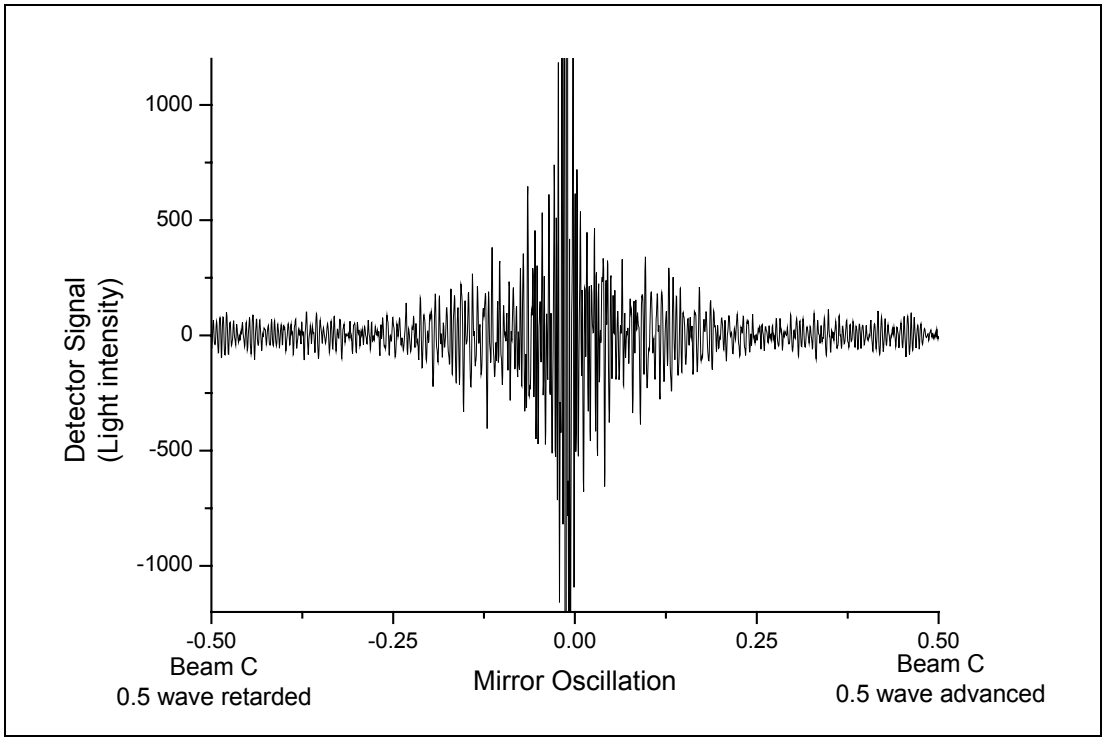


Fig 11 The Interferogram produced by a single mirror oscillation

ix) The signal illustrated in Fig 11 is now subjected to a mathematical procedure called a Fourier Transform. This extracts the light intensity versus wavelength (wavenumber) information. This is stored as presented in Fig 12A.

x) With no reference or sample in the beam, this measurement is the background or I_o spectrum which is stored in the computer to be used as the I_o for all subsequent transmittance (I_t/I_o) or absorbance ($\log(I_o/I_t)$ measurements during the working session.

xi) A measurement is now made with the sample in place. A similar interferogram is created. This is also subjected to a Fourier transformation to produce the sample, light-intensity throughput spectrum as presented in Fig 12B.

xii) Subsequent data manipulation in the computer produces the transmission or absorption spectrum of the sample as illustrated in Figure 12C & 12D.

xiii) A laser beam (typically He/Ne, 633nm) is often included in the optical train. The laser beam passes through the sample as a visible red beam and is monitored to ensure that the optics are kept optimal maintaining the precise wavelength and spectral bandwidth characteristics of the spectrometer. The laser beam is not itself an integral part of the measurement.

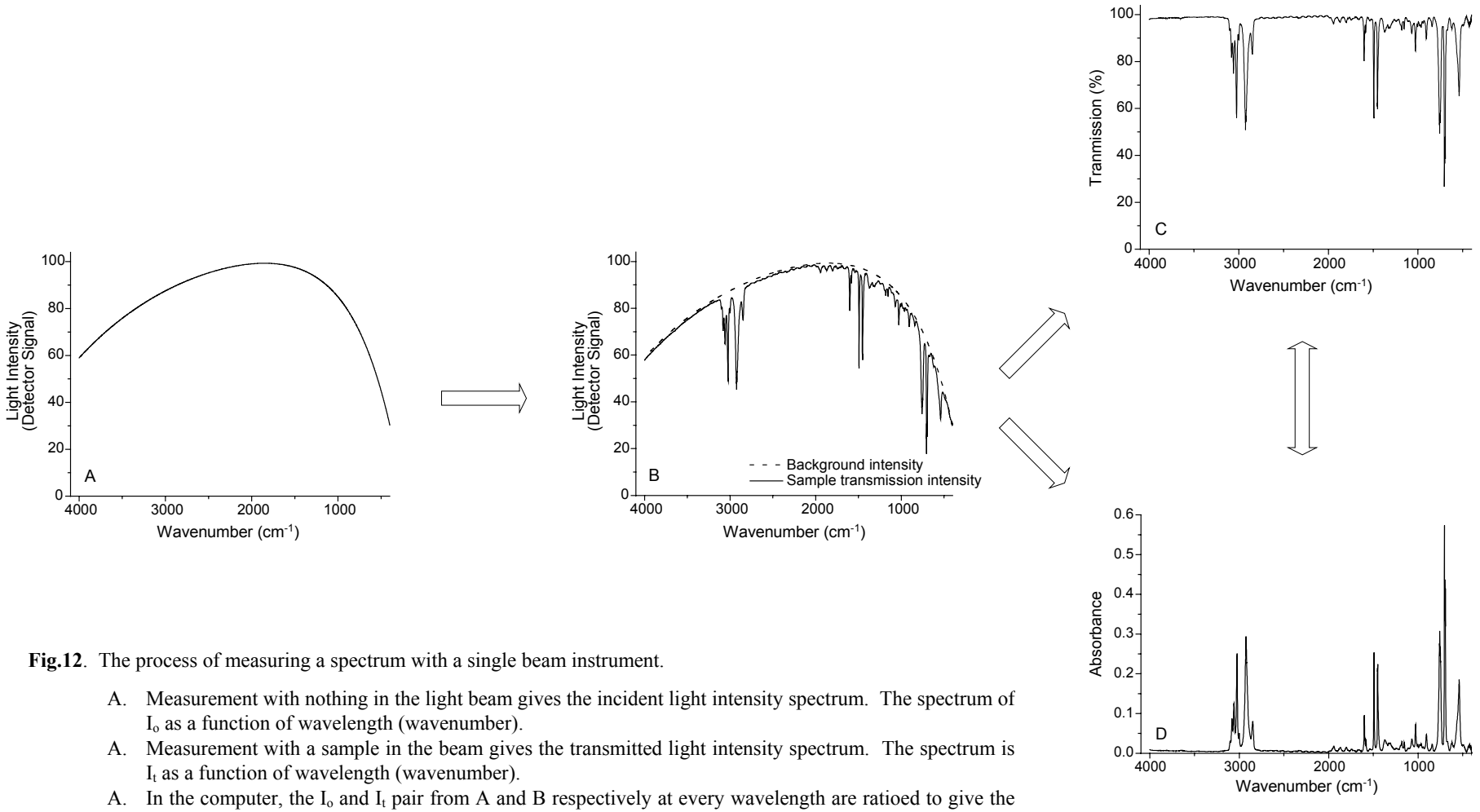


Fig.12. The process of measuring a spectrum with a single beam instrument.

A. Measurement with nothing in the light beam gives the incident light intensity spectrum. The spectrum of I_o as a function of wavelength (wavenumber).
A. Measurement with a sample in the beam gives the transmitted light intensity spectrum. The spectrum is I_t as a function of wavelength (wavenumber).
A. In the computer, the I_o and I_t pair from A and B respectively at every wavelength are ratioed to give the Transmission spectrum.
A. In the computer, the log ratio of the I_o and I_t spectra, $\log(I_o/I_t)$, the difference of the logarithms, $(\log(I_o)-\log(I_t))$, or the expression [A=2-log(T%)] gives the absorption spectrum.

Technical details

- The IR source is a Globar or similar proprietary "hot" element operating at ~1300K.
- The detector needs to have a fast response and have low inherent noise. The TGS (deuterated triglycine sulphate) detector remains the most widely employed for routine use. Pyroelectric devices based upon Lithium tantalate are becoming popular as they are less expensive, have greater ordinate linearity and present better temperature stability (TGS linearity falls off above ~32^{o}C). For high precision work with lower noise, liquid nitrogen cooled semiconductor detectors are available based on Indium/Antimonide (In/Sb), Indium-Gallium-Arsenide (In/Ga/As) or Mercury-Cadmium-Telluride (Hg/Cd/Te).
- The essential elements of the interferometer, the beam splitter and the mirror movement, will have manufacturer specific features. Some spectrometers are designed to give optimum performance over all wavelengths. Some instruments will be optimised for measurements in a specific wavelength region (eg 1800 to 1400 cm^{-1} for protein studies).
- Fourier transform infrared spectrometers have advantages over dispersive instruments. The interferometer offers greater light collection and throughput (Jacquinot advantage). More light means less noise and greater sensitivity. The Felgett advantage (multiplex advantage) concerns the very nature of the interferometric measurement. All wavelengths are observed for a single scan at the same time. In a dispersive instrument, only one wavelength is detected at any one time. In the interferometer, more time is spent effectively measuring each wavelength even though the total scan time may be the same. More time to measure each wavelength means lower noise and greater sensitivity.
- Two options are available to the FT technique : either a spectrum can be scanned much faster than a dispersive instrument or the same time as the dispersive instrument can be spent in measuring a spectrum to present lower noise results. In practice, a single mirror oscillation in the interferometer produces a spectrum scan in a fraction of a second. A dispersive instrument scan can take up to 10 minutes. Taking 10 minutes over an FTIR measurement allows the averaging of very many scans. The signal-to-noise (S/N) in an IR spectrometer is proportional to $\sqrt{}$(number of scans). Accumulating and averaging 1, 4, 16, 64, 256 or 1024 scans produces a signal –to-noise improvement of 1, 2, 4, 8, 16, 32 fold respectively.
- The level of stray light associated with FTIR is low, typically less than 0.02% due to the nature of the technique being devoid of imperfect gratings and signals being selected only associated with the oscillating mirror movement. Absorbance values remain linear up to two absorbance units, which in turn leads to more accurate quantitative measurements even with strongly absorbing bands.
- The question of spectral resolution is set by the precision of the mirror movement and the user set parameters in the mathematical Fourier transformation. Resolution is generally excellent over the whole spectrum with an effective measurement spectral bandwidth of 1cm^{-1} being readily achieved.
- The interferometer advantages in the IR accrue because background (stray) light signals and noisy detectors limit detection. In the UV, there is little background stray light and the detectors are excellent. The detection of UV/Vis spectra is limited only by the intensity of the measurement light level. Any oscillating mirror movements in the UV/Vis will need to relate to wavelengths of the order of UV/Vis light (ca 400nm) and not the 50,000nm encountered in the IR; demands on mirror movements in the UV/Vis are obviously higher. In the UV/Vis region of the electromagnetic spectrum, the interferometer advantages are NOT so apparent except perhaps for very high spectral resolution work. Dispersive instruments are preferred in the UV/Vis perhaps with diode array detection to gain the multiplex advantage.

IR spectrometer Calibration

Modern IR spectrometers are generally interferometeric instruments run in a single beam mode. I_o and I_t are measured in separate scans. It is important that the intensity of the IR incident on the sample should not change during the process of studying the sample. The I_o spectrum (background) should remain relevant to the sample measurements being made. The I_o spectrum (background) may need to be re-run immediately prior to critical sample measurements. Double beam instruments monitor I_o continuously, and transmittance/absorbance values are therefore inherently more stable.

Six factors need to be considered when calibrating any optical spectrometer.

i) Wavelength (wavenumber) scale
ii) Absorbance scale
iii) Stray light
iv) Spectral resolution
v) Data resolution
vi) Time scale of measurement (time constant)

i) Wavelength (wavenumber) scale

A card carrying an accredited transparent film of polystyrene (0.04mm in thickness) is used to calibrate the wavelength (wavenumber) scale. After running a background scan with nothing in the sample compartment to ensure the reference and sample beams in a double beam instrument are balanced or to provide a fresh I_o spectrum (background) for a single beam instrument, the spectrum of the polystyrene film is measured. It should have the form illustrated in Fig 3 and 13. Peak locations and tolerances recommended in the British Pharmacopoeia are listed in Table 1.

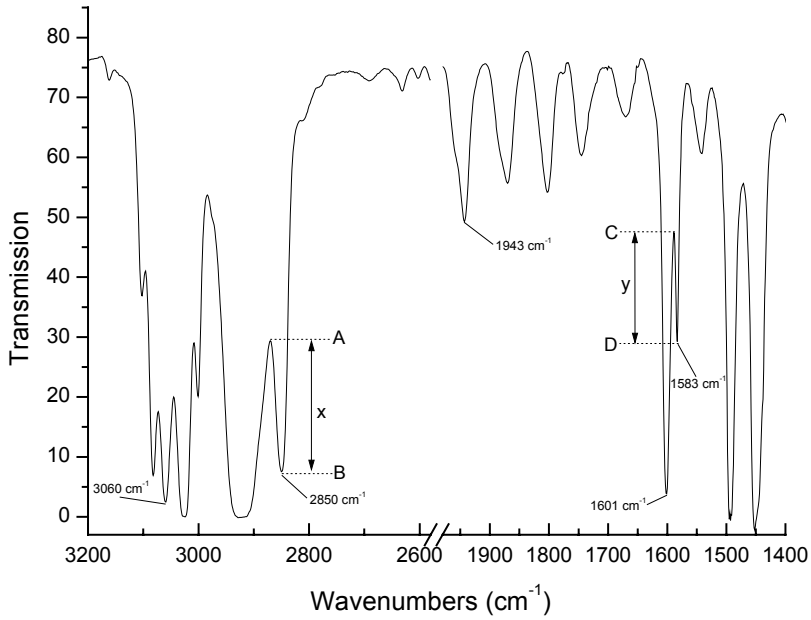


Table 1 : Transmission minima (absorbance maxima)
3060.0 (±1.5cm^{-1})
2849.5 (±1.5cm^{-1})
1942.9 (±1.5cm^{-1})
1601.2 (±1.0cm^{-1})
1583.0 (±1.0cm^{-1})
1154.5 (±1.0cm^{-1})
1028.3 (±1.0cm^{-1})
Accepted tolerances in brackets

Fig 13 Zoomed in regions of the IR spectrum of a 0.04mm thick polystyrene film indicating the location of critical parameters. A whole spectrum is illustrated in Fig 3.

ii) Absorbance scale

In the UV/Vis region of the electromagnetic spectrum, a defined 0.02% (w/v) solution of potassium dichromate in 0.005M sulphuric acid is used to calibrate the absorbance scale. Unfortunately, there are no internationally recognised standards for the IR. The absorbance scale is set in the factory with the aid of complicated optics. Filters are available in the 4000 to 2000cm^{-1} range.

In the UV/Vis region, a sample absorbance of A=0.864 is considered to produce results with the lowest signal-to-noise. In the IR due to the high background blackbody radiation falling on the detector from sources other than the sample, the ideal absorbance for good signal-to-noise is A~0.4.

iii) Stray light

Stray light can be tested by introducing a neat solvent or very high concentration sample into the sample beam so that at the wavelength (wavenumber) of interest the expected absorbance is in excess of A = 5. For the present purpose, it can be argued that all the relevant light at this wavelength (wavenumber) has been effectively absorbed. Any recorded light transmittance less than 99.9% or absorbance less than A=5 are now apparently reduced due to the presence of stray light.

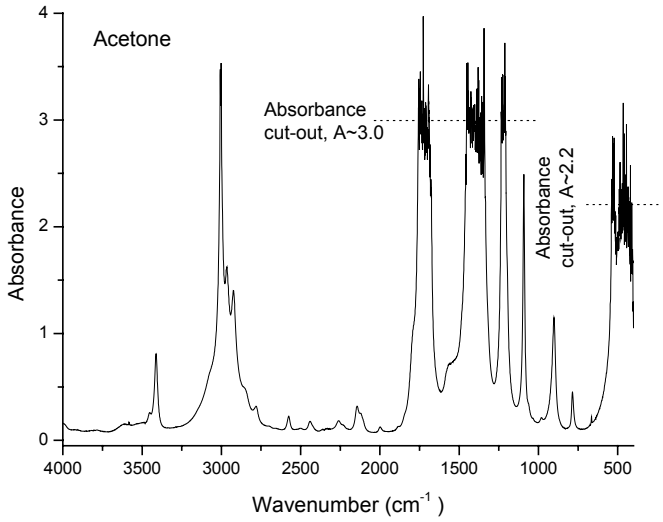


Fig 15 The IR spectrum of neat acetone in a 0.1mm NaCl cell

The maximum absorbance of neat acetone in a 0.1mm cell will exceed A=5.0 in the 2000 to 1000 cm^{-1} region. However, the measured values never exceed A=3.0. An absorbance of A=3.0 corresponds to a percentage transmittance of 0.001%. Only absorbances up to A~2.0 are reliable in this case. If the sample is being measured in solution, allowing for a solute absorption up to 1.0 means a solvent background of only A=1.0 can be used reliably. An example is given of acetonitrile measured in a 0.1mm NaCl cell (see Fig 16). The shaded sections represent areas where IR measurements of solutions cannot be made in a 0.1mm cell. Meaningful measurements can only made in the *windows* of low solvent absorption.

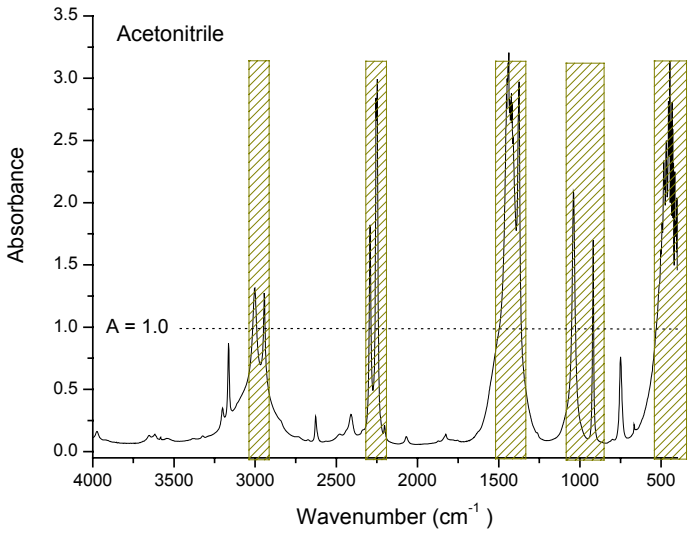


Fig 16 The IR spectrum of neat acetonitrile in a 0.1mm NaCl cell. Solute measurement is not possible in the shaded areas.

iv) Spectral resolution (Spectral Bandwidth, SBW)

In dispersive instruments, the spectral bandwidth is set by the entrance and exit slits of the monochromator. In the FTIR spectrometer, the "depth" of the Fourier Transformation sets SBW with typical values of $1cm^{-1}$, $2cm^{-1}$ and $4cm^{-1}$ available in the spectrometer control software. The narrower the SBW the more faithfully are sharp absorption bands registered. However, the noisier will be the spectrum (less light) requiring more time (accumulations) to keep the noise level down.

The British Pharmacopoeia recommends the following to ensure good spectral resolution :

Record the spectrum of a polystyrene film 0.04mm in thickness. The difference x (see Fig 13) between the percentage transmittance at the transmission maximum A at $2870cm^{-1}$ and that at the transmission minimum B at $2849.5cm^{-1}$ should be greater than 18. The difference y between the percentage transmittance at the transmission maximum C at $1589cm^{-1}$ and that at the transmission minimum D at $1583cm^{-1}$ should be greater than 12.

This recommendation is very specific for a 0.04mm thick film of polystyrene.

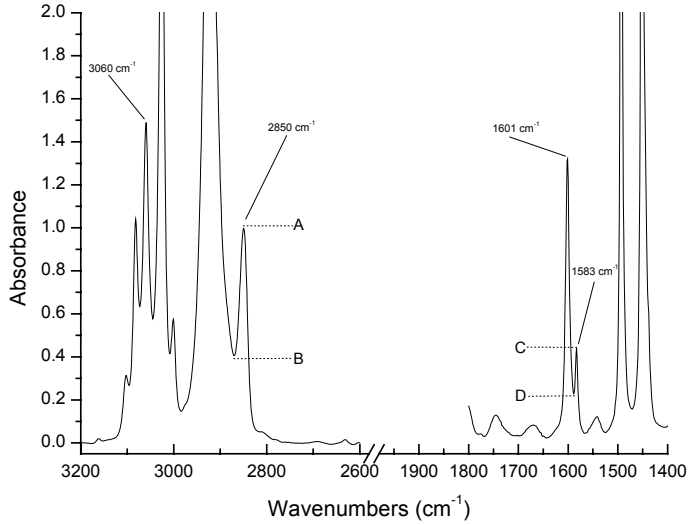


Fig 14 Zoomed in regions of the IR spectrum of a 0.04mm thick polystyrene film indicating the location of critical parameters. The spectrum is plotted in terms of Absorbance and should be compared with Fig 13A. The whole spectrum is illustrated in Fig 3.

iv) Data resolution

In a computer, spectra are stored as lists of wavenumber/transmittance (absorbance) data pairs. These X,Y data pairs are plotted on demand. A sufficient number of data points is required to give an undistorted picture. Too many points may be unnecessary requiring excessive memory allocations. Figure 16 illustrates the effects of spectral and data resolution on the spectral integrity of the IR spectrum of a 0.04mm thick polystyrene film.

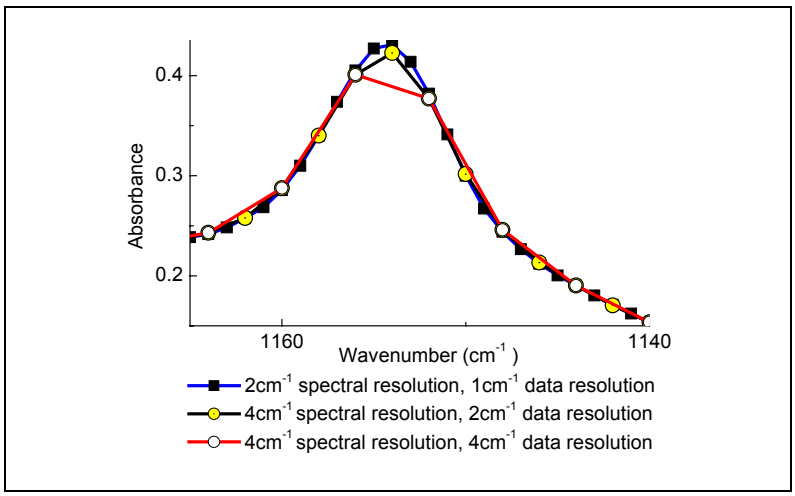


Fig 15 Zoomed in region of the IR spectrum of a 0.04mm thick polystyrene film illustrating the effect of spectral bandwidth and data resolution.

The terms spectral resolution and data resolution are often confused. Some instruments in modifying the Fourier transform mathematics to reduce the spectral bandwidth also produce a change in the data resolution of the spectra computed. The 1154cm^{-1} band of polystyrene is reproduced in Fig 15 (cf Fig 14) measured with different SBW and data resolution. Viewing the spectra presented in Fig 15 indicates that data resolution can be a more important issue than spectral resolution. In general, for typical drug molecules, a spectral bandwidth of 4cm^{-1} is probably sufficient to faithfully describe the natural bandwidth of a vibrational absorption band. However, a data resolution of 1cm^{-1} is preferred to ensure that the measured data is a faithful representation of the capability of the spectrometer. Increasing SBW, reducing data resolution or both can reduce noise.

vi) Time scale of measurement (time constant, averaged scans

In analogue dispersive instruments, the scan speed refers to the rate of rotation of the monochromator diffracting grating, which in turn controls the rate-of-change of the wavelength (wavenumber) coming from the monochromator exit slit. The instrument has an inherent controllable response time (time constant). Scanning too fast means that measured peaks will be distorted and flattened (reduced intensity); the instrument is being run too fast for the electronics to cope with the changing signal from the detector. A scan should be fast enough to avoid time wastage yet slow enough to leave the spectrum undistorted. The lower the response time (time constant), the lower the noise yet the longer the time required to measure spectra. The choice of time response/scan speed is sample dependent and will need to be selected by experience.

In a FTIR spectrometer, the scan speed is set by the oscillation rate of the moving mirror and the computer Fourier Transformation speed. A typical spectrum can be accumulated in a second or so. The instrument operator has no control of this. The time to measure an FTIR spectrum is set by the number of scans accumulated and averaged, and the speed of the computer (see technical details).

DATA PROCESSING

All modern IR spectrometers are computer controlled with measured data stored digitally. Computer control simplifies the process of running instruments and will allow the easy implementation of standard-operating-procedure (SOP) routines. Although software will in the main be manufacturer specific, companies such as Galactic Industries have produced software (GRAMS/AI from Thermo Galactic, 395 Main Street, Salem, NH 03079 USA) that will operate many instruments and certainly accept data from effectively all spectrometers on the market.

Computers readily allow changes between spectral units. In the early days, the infrared spectrum of a compound was reported as percentage transmittance as a function of wavelength in microns; by the 1970's percentage transmittance as a function of wavenumbers became the preferred form. The computer allows the ready conversion between transmittance and absorbance and between microns and wavenumbers. Presentation in terms of absorbance/wavenumber is likely to become increasingly more familiar.

Computers readily allow spectra accumulation. The spectrum of a weak sample can be scanned repeatedly to give an averaged spectrum with appreciably reduced noise with an improvement in sensitivity. For example, there is little difference between the spectra of carbon disulphide and of benzocaine in carbon disulphide shown in Fig. 16, but with spectrum manipulation, a good spectrum of benzocaine is readily obtained (Fig. 17). The amount of benzocaine in the cell was approximately 4µg but only about one quarter of this was in the infrared beam. Each spectrum was recorded in less than 30 seconds.

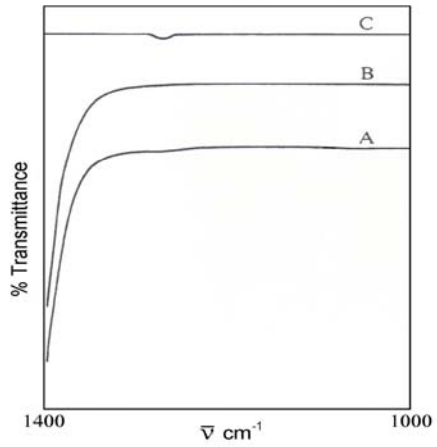


Fig 16 IR spectrum of A, benzocaine in carbon disulphide; B, carbon disulphide; C, the difference spectrum (A-B). (Courtesy of Perkin-Elmer Ltd)

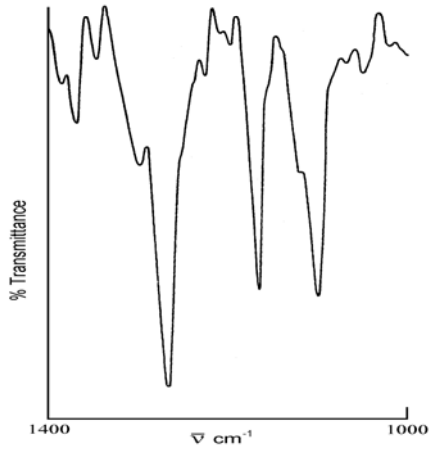


Fig 17 The difference spectrum of benzocaine shown in Fig 16 smoothed, corrected for baseline contribution, % Transmittance scale expanded 200 times. (Courtesy of Perkin-Elmer Ltd)

Digitised spectra can be readily corrected for solvent absorption or the presence of impurity. Various mathematical procedures can be applied including baseline corrections & levelling, smoothing, the determination of peak bandwidths and the calculation of absorption band areas. Derivative spectra can be produced to help distinguish the contributions of overlapping components in an absorption band.

The identification and interpretation of spectra is greatly assisted by computer analysis. Spectra can be readily overlaid for comparison. The spectrum of a sample can be compared with a library of spectra (database) and a list of the compounds of best fit can be either displayed on a screen or printed out. The presence of certain functional groups can also be confirmed.

PURIFICATION OF SAMPLES

A major advantage of IR spectroscopy is the ability to measure relatively heterogeneous materials and poorly characterised samples particularly in condensed phases (e.g. creams, powders, crystalline materials, ….etc.). By their nature, these samples are often not chemically pure and IR spectroscopy is being used to identify or confirm the existence of major constituents. IR spectroscopy is often used to demonstrate that a sample is concordant with expectation.

Nevertheless, it is essential to have pure samples to act as standards for infrared spectroscopy. A major difficulty can be that of purifying and handling a few micrograms of material without substantial losses, although these problems have largely been overcome by using fractional crystallisation or chromatography as a prelude to IR spectroscopy. On-line FT IR spectroscopy is possible but is largely a research tool. Nowadays, the identification of samples in minute amounts is achieved by other techniques such as Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS). This is particularly the case with hyphenated techniques, which involve the use of spectrometric methods on-line with a chromatographic process. Nevertheless, IR has an important role to play in identifying functional groups.

However, the isolation of pure samples of an analyte for IR spectroscopy can still be an important issue. This section reproduces the content of the previous edition of this chapter produced by Prof. D. Chapman. Practical advice is given on the purification of microgram amounts of a sample. It is assumed that the starting material is a residue from the evaporation of a solvent extract of urine, blood, tissue, or other material. For the toxicologist, the most suitable method of purification is some form of chromatography.

Paper and Thin-layer Chromatography

Suitable systems for thin-layer chromatography are described elsewhere in this book. Any of these systems is potentially useful where it is required to elute a spot, but reversed-phase systems should be avoided because it is difficult to remove the spot without the stationary phase, which would interfere with the infrared spectrum. Furthermore, location reagents must be chosen with care, and a destructive reagent such as the Marquis reagent for alkaloids, should not be used. Non-destructive reagents such as iodoplatinate solution can be used because the coloured complex is decomposable to yield the original compound. However, even this procedure may introduce extraneous peaks into the spectrum and, ideally, location reagents are best avoided. If the compound cannot be detected under ultraviolet light, it could be applied to the paper or thin-layer plate twice and only a portion of the chromatogram sprayed, thus allowing the unsprayed portion to be eluted.

Elution from cellulose thin-layer and paper chromatograms is generally more efficient than from silica gel thin-layer chromatograms; quantitative recoveries from silica gel are rarely attainable. However, thin-layer chromatographic systems produce results more quickly than paper chromatographic systems and will often give better resolution of spots. Thin-layer plates are available with fluorescent additives, which facilitate location of spots without altering them chemically. Only experience can determine the best chromatographic system and eluting solvent for any particular compound.

The use of aqueous acid or alkali to elute the compound from the thin-layer plate or paper chromatogram, followed by solvent extraction of the aqueous solution, is more efficient than direct solvent extraction of the adsorbent.

In one method of direct extraction, the adsorbent is scraped from around the spot, the glass adjacent to the spot is carefully cleaned, and the adsorbent is eluted *in situ* directly on to a wall of potassium bromide built around the tip of the spot. The potassium bromide is then pressed into a disk. This technique is only suitable for well-resolved spots.

Elution of the spot sideways will reduce contamination from compounds that are not as well resolved. The recovery of material from chromatograms varies from nil to over 70%. Compounds containing hydroxyl and carboxyl groups, which can readily form hydrogen bonds with the solid support, tend to be recovered in low yield. Considerable interference in the 1100 cm^{-1} region is found with some adsorbents and compounds.
In a variation of this method, the thin-layer adsorbent is placed in the bottom of a glass vessel together with a triangular `wick' of compressed potassium bromide. Solvent is added and it rises up the wick and evaporates from the upper region. The compound is conveyed up the wick by the solvent and accumulates at the tip of the triangle which is then cut off, dried, and used to prepare a disk. About 10µg of compound is required to produce a satisfactory spectrum. The advantage of this technique is that the lower part of the potassium bromide wick acts as a filter and removes finely divided adsorbent, which can give rise to spurious peaks.
In a further method, the thin-layer adsorbent is scraped onto a small amount of potassium bromide powder in the hub of an 18-gauge metal hypodermic needle. A 1-ml glass syringe is filled with pure solvent and connected to the needle, and the compound is eluted dropwise onto a mound (10mg) of dry potassium bromide powder. Each drop of solvent is allowed to evaporate completely before the elution of the next drop. The powder and solute are then mixed and pressed into a disk.
Eluted material almost always includes unwanted extraneous matter co-extracted from the paper or thin-layer chromatogram. Thus it is advisable to use the eluent from a `blank' area as a reference solution. Contamination from plasticisers, solvents, and dirty glassware can also be a serious problem when a spectrum has to be obtained from a few micrograms of a compound. Even momentary contact of dry adsorbent with plastic tubing can remove appreciable quantities of plasticisers. Hence the following precautions should be taken: (a) use the minimum amount of the purest adsorbent available; (b) elute with less than 1ml of a solvent containing less than 0.0001% (1ppm) of non-volatile residue; (c) keep sample handling to a minimum; (d) clean all glassware with an efficient detergent in an ultrasonic bath; (e) avoid contact of materials and samples with plastics.

Gas Chromatography

Gas chromatography can provide a very convenient method of obtaining pure samples for infrared spectroscopy. However, the sample can still be contaminated with impurities eluted from the stationary phase. The effluent from thechromatograph is a hot vapour and the problem is to obtain small quantities in a form suitable for presentation to the spectrometer. The spectrum of the vapour can be recorded directly or the compound can be trapped and then its spectrum recorded. Unfortunately, there is no entirely satisfactory method for the direct coupling of a gas chromatograph to a standard dispersive infrared spectrometer. The outlet of the gas chromatograph can be split and one part connected to a heated cell (or light pipe) placed in the beam of an infrared spectrometer. The gas flow is then stopped, trapping the sample in the cell, and the spectrum is recorded in the vapour phase. This technique can provide acceptable spectra of volatile compounds such as butyl acetate, which has a strong carbonyl band, but spectra of less volatile compounds such as caffeine and phenylbutazone are more difficult to obtain. The temperatures of the connecting pipe and cell are clearly of great importance to keep the compounds as vapours. The coupling of a gas chromatograph to a Fourier transform instrument is much more satisfactory because the speed of scanning is sufficiently rapid to enable the spectrum of a compound to be recorded as it is eluted. Nevertheless, the temperatures of the cell and pipework are still of critical importance.

The method used for trapping a compound will depend on whether it is a solid or a liquid and, if the latter, on its volatility. Ways in which small samples can be obtained from a gas chromatograph in a form suitable for presentation to the spectrometer are given below.

Cooled Tubes
Most techniques of collecting the effluent employ cooled tubes of glass or metal, but it is difficult to obtain good recoveries of a few micrograms of compounds of differing volatilities by any one technique. Drugs such as barbiturates and phenothiazines can be recovered in 50 to 70% yields in glass or metal capillary tubes held at room temperature, whereas more volatile drugs such as amphetamines require cooling in liquid nitrogen or solid carbon dioxide (A. S. Curry *et al., J. Chromat.*, 1968, *38*, 200–208; A. De Leenheer, *J. Chromat*., 1972, *74*, 35–41).

Silver Chloride Microcells
Microcells extruded from silver chloride and known as `Extrocells' can be fitted with a silicon rubber serum cap and connected to the outlet of the gas chromatograph by means of a hypodermic needle. The sample collects in the neck of the cell, which is then centrifuged in order to force the sample into the main body of the cell. Solvents can be added if solution spectra are required and are, of course, necessary if the compound is a solid. The cells can be used at room temperature or they can be cooled in freezing mixtures or in liquid nitrogen; they can be used many times provided that they are stored in the dark. The cells are available with path-lengths from 0.1 to 0.01mm and volumes of 2 to 0.2µl. The main disadvantage is that they have to be centrifuged for cleaning as well as for filling and emptying.

Membrane Filters
Membrane filters composed of cellulose esters can be used to collect samples. The filters, about 25μm thick, are supplied in matched pairs to compensate for their absorption in a double-beam instrument.
The filter is placed in a specially designed metal and PTFE holder. The PTFE inlet section reduces condensation of the effluent before it reaches the filter and the metal outlet section allows the filter to be cooled. A Luer fitting just before the sample emerges connects the filter holder to the outlet of the chromatograph. When the sample has been collected, the filter is removed and placed in the spectrometer. The method is simple and easy to use and is capable of giving extremely good results.

Alkali Halide Tubes
A straight tube containing a plug of powdered alkali halide is connected to the outlet of the chromatograph. The effluent condenses on the halide, which can then be pressed into a disk. This technique is most useful for compounds, which are solid at room temperature.

The main difficulty, common to all these methods of collecting fractions, is to determine the optimum temperature of the outlet tube from the chromatograph and the temperature of the collecting device. This problem can only be solved by trial and error.

High Pressure Liquid Chromatography

High pressure liquid chromatography provides a very convenient method of purification, particularly where gas chromatography is either inapplicable or where derivatisation of the compound is necessary. Unlike gas chromatographs, liquid chromatographs are usually operated at or slightly above ambient temperature, and most types of detector are non-destructive. Thus, the appropriate fraction of eluate can be collected by holding a test-tube under the exit port.
The method used to retrieve the sample from the eluate for presentation to the spectrometer will depend upon whether the compound is a solid or a liquid and, if the latter, on its volatility as well as the quantity present. All the common solvents absorb in the infrared region. However, with the data processing facilities of modern infrared spectrometers, this is not a great disadvantage. The spectrum of the solvent can be recorded and then subtracted from the combined spectra of the compound and solvent to give a difference spectrum. If the concentration of the sample is low, the difference spectrum can be enhanced either by repetitive scanning and signal averaging or by expansion of the ordinate scale (see Figs 16 and 17). In many cases, however, the amount of material will be too small to enable the compound to be collected and transferred to standard cells. The use of microcells such as `Extrocells' may be advantageous.
Alternatively, the compound can be recovered by evaporation of the solvent. However, evaporation will also concentrate any non-volatile impurities in the solvent and the use of pure solvents is essential. Another possible source of contamination is the packing material used in liquid chromatography columns. Many of these materials are based on silica gel and appreciable amounts of silica may be dissolved by certain solvents.

Microsublimation

This simple technique can be highly effective in purifying certain compounds (Fig 18). Drugs may be sublimed from an evaporated solvent extract in the tube on to the cold finger of the apparatus, and the sublimate transferred by grinding the potassium bromide powder gently with the cold finger.

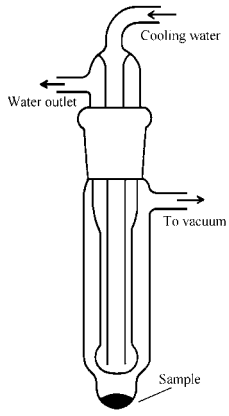


Fig. 18. Apparatus for microsublimation

SAMPLE PRESENTATION

Infrared spectra can be measured in the gas, liquid or solid phase. However, most compounds of interest are solids at room temperature. In principle, an infrared spectrum can be obtained from as little as 1μg of a compound. From a practical point of view, quantities of the order of 0.2 to 1.0mg are much easier to handle. Very small quantities require greater sensitivity to be achieved with micro cells placing as much material as possible in the IR beam. Micro cells require beam-condensing optics to focus as much light as possible through the micro cell assembly.
Glass and silica contain SiO–H and Si–O bonds that can vibrate and strongly absorb IR radiation. Therefore, cell windows need to be fabricated from ionic materials containing bonds that do not have vibrations. Crystal lattice vibrations will cause absorption in the far IR. BaF_2 (and CaF_2) are excellent for aqueous media although window absorption prevents measurements below $1000cm^{-1}$. AgCl windows are water-resistant, will allow transmission down to $400cm^{-1}$ but are friable. The most popular IR window material is NaCl, which is water soluble but transparent down to $600cm^{-1}$.

Gases

In normal laboratory experiments, IR spectroscopy is only rarely used for analysis in the gas phase. Gases are likely to be at a very low concentration and special long-path, airtight gas cells are required. These cells normally have NaCl windows and mirrors may be used to reflect the light through the gas cell several times to achieve a very long pathlength. The detection of environmental gases is a typical application of gas phase IR spectroscopy.

Liquids and Solutions

Neat liquids have a very high molarity. Thus chloroform, with MW=119.4 and density 1.48g/ml, can be said to be 124M. According to Beer's Law, to detect the C-H stretch (ε~10) with absorption A=0.4, the pathlength required, given by $l=(A/(\varepsilon.c)$, is ~3.10^{-4}cm.

Non-volatile, neat liquids can be measured simply by placing a drop between two IR transparent plates and pressing the plates together to ensure a narrow measurement pathlength ($\leq$ 0.1 mm). Volatile liquids may need proper sealed liquid cells.

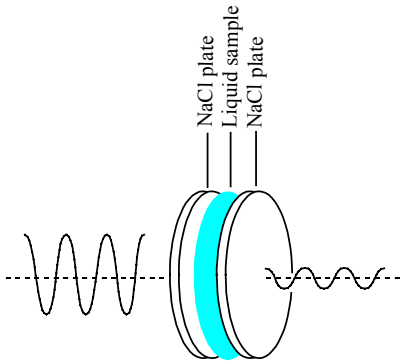


Fig 19 : Liquid sample or nujol mull sandwiched between two NaCl plates.

Liquid cells usually consist of two parallel transparent windows (0.1 to 0.01mm apart) separated by a precise gasket made of teflon or lead and fitted with inlet and outlet ports. Variable path-length cells are also available, in which one window is retained on a screw that can be finely adjusted to give a precise pathlength in the 0.1 to 0.01mm range. These cells are particularly useful to vary pathlength to accommodate for solvent absorption and variations in concentration ranges. Solvent absorption can be accounted for in a computer by comparison of the solution and solvent spectra
The number of solvents suitable for IR spectroscopy is limited. Solvents absorb infrared at their characteristic wavenumbers. Therefore, the measurement of the IR absorption of a solute is only possible in a spectral range where the solvent is relatively transparent (eg see the spectrum of 4%(w/w) liquid paraffin in toluene in a 0.1mm NaCl liquid cell, Fig 18). Carbon tetrachloride and carbon disulphide, lacking hydrogen and containing a minimal number of bond types, are often suggested to be the most useful solvents as they have relatively few absorption bands in the infrared region. However, they have poor solubilisation characteristics. In practice, solvent choice is based upon solubility and IR transparency at the wavenumber of interest. Deuterated solvents can help open regions for analysis. $CHCl_3/CDCl_3$,

CH_3CN, H_2O/D_2O, toluene, dioxane are good solvents to consider.

To overcome the inherent absorption of solvents and the relatively low extinction coefficient of a vibration, pathlengths are narrow and concentrations are high. The concentration of the test compound is usually about 5 to 10%, but concentrations up to 20% w/v can be employed. With these high concentrations, hydroxyl and amino compounds often exhibit bands due to intermolecular hydrogen bonding. Interactions between the compound and the solvent can occur. This may result in changes in the intensity and wavenumber of bands in different solvents and the breakdown of Beer's law.

IR solvents are often volatile requiring very short pathlengths. This combination can lead to solvent evaporation producing serious concentration changes. In the older style IR spectrometers, the heat of IR radiation could cause evaporation – this is less of a problem with FT systems with the sample after the interferometer. An example is presented here of the determination of the amount of dimethicone in a cream formulation.

Dimethicone can be extracted from creams with 4%(w/w) liquid paraffin in toluene and quantified by reference to standard solutions based upon the Si-O stretching vibration at 1260cm^{-1} in a 0.1mm NaCl liquid cell, Fig 20.

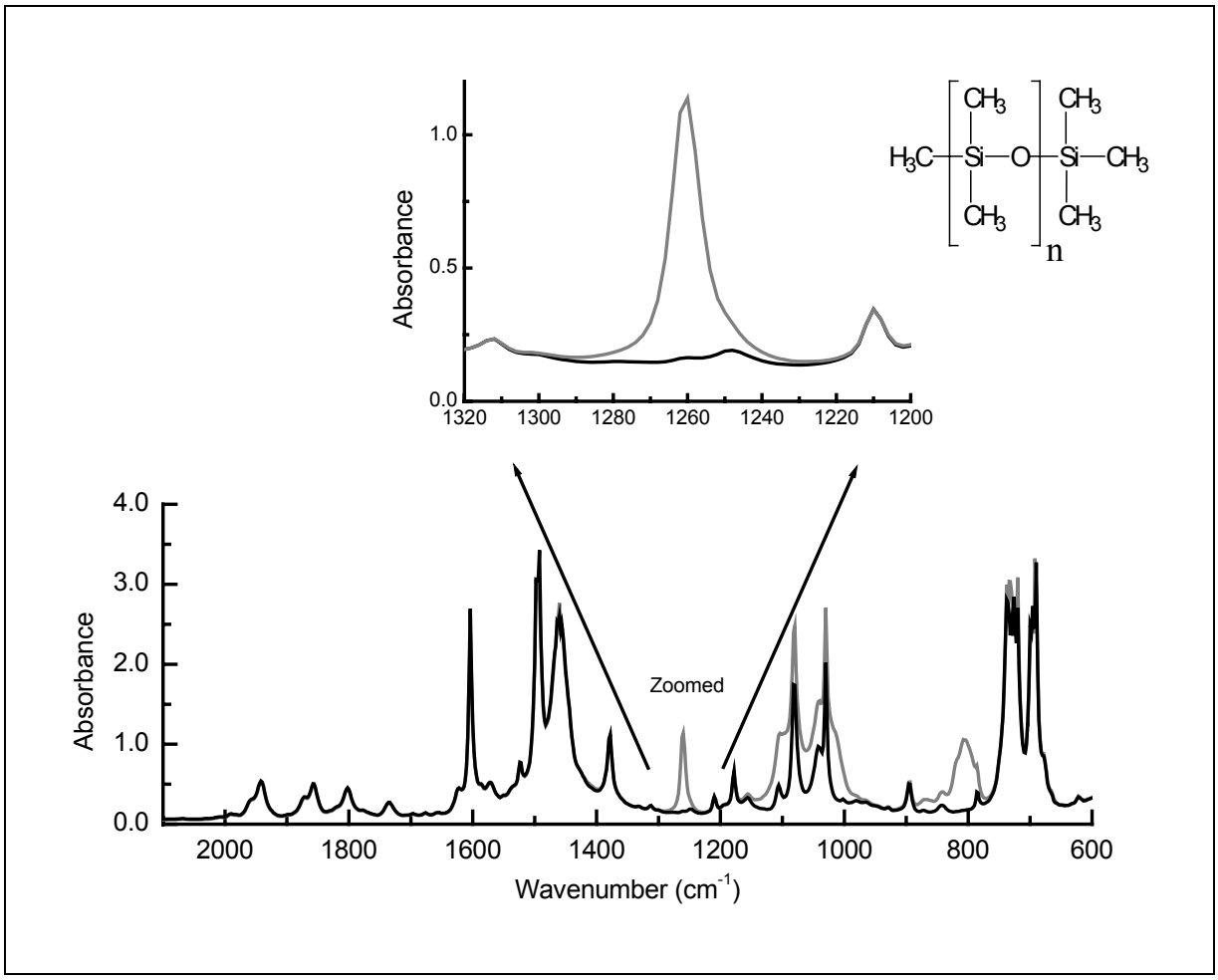


Fig. 20 The IR spectrum of dimethicone dissolved in 4%(w/w) liquid paraffin in toluene. (——) 4%(w/w) liquid paraffin in toluene; (——) 1.5%(w/v) dimethicone dissolved in 4%(w/w) liquid paraffin in toluene.

Solids

Solids are generally examined either as thin films or as dispersions in either liquids or solids. The ideal sample for IR transmission measurements is clear, visually transparent and homogeneous. This can be difficult to achieve with solids. Heterogeneous samples that are optically poor with large sample particles can introduce light scattering and the Christansen effect. Light scattering becomes significant when the particle size is $\geq 1/20^{th}$ the wavelength of the incident light. Light scattering produces a spectrum offset that is curved with high light scattering at shorter wavelengths/higher wavenumbers and lower light scattering at longer wavelengths/lower wavenumbers. Samples must be ground until particle sizes are ≤ 1 micron. The Christansen effect, due to severe refractive index changes at the sample surface, leads to distorted band shapes. Peaks take on an S-shape with apparent reduced (or even negative) absorption at the longer wavenumber edge. As a result, asymmetric bands may be observed which vary in position and intensity from true values. For transmission measurements, the sample must ideally have the appearance of a "perfect glass". To reduce the light scattering and the Christansen effect, all components of the sample must have a very small particle size (≤ 1 micron) and they must be dry.

The polymorphic form of a solid sample can effect IR. This is an important issue in the pharmaceutical industry when the rate of dissolution of a solid drug can depend upon its crystal morphology.

Sucess has been achieved by simply crushing the sample between two diamond windows in device known as a Diamond anvil (Fig 21).

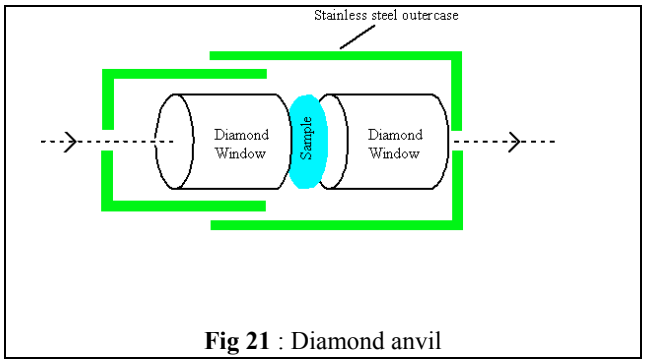


Fig 21 : Diamond anvil

Mulls

Solid compounds are dispersed in a liquid, e.g. liquid paraffin (Nujol). The finely powdered compound (about 1 to 10 mg) is mixed with one drop of the liquid and ground in an agate mortar. The test sample must have the constituency of a smooth thin cream. The mull is spread onto an alkali halide plate, usually sodium chloride or potassium bromide, and another plate placed on top, taking care to exclude air bubbles (cf Fig 19). The plates are pressed together strongly. A disadvantage of this method is that the spectrum of the mulling agent will be superimposed upon that of the sample. Consequently, liquid paraffin cannot be used if the C-H stretching vibrations are to be examined and a halogenated liquid such as `Fluorolube' (a fluorinated hydrocarbon) or hexa-chlorobutadiene must be employed

Alkali Halide Disks

The technique of dispersing the compound in an alkali halide has been used widely in the identification of drugs. Originally, potassium bromide was used and the technique is still often referred to as the `KBr technique'. However, potassium chloride is superior to potassium bromide because it is less hygroscopic. Storing the alkyl halide in an $80^{o}C$ oven helps ensure anhydrous conditions.

The finely powdered dry test compound (about 1mg) is mixed with the alkali halide (about 250mg) and ground either mechanically in an agate ball mill or by hand in an agate mortar. A texture approaching talcum powder is a good constituency. The mixture is pressed under ~10 tons pressure in a purpose designed press to produce an optically good, thin disk (see Fig 22). A vacuum helps to retain dry conditions and smooth disc formation. The pressure is applied for 10 minutes.

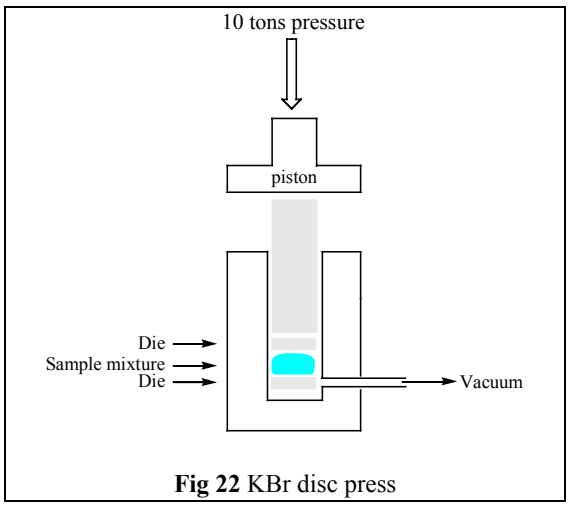


Fig 22 KBr disc press

If only small quantities of the compound are available (about 200µg), a thin cardboard mask with a slot in the centre can be used. The mask is placed in the die and the slot filled with the mixture before pressing. A mask is often employed routinely because it provides a support for the alkali halide and so enables the disk to be handled more easily. Microdisks of diameter down to 0.5mm can be prepared by using metal (lead or stainless steel) disks of 13mm diameter with the appropriate size hole in the centre. The hole is filled with potassium bromide (about 1mg) containing from 0.05 to 0.2% of the sample, which is then pressed in the usual way. The metal disks should be washed before use in both polar and non-polar solvents and finally in good quality acetone to remove traces of oil and grease, which may produce artefacts in the C-H region of the spectrum. The method may fail if excessive pressure is used, causing deformation of the lead disk.

Another useful technique consists of dissolving the compound in a small volume of chloroform and drawing it into a Hamilton-type syringe held in a repeater holder. A small cluster of fine potassium bromide particles is picked up on the end of the needle by a trace of chloroform expressed from the needle. The solvent is gently evaporated and the rest of the solution is fed into the potassium bromide from the syringe as it evaporates. A disk is then made from the powder. It is important that the end of the needle is cut at right angles to the shaft and ground flat; those supplied for use with liquid chromatographs are suitable. For bases one must decide whether to evaporate solvents without the addition of hydrochloric acid, and accept the consequent loss of certain amines by volatilisation, or to add hydrochloric acid and accept the reduced solubility of the amine hydrochlorides in chloroform. Considerable losses of the sample by evaporation may occur for other types of compound (e.g. phenols), particularly when dilute solutions are used.

Because potassium bromide is hygroscopic and it is sometimes difficult to remove the last trace of water, silver chloride may be used instead. An indentation about 0.8mm deep and slightly wider is made in the centre of a small piece of silver chloride sheet, and a solution (about 0.1µl) containing as little as 500ng of substance is placed in the indentation and gently warmed to evaporate the solvent. The sheet is then placed in a die, which produces a cone of silver chloride with the sample embedded in it. A similar cone of plain silver chloride is mounted in the reference beam. Excellent spectra can be obtained with this technique.

The alkali halide disks can be stored in a dry environment and give good spectra several years after preparation. A well-prepared disk should have over 80% transmittance in regions where the sample does not absorb, although it will not necessarily be visually clear. It is not always easy to obtain a good disk when a very small amount of a recovered drug is available. In these circumstances, attenuation of the reference beam can `sharpen' the spectrum. Another technique is to heat the alkali halide disk to about 80º for 30 to 60 minutes with an infra-red lamp to evaporate any absorbed water. However, the high temperature accentuates the disadvantages of the alkali halide disk technique. In addition, the following artefacts have been observed: (i) formation of anhydrides from carboxylic acids, (ii) ketals and cyanohydrins reverting to the parent ketone, and (iii) loss of water from secondary alcohols.

There are several disadvantages inherent in the alkali halide disk technique. The alkali halides, which are generally used, are hygroscopic and it is very difficult to exclude all traces of water. This often results in an O–H band in the spectrum. A number of compounds containing O–H groups either form hydrogen bonds with the alkali halide or are adsorbed on its surface, so the method is unsuitable if the O–H band is to be examined. In such cases, polytetrafluoroethylene (PTFE) powder can sometimes be used in place of the alkali halide. Polymorphism occurs in many compounds and the grinding and pressing can alter the crystal form and consequently the spectrum. Splitting of bands also frequently occurs. Another disadvantage is the possibility of chemical changes occurring during the preparation of the disk. For example, double decomposition can occur:

$$\text{Base HCl} + \text{KBr} \rightarrow \text{Base HBr} + \text{KCl}$$

Hence, hydrochlorides should preferably be examined in potassium chloride. Bromide may be oxidised to bromine by some compounds, particularly strong oxidising agents, and this may lead to a disk becoming either discoloured or having yellow-brown spots.
If the sample is a potential oxidising agent, other techniques of sample preparation should also be used in order to check the reliability of the spectra obtained from the alkali disk.
Organic compounds that contain nitrogen in a functional group should not be used with plates which are made of thallium bromide and thallium iodide (KRS-5 plates) as they appear to react with the plates.
Despite these disadvantages, the technique is still a most useful one for solid drugs. The advantages are that, besides being easy to use, the absorption of the alkali halide is very low and the quantity of compound required is small. The disks can easily be stored for reference purposes or the compound can be recovered if required.

Thin Films
This method is of use where it is necessary to obtain spectra free from dispersing media. The film can be prepared either by melting the solid and pouring it on to a suitable plate or by evaporation of a solution on an IR transparent plate. This method is not widely used.

Measurement of Strongly Absorbing or Strongly Light Scattering Samples

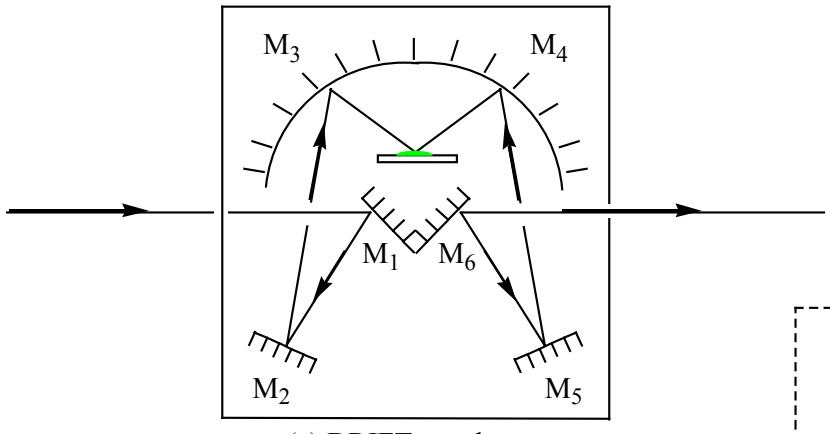
IR light incident on solid, powders or other materials such as creams is only poorly transmitted, if at all, due to long pathlengths and light scattering. Neat liquids and solutions need very narrow pathlengths to overcome solvent absorption. However, scattered light or reflected light can be monitored in these cases.

Light scattering

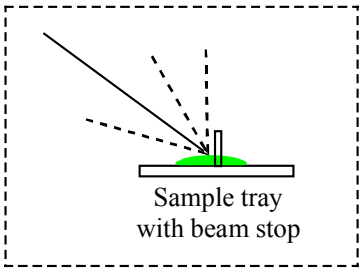
IR light falling on a powder can be reflected in two ways. The light can be truly reflected in the sense of mirror reflection (angle of incidence equals the angle of reflectance); this is known as specular reflectance. Alternatively, the IR light can be scattered in the Rayleigh scattering sense over all angles with a scattering intensity related to particle size; this is often referred to as diffuse reflectance. Specular reflectance is related to the refractive index of the sample and intensity versus wavenumber data is difficult to interpret. Diffuse reflectance on the other hand is more simply related to light intensity; if the light is absorbed at the surface it cannot be scattered back. A typical apparatus, often given the acronym DRIFT (diffuse reflectance infrared fourier transform spectroscopy) accessory is illustrated in Fig. 23. The sample is placed on a sample tray located beneath two ellipsoidal mirrors M_3 and M_4. Heterogeneous powders and fibres often benefit from being ground and "diluted" with KBr. Pure KBr can now be used as the reference material in a separate measurement for the reference spectrum. A good sample can be produced by rubbing a solid sample with English Abrasives paper P220C silicon carbide to produce a sample of ~150μgrams over a 35mm^2 area.
Diffuse reflectance is a measure of intensity versus wavenumber data normally in a single beam configuration. To ensure measurements are at least approximately proportional to concentration, a Kubelka-Monk transformation can be applied.

$$\text{Kubelka-Munk spectrum} = \left[\frac{\left[1-\left[\frac{\text{Sample spectrum}}{\text{Reference spectrum}}\right]\right]^2}{\left[2\cdot\left[\frac{\text{Sample spectrum}}{\text{Reference spectrum}}\right]\right]}\right]$$

Alternatively, data can be presented as [– log(reflectance spectrum)] versus wavenumber.



(a) DRIFT attachment



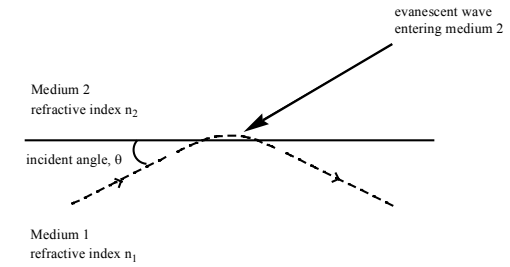
(b) Zoomed view of sample tray

Figure 23 : Drift attachment : IR from the interferometer strikes mirror M_1 and is directed onto the sample tray by mirrors M_2 and M_3. In (a) the pure reflection path is highlighted. In practice, a beam stop (see (b)) blocks the specular reflectance and only the diffuse reflected (scattered) beam is collected by mirrors M_3 and M_4 and directed towards the detector by mirrors M_5 and M_6.

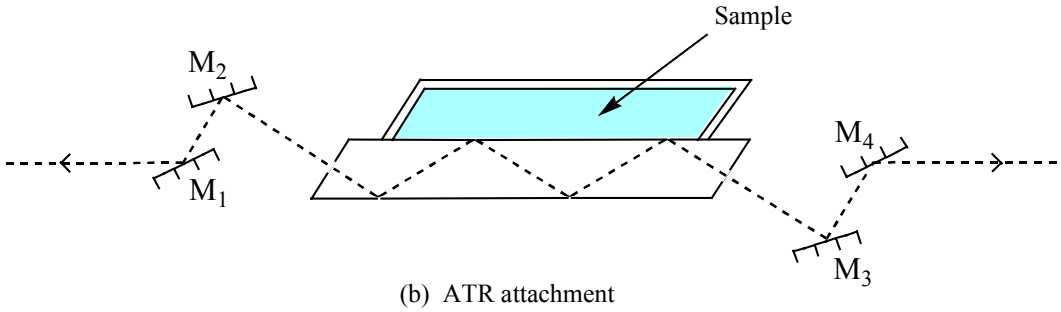
Attenuated Total Reflectance

Light arriving, at an appropriate angle to the boundary between two media (or materials) with appropriate refractive indices n_1 and n_2, can be reflected back into the first medium (see Fig 24(a)). This is known as internal reflectance. For this to happen the light beam must have at least sampled the second medium if only to a depth of ~10microns. The light in this fine slice of the second medium is referred to an evanescent wave. If the second medium has absorption properties this will be sensed by the evanescent wave and the reflected beam will have reduced (attenuated) intensity (ATR – attenuated total reflectance). The detected beam will now provide intensity versus wavenumber characteristics that are effectively the absorption spectrum of the second medium.

There are several proprietary attachments on the market based upon a single rhomboid prism, an example is illustrated in Figure 24. Suitable optical materials for medium one are zinc selenide (ZnSe), germanium and diamond. A sample well is created on the side of the rhomboid optical block. A typical ZnSe block is 50mm x 1 or 2mm giving 15 to 45 reflections depending on rhomb angles. In this case, 5 to 10 microns sections of medium 2 are sampled 7 to 22 times, giving an effective optical pathlength of the order 35 – 220 microns. The pathlength is reproducible and samples are easy to change in comparison to the equivalent simple transmission spacer cell although the latter may be preferred for simple solutions. Any material forming a good optical contact with the prism can in principle be measured (solutions, oils, waxes, creams, pastes, powders and films).



(a) Evanescent wave at a reflecting surface



(b) ATR attachment

Figure 24 : (a) Illustration of the evanescent wave sensing medium 2. (b) The ATR attachment with light directed by mirrors M_1 and M_2 entering the rhomboid prism at 90^o. In this illustration the sample is measured twice (two internal reflections) before the light exits the prism at 90^o and directed to the detector by mirrors M_3 and M_4. In practice, there can be as many as 45 reflections.

More sophisticated devices exist such as such the DuraSamplIR attachment (Fig 25) supplied by SensIR Technologies, Warrington, UK. The IR radiation from the interferometer is directed into a ZnSe prism by mirror M_1. (see Fig 25). Subsequent internal refection directs the IR radiation through a diamond prism where the evanescent wave is reflected back through the ZnSe prism and then onto the detector via mirror M_2. In principle, a powder sample, with NO sample preparation is placed on the diamond prism surfaces where it is compacted by a plunger. Powders, films, solutions …etc can all be measured with equal ease with no sample pre-treatment. This device produces excellent results; baselines are flat, as the technique is not dependent on light scattering.

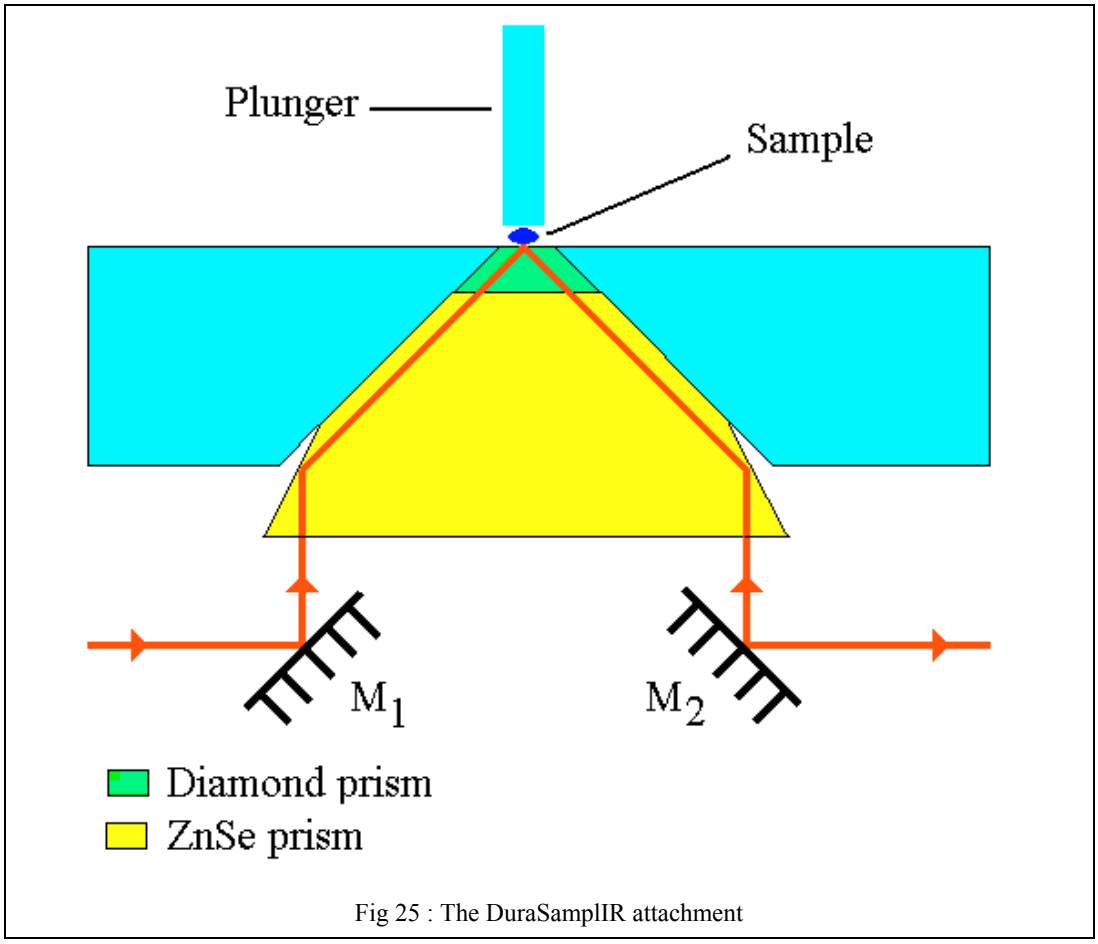


Fig 25 : The DuraSamplIR attachment

SAMPLE IDENTIFICATION AND THE ANALYSIS OF SPECTRA

A non-linear molecule has (3N-5) fundamental (normal) modes of vibrating. This excludes overtones and combinations. Thus a molecule such as Paracetamol (see Fig 28) with the formula $C_8H_8NO_2$ has (3x19-5)=52 fundamental (normal modes of vibrating). Assigning 52 peaks in an IR spectrum will be a daunting task at the very least. Therefore, the total molecular structure of a drug is unlikely to be determined directly from IR spectra information alone.

There are three aspects to identifying a chemical entity. In the first instance, the properties (biological, chemical and spectroscopic) of a drug will be assessed and the drug will be classified according to its type (eg non-steroidal inflammatory drug, barbiturate, steroid ….etc.). The analyte may be a previously characterised compound in which case a comparison of data from the unknown with reference data, often termed fingerprint identification, will confirm the identity of the compound. This may be possible with computer matching of spectra. The molecular structure of a new chemical entity will most likely need to be determined by NMR spectroscopy perhaps in combination with Mass spectrometry. However, information such as the existence of specific functional groups or the elimination of putative structures is a great help in processing the NMR information.

There are three broad applications of IR spectroscopy *viz*. functional group identification, fingerprint sample identification and concentration determination of various species in the sample.

Functional group identification

A small table listing important IR vibration frequencies is worth committing to memory

$\overline{\nu}$ cm^{-1}	Vibration
3600 to 2500	O–H stretch, broad, strong (prominent)
3400	N–H stretch, broad, strong (prominent)
3000	C–H aromatic stretch
2900	C–H aliphatic stretch
1800 to 1650	C=O stretch strong (prominent) Ester R–O–C=O, ~1740cm^{-1} Ketone C=O, ~1715cm^{-1} Carboxylic acid HO–C=O, ~1705cm^{-1} Amide H_2N–C=O, ~1650cm^{-1}
1300 to 1000	C–O stretch, strong
1800 to 400	Forest of vibrations – fingerprint region

Table 2 Important vibrations and IR frequencies

The precise location of a band will often give an indication of the structural environment of the group (*e.g.* C=O group in cyclohexanone and cyclopentanone, amide bond in an α-helix or β-sheet polypeptide chain). A more substantial Table is included as an appendix.

IR spectra matching and fingerprint identification

In the simplest case two spectra printouts, one of the reference and the other of the analyte can be overlaid on a light box and spectral features related by eye. Overlaying spectra on the computer screen achieves the same objective.

In recent years, infrared spectra databases have been created held in computer memory. The spectrum of the analyte is presented to the database and the computer attempts to match the spectrum with one already held in the database. A report is made of the best matches. The computer program will list the most likely hits in order of a *closeness of fit*. Many spectra compilations (databases) are private collections held typically by individual pharmaceutical companies, some can be purchased and a few are in the public domain.

The number of compounds whose IR spectra have now been measured is massive. Potentially, the greater the number of spectra in a database, the greater is the probability of making a good match for the unknown sample. However, the probability of making a mismatch is also greater as more spectra with fine differences are available for comparison. The computer is simply matching "pictures" covering the number of peaks, their positions and their relative intensities. The best the computer fitting can do is to indicate a mathematical similarity. It is important to qualify a computer search.

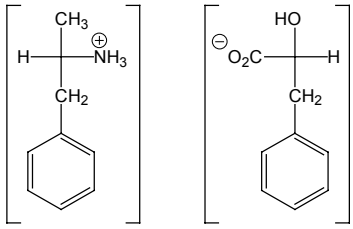
- A visual overlay of the test compound spectrum and the hit spectrum will ensure that the search has not chosen a match that is mathematically acceptable but chemically not acceptable.
- Knowledge of the class of a compound can help restrict the search to a more refined reference set (database).
- Other properties of the sample and the reference compound should match. These include factors such as chromatographic retention times, chemical and colour reactions and functional group assignments.
- The computer can only select spectra that are in its library and if the spectrum of the compound under investigation is absent, then it will select those giving the next best fit.
- Different forms of the same compound will give different infra-red spectra (different polymorphs, racemate/enantiomer, ionisation status, cations and anions).
- If the spectra have been recorded on different instruments then they may, superficially at least, appear very different. In this case a more detailed study of band frequencies and relative intensities must be undertaken.

If the matching procedure fails and in cases where the type of compound is unknown or can only be allocated to a certain class (e.g. a phenothiazine or a barbiturate), reference may be made to the index of Infra-red Peaks in Part 3 and

to the information in the individual monographs. Comparison of the spectrum of the unknown with that of the suspected compound should either confirm or disprove the tentative identification. If the two spectra have been recorded under similar conditions on the same type of instrument then they should be very similar in appearance.

The IR spectra of Amphetamines

The IR spectra of amphetamine base and the hydrochloride have many similarities, but the hydrochloride spectrum shows much finer detail (Figs 26A and 26B). The IR spectra of the hydrochloride and mandelate salts show differences (Figs 26B and 26C) due to the absorption of the mandelic acid. However, the spectra of the hydrochloride and sulphate salts (Figs. 26B and 26D) are very similar since they both have inorganic anions. The only major difference is the absorption band due to the sulphate at 1110 cm^{-1}.



Amphetamine Anion of Mandelic acid

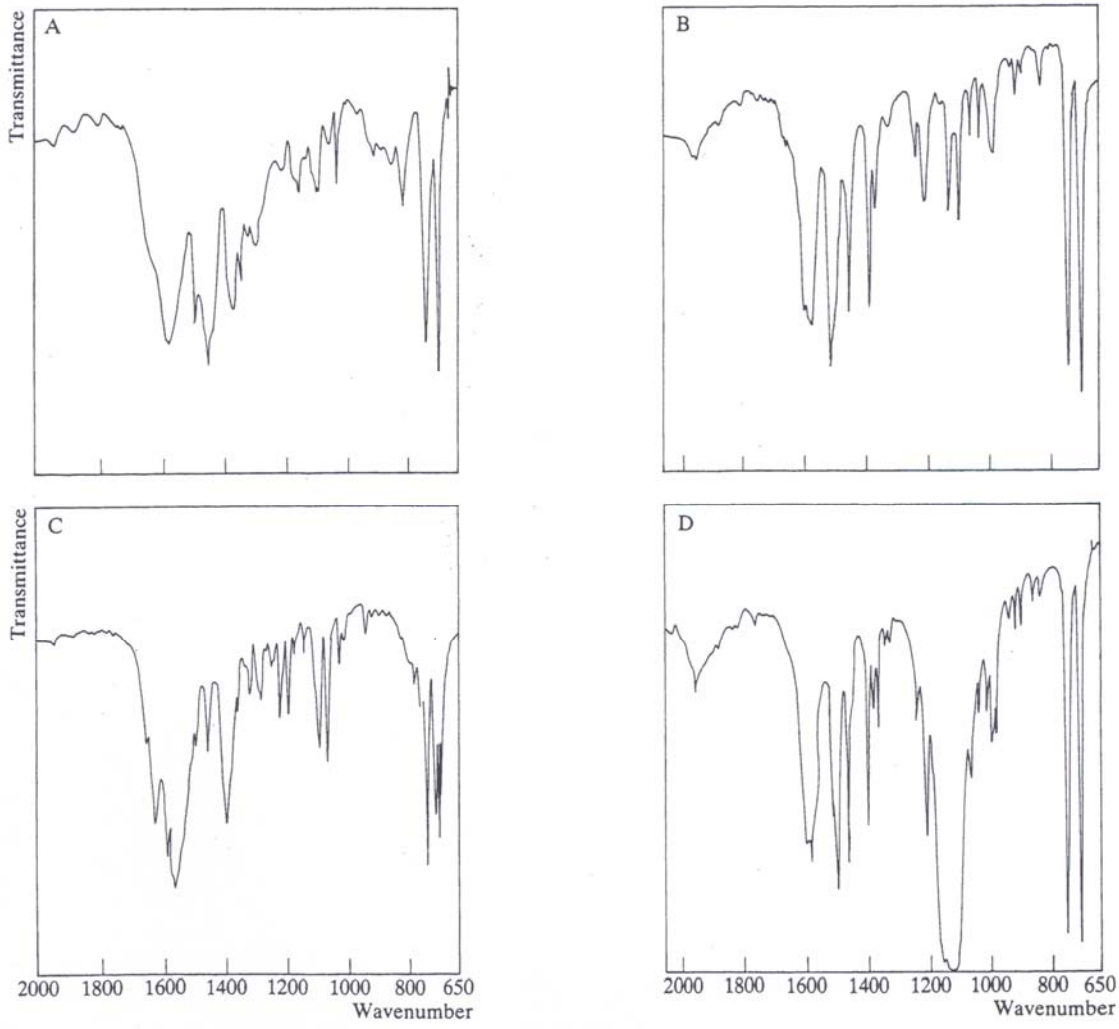
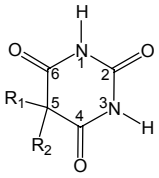


Fig 26 Infrared spectra of A, amphetamine base; B, amphetamine hydrochloride; C, amphetamine mandelate; D, amphetamine sulphate.

The IR spectra of Barbiturates

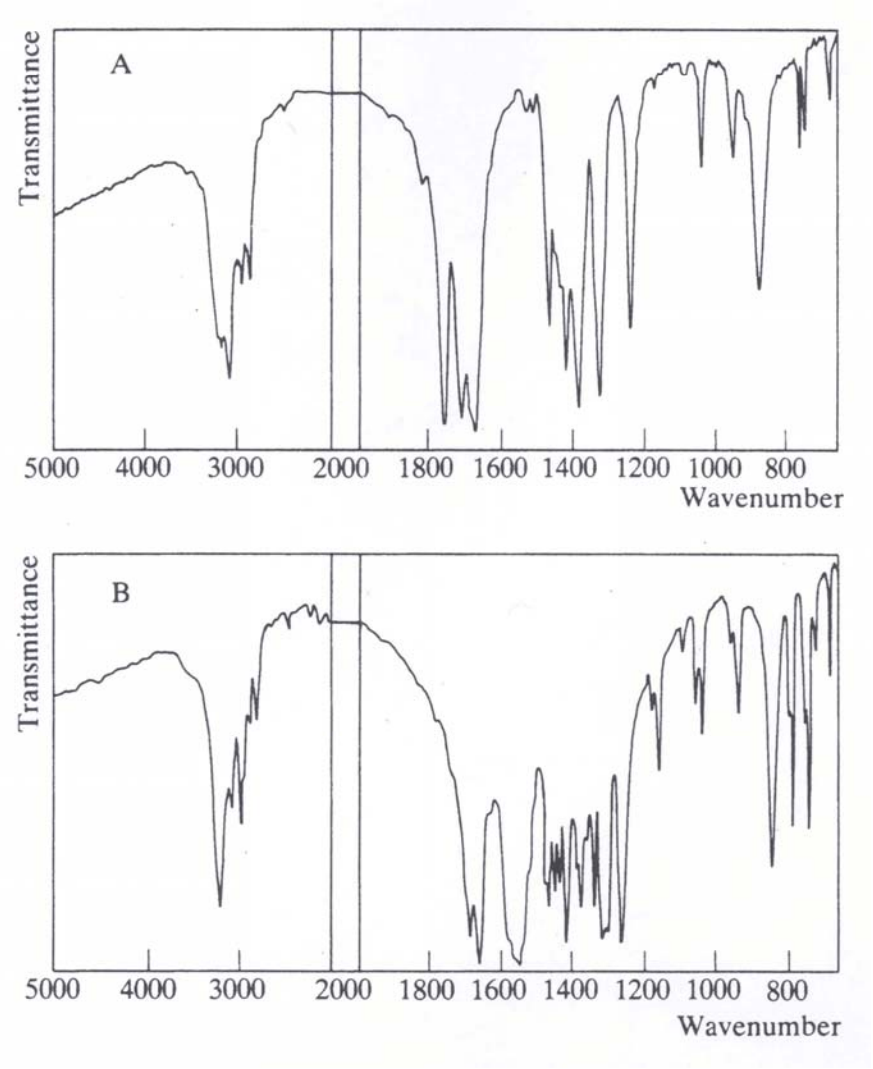
Important derivatives of malonylurea (barbituric acid) have two substituents at position 5. Others are also substituted at position 1 and in others the oxygen atom attached to position 2 is replaced by sulphur to form thiobarbiturates.

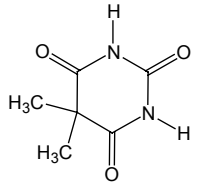


Malonylurea (barbituric acid)

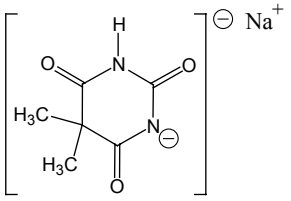
The barbiturates can be classified chemically into three classes: 5,5-disubstituted barbituric acids, 1,5,5-trisubstituted barbituric acids, and 5,5-disubstituted thiobarbituric acids. These classes can be further divided depending on whether the substituents in position 5 are alkyl, alkenyl, aryl, or cycloalkenyl. In most common barbiturates, one of the 5-substituents is either ethyl or allyl and the other is either a straight- or branched-chain alkyl or alkenyl group with five or fewer carbon atoms. Some barbiturates are available as sodium salts. The infrared spectrum of a barbiturate will therefore depend on the class of compound, the nature of the substituents, and whether it is the free acid or the sodium salt.

The various polymorphs of a compound often exhibit different infrared spectra. The barbiturates are notable for the extent to which they exhibit polymorphism, including many metastable forms found only in mixtures. Spectral differences between polymorphs are associated with different types of hydrogen bonding, and there is a correlation between hydrogen bond strength and duration of action of the barbiturates on the central nervous system. The crystalline structure of barbiturates can be affected by grinding with an alkali halide or in preparing a mull, but if precautions are taken to ensure reproducibility, the spectra of the barbiturates are sufficiently different to be used for identification purposes.
With the exception of phenobarbitone and barbituric acid, the free barbiturates do not absorb appreciably above 3300cm^{-1} (e.g. barbitone, Fig. 10A), a feature which distinguishes them from the ureides; a weak band of unknown origin occurs sometimes between 3500 and 3400cm^{-1}. All the barbiturates have two bands, which occur near 3200 and 3100cm^{-1} and are due to N–H stretching vibrations. In the 5,5-disubstituted compounds, the relative intensity of the two bands is similar although that at 3100 cm^{-1} is usually slightly less intense. In compounds substituted on the nitrogen atom at position 1, the intensity of the band at 3100 cm^{-1} may be greatly reduced and is often present only as a shoulder on the band at 3200cm^{-1}, e.g. metharbitone. Methylphenobarbitone appears to be an exception in that the band at 3100cm^{-1} is the most intense one in the region. A similar phenomenon occurs with the sodium salts since here again one of the hydrogen atoms in either position 1 or 3 has been replaced.





Barbitone



Barbitone sodium

Fig 27 Infrared spectra of A, barbitone and B, barbitone sodium

A series of up to four medium to intense bands occurs in the region 3000 to 2800cm^{-1}, and is due to alkyl C–H stretching vibrations of the substituents in positions 1 and 5. The intensity of the bands gives a very approximate indication of the number of C–H bonds and hence the number of carbon atoms in the chain. This does not appear to apply to the sodium salts, in which the band occurring at 3000 to 2950cm^{-1} is usually increased in intensity, compared to that of the free acid, and becomes the strongest band. Compare, for example, the spectra of barbitone (Fig. 10A) and barbitone sodium (Fig. 10B).

The barbiturates have up to three strong bands in the region 1765 to 1670cm^{-1} which are due to C=O stretching vibrations. Knowledge of the origin of these bands is useful in helping to understand the differences in the spectra of the various types of barbiturate.

In symmetrical molecules, the three bands are all of similar intensity. In asymmetrical molecules, the band at the highest frequency is often less intense than the other two, and this is particularly so when the molecule is substituted in position 1. The sodium salts of the barbiturates have only two bands in this region, since the molecule is no longer symmetrical, and these occur at a lower frequency, between 1700 and 1650cm^{-1}. In addition, a broad strong band occurs between 1600 and 1550cm^{-1}; the free barbiturates show practically no absorption in this region. The sodium salts of the thiobarbiturates exhibit only the lowest of the three C=O vibrations in the region 1700 to 1680cm^{-1}. They do, however, exhibit the broad, strong band which occurs between 1650 and 1600cm^{-1}. Therefore, the number, position and intensity of the bands between 1800 and 1500cm^{-1} give a very good indication of whether the barbiturate is the free acid, the salt, or a thiobarbiturate.

Most barbiturates have a number of strong bands between 1460 and 1250cm^{-1}, and some of these are due to C–H

deformation and C–N stretching vibrations. The sodium salts of the thiobarbiturates have a broad strong band between 1500 and 1480cm^{-1}, which is believed to be due to C–N stretching vibrations of the carbon atom attached to sulphur. This band is not present in the ordinary barbiturates and therefore provides another means of distinguishing those containing sulphur. Many barbiturates exhibit a few weak to medium intensity bands in the region 1150 to 900cm^{-1}. The 1-substituted barbiturates exhibit a greater number of sharp bands of medium intensity. Those compounds, which contain an allyl group, exhibit bands at about 1000 to 960cm^{-1} which are due probably to C–H deformation vibrations. The sodium salts of the thiobarbiturates show a band of medium intensity between 1020 and 1000cm^{-1}. Finally, many barbiturates, but not the thiobarbiturates, exhibit a broad band of medium to strong intensity between 900 and 800cm^{-1}.

IR spectra of Nujol, Paracetamol and Aspirin

The spectra of paracetamol, aspirin and the nujol mull agent are reported in Fig. 28 The figure illustrates the differentiation of N-H, O-H, ester, carboxylic acid, and amide. In particular the effect of nujol on the drug spectra is apparent.

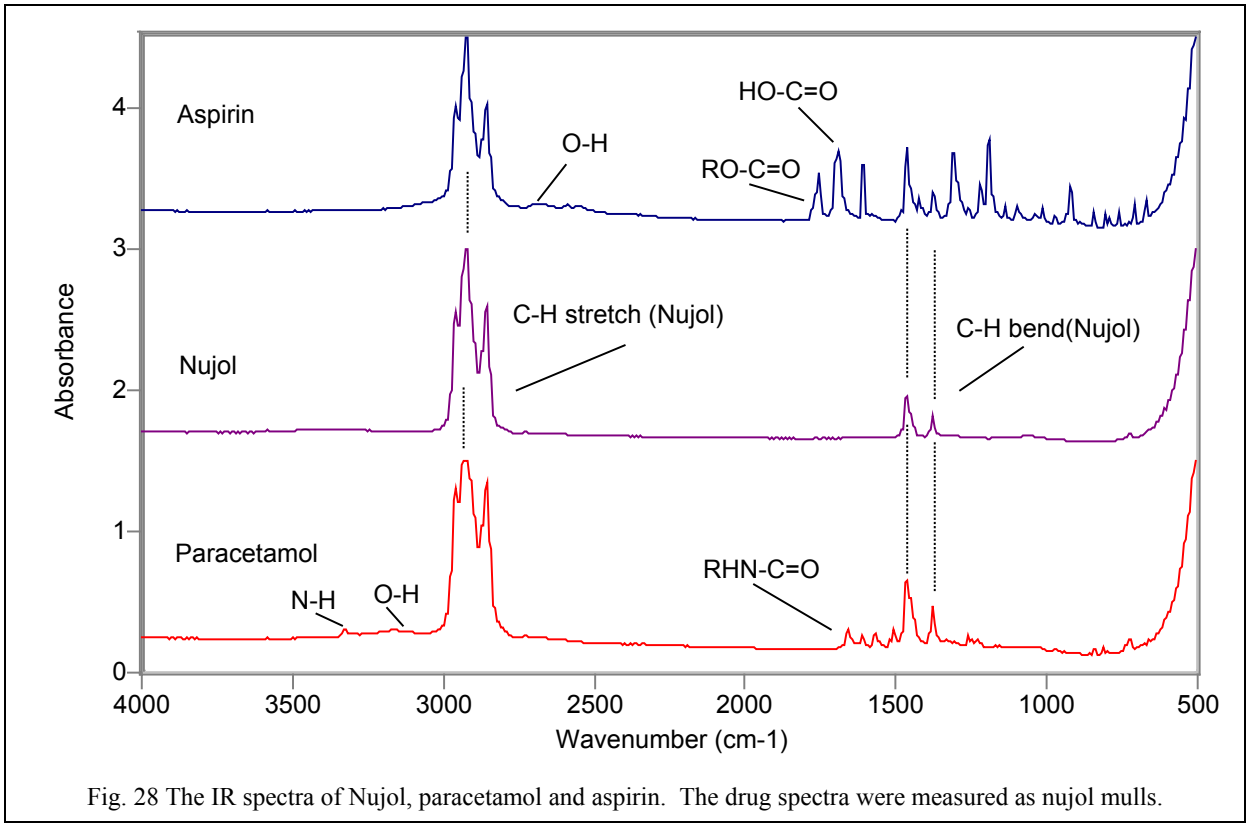


Fig. 28 The IR spectra of Nujol, paracetamol and aspirin. The drug spectra were measured as nujol mulls.

Interferences

Spurious bands can occur readily in infrared spectra, particularly when a biological sample has undergone several purification procedures. Traces of plasticisers, surfactants, and oils left on glassware can all give rise to spurious infra-red bands. A useful list has been compiled by Szymaski. (after H. A. Szymanski, *A Systematic Approach to the Interpretation of Infra-red Spectra*, Buffalo, New York, Hertillon Press, 1971).

Wavenumber (cm^{-1})	*Assignment*	*Comments*
3800–2500	H_2O	Bound or unbound water in a molecule can give rise to sharp or broad bands. In alkali halide disks a water band at 3350cm^{-1} may appear
3300–3000	NH_3^+	Lens tissues
1810–1600	C=O	Impurities containing the carbonyl group, e.g. phosgene in chloroform, plasticisers
1750–1500	H_2O	Bound or unbound water can give rise to sharp or broad bands
1610–1515	COO^-	Alkali salts (which also have a weaker band at 1425 cm^{-1}) can be produced from alkali halides
1400	NH_3^+	Lens tissues
1265	Si—CH_3	Stopcock grease or silicone oil
1110–1050	Si—O—Si	Glass or hydrolysed Si compounds
730 & 720	Polyethylene	Polyethylene laboratory ware
700	Polystyrene	Polystyrene laboratory ware

Infra-red Data in Monographs

Modern spectral identification by reference to computer databases involves sophisticated chemometric algorithms to compare all the digitised points in a test spectrum with a set of reference spectra. Spectra that are judged to be most similar are said to be a match allowing the identity of the test spectrum to be established. This type of work requires specialised database reference sets and computer programs. Much of this work is proprietary and related to the software of the spectrometer being used.

However, it has been shown (A. S. Curry *et al*., *J. Pharm. Pharmac*., 1969, *21*, 224–231; P. H. B. Ingle and D.W. Mathieson, *Pharm. J.*, 1976, *216*, 73) that an infra-red spectrum of a particular substance can be retrieved from a collection, with some degree of confidence, by reference to its six major absorption bands. This forms the basis for a system of identification.

Data consisting of six major absorption bands which have been selected from the recorded spectrum over the range 2000 to 650cm^{-1} (5 to 15µm) are included in the monographs in Part 2. In many cases, the spectrum is also reproduced in a reduced size. The selected peaks are the six most intense peaks, except that peaks in the region where Nujol absorbs (1490 to 1320cm^{-1}, 6.7 to 7.6µm) have been omitted. The peaks are arranged in descending order of amplitude. It should be noted that, because of variations in instruments and conditions, other determinations of the spectrum might not give peaks with the same relative intensities.

The principle peaks are also listed in descending order of amplitude in Part 3.

Concentration of Molecular Species

FTIR spectrometers are now very stable instruments and coupled with computer control and data manipulation should be as easy to operate as UV/Vis spectrophotometers. They can operate routinely in the absorbance mode, which is required for concentration determinations. However, relatively high concentrations are required given the restriction of solvent absorption, the need for narrow pathlengths and the low extinction coefficients of vibrations. Assuming Beer's law is obeyed, absolute concentrations can be determined in solution from specific bands in windows of solvent transparency. In the solid state, the relative amounts of two components can be readily estimated from the relative intensities of two specific absorption bands.

$$A_{1,\lambda_1} = \varepsilon_{1,\lambda_1}.c_1.l$$

$$A_{2,\lambda_2} = \varepsilon_{2,\lambda_2}.c_2.l$$

$$\frac{A_{1,\lambda_1}}{A_{2,\lambda_2}} \propto \frac{c_1}{c_2}$$

where A_{1,λ_1} , A_{2,λ_2} , $\varepsilon_{1,\lambda_1}$, $\varepsilon_{2,\lambda_2}$ are respectively the absorbances and extinction coefficients of species 1 and 2 at the corresponding wavelengths λ_1 and λ_2. The concentration of species 1 and 2 are c_1 and c_2, the pathlength is *l*.

Published Spectra

GENERAL COLLECTIONS

Compilations of IR spectral data are available in two forms either as pictures or as digital absorbance/wavenumber computer data sets in databases. Pictures are available in book or computer form and are suitable for visual inspection. Spectral characteristics can be determined with the aid of a ruler. Computer databases are available in computer memory for data manipulation and spectral matching. Third party software for the computer manipulation of IR spectral data include :
GRAMSAI® version 7 and Spectral ID® available from Thermo Galactic, 395 Main Street, Salem, NH 03079 USA.
Aldrich® Spectral Viewer™ available from Sigma-Aldrich Company Ltd., The Old Brickyard, New Road, Gillingham, Dorset, UK SP8 4XT or their web site http://www.sigmaaldrich.com.
Thermo Nicolet OMNIC® FT-IR available from Thermo Nicolet Corporation, 5225 Verona Road, Madison, WI 53711-4495, United States.
Other instrument manufactures can provide suitable software.

Sadtler Collection. The most comprehensive collection of published spectra is that of the Sadtler Research Laboratories. This is available through Bio-Rad, Informatics / Sadtler group, 3316 Spring Garden Street, Philadelphia, PA 19104-2596, USA. The total database covers 220,000 spectra which are divided into specialized groupings : Polymers and Related Compounds, Pure Organic Compounds, Industrial Compounds, Forensic Sciences, Environmental Applications ,Inorganics and Organometallics. Sub-groups are preferred to ensure more secure compound matching. Bio-Rad also supply appropriate database software.

The Chemical Rubber Company collection of 12000 spectra: J. G. Grasselli and W. M. Ritchey, *CRC Atlas of Spectral Data and Physical Constants for Organic Compounds*, 2nd Edn, CRC Press Inc., USA, 1975 is now out of print and has been replaced by :
CRC Handbook of Data on Organic Compounds : D.R Lide and G.W.A. Milne, CRC Press Inc., USA, 1993 ISBN: 0849304458

A collection of 3000 FT-IR spectra of standard chemical compounds available from E. Merck, Darmstadt, with:

- Absorbance spectra are presented, hereby breaking with tradition, with the advantage that band intensities are directly proportional to concentration.
- Spectra of solid compounds are recorded in a KBr matrix. Hence, no interfering peaks from the matrix are included.
- All spectra are augmented with the following data: list of nine strongest bands, structure, molecular formula, molecular weight, melting and boiling points, CAS registry numbers, sample preparation and Merck-Schuchardt catalog number.

Merck FT-IR Atlas: Eine Sammlung von FT-IR-Spektren Teil I/II (A Collection of FT-IR Spectra Part I/II), H-U Gremlich, K.G.R. Pachler, F. Matlok, ISBN 3-527-26459-0, John Wiley & Sons Inc, (Year 1988).

The Sigma-Aldrich company offer several collections of IR spectral data from printed books to computer based systems. The details of Sigma-Aldrich products are available from Sigma-Aldrich Company Ltd., The Old Brickyard, New Road, Gillingham, Dorset, UK SP8 4XT or their web site http://www.sigmaaldrich.com.

The Aldrich Library of FT-IR Spectra, 2nd ed., 3-volume set, 1997 (ISBN 094163339X), 1997, contains over 18000 IR spectra.

The Aldrich Library of FT-IR Spectra: Vapor Phase, Volume 3, (ISBN: 0941633195) C.J. Pouchert, Aldrich Chemical Co., Milwaukee, WI, 1989, contains 6,550 vapor phase spectra.

The Aldrich Library of Infrared Spectra, 3rd Ed. (No ISBN) C.J. Pouchert, Aldrich Chemical Co., Milwaukee, WI, 1981, containing more than 12,000 spectra classified by functional group and structural presentation.

A set of over 10,400 FT-IR spectra of biochemicals and related organics. : *Sigma Library of FT-IR Spectra, Volumes 1 and 2* , R.J. Keller, ISBN: 0-941633-10-1, Sigma Chemical Company, St. Louis, MO, 1986, Product number S2639

The computer based systems include the IR collection of over 1100 compounds in the Aldrich® Spectral Viewer™, Product Number: Z54028-5, (Year 2002).

667 spectra of commercial materials with frangrance or flavor-enhancing properties are included in the *Nicolet/Aldrich Flavor & Fragrances Vapor Phase Library*, Product Number Z27,303-12003.

5,010 gas phase spectra collected by Aldrich using a GC interface to ensure chromatographically pure samples : *Nicolet/Aldrich Vapor Phase Library,* Product number Z27,296-5

A spectral collection of 10,411 of the most common chemicals found in the Sigma Chemical catalog representing a wide range of chemical classes of particular interest to those in biochemical research or QC *: Nicolet/Sigma Biochemical Condensed Phase Library*, Product Number Z27,297-3

Collection of 3,011 spectra of a wide variety of natural and synthetic steroids organized by skeletal type and degree of substitution. The steroid spectra were acquired by Sigma and the United Kingdom Medical Research Council : *Nicolet/Sigma Steroids Condensed Phase Library*, Z27,298-1

Coblentz Society Collection. A library of over 900 spectra: *The Coblentz Society Desk Book of Infra-red Spectra*, The Coblentz Society, Kirkwood, Mo., USA,. Manager for Solicitation and Collection of Spectra : K.S. Kalasinsky, Armed Forces Institute of Pathology, Division of Forensic Toxicology, 1413 Research Blvd., Rockville, MD 20850, USA.
Computer based collections are also available

British Pharmacopoeia Collection. A collection of over 400 spectra, Volume II of the British Pharmacopoeia 2001 (London : The Stationary Office).

Fiveash Data Management Inc. offers software and reference spectra databases through their web site http://www.fdmspectra.com. The databases include the Fluka FTIR collection.

The spectra of 1044 organic compounds are presented as pictures in the *Raman/Infrared Atlas of Organic Compounds*, 2nd Edition, B. Schrader, ISBN: 3-527-26969-X, John Wiley & Sons Inc. 1989.

Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules : D. Lin-Vien ,N.B. Colthup, W.G. Fateley , J.G. Grasselli , ISBN 0124511600, Academic Press, 1991

The Handbook of Infrared and Raman Spectra of Inorganic Compounds and Organic Salts (4 Volume Set) : R.A. Nyquist , R.O. Kagel , C.L. Putzig , M.A. Leugers , ISBN 0125234449 ,Academic Press 1996

NIR, IR, Raman, and UV-Vis Spectra of organic compounds, polymers and surfactants are presented in the *Handbook of Organic Compounds* (3 Volume Set) : edited by J. Workman, Jr., ISBN 0127635602, Academic Press, 2000.

Tables and charts of IR Spectra can be obtained from :
Infrared and Raman Characteristic Group Frequencies: Tables and Charts, 3rd Edition, G. Socrates, ISBN: 0-471-85298-8, John Wiley & Sons Inc, 2001.

SPECIALISED COLLECTIONS

Alkaloids. 1000 spectra with other physical data: J. Holubek and O. Strouf, *Spectral Data and Physical Constants of Alkaloids*, London, Heyden and Sons Ltd, 1965–1973.

Antibiotics. A comprehensive collection published by the International Centre of Information on Antibiotics: C. Lenzen and L. Delcambe, *ICIA Inf. Bull.*, 1972, *10*, 78–160; 1973, *11*, 1–157; 1975, *12*, 1–178; 1976, *13*, 1–178; 1977, *15*, 1–208; 1977, *16*, 1–212; 1978, *17*, 1–248; 1979, *18*, 1–268.

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