Infra–red (IR) spectroscopy is the study of the scattering, reflection, absorption or transmission of IR radiation in the spectral range 800 nm to 1 000 000 nm (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:

The IR spectrum can be divided into three sub–regions, 12 500 to 4000 cm−1 (0.8 to 2.5 µm; near IR), 4000 to 400 cm−1 (2.5 to 25 µm; mid IR), and 400 to 10 cm−1 (25 to 1000 µm; far IR). Only the mid IR region (often referred to simply as infra–red) is considered here because it is the region widely used in the analysis of drugs and pesticides. However, some instruments scan from 5000 to about 200 cm−1; the extension to the far IR is useful for halogenated compounds and for inorganic substances.

The energy associated with electromagnetic radiation is given by Planck's equation:

where E is the energy, h is Planck's constant, C is the velocity of light and λ is the wavelength of light.

IR radiation can 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. Provided the incoming IR radiation has the appropriate energy (wavelength, wavenumber), resonant absorption occurs to excite the molecule to a particular higher vibrational state. Vibrational transitions give rise to an absorption spectrum characteristic of the compound. Several factors characterise this absorption spectrum: the number of absorption features and their associated wavenumbers, the strength (intensity) of the absorption features and the sharpness of these features.

Sommaire

Energy and wavenumber of infra–red absorption by molecular vibration

Molecules can undergo two types of vibrations, namely stretching vibrations that involve changes in bond length and bending vibrations that involve changes in bond angles. The vibrational modes associated with the methylene CH2 group are illustrated in Fig. 22.1.

Molecular vibrations of the methylene CH 2 group (courtesy of Pavia et al. 1996).

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 to 400 cm−1 (mid IR) to become excited. Not all vibrations in a molecule are assignable; generally, only the most prominent are readily assigned to a given vibrational mode. These characteristic vibrations are a good way to detect the existence of functional groups in a chemical compound. The precise wavenumber at which 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 are given in Table 22.1.

Important vibrations and IR frequencies

In addition, there are overtones (the excitation of a vibration to a double or higher frequency) and combinations that are the sum or difference of two or more fundamental bands. No fundamental vibrations require energy greater than 4000 cm−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 (e.g. Williams and Fleming 1995, and others listed in the Further reading).

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 400 cm−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 assigned directly 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.

Strength of molecular vibration absorption

Traditionally, an IR spectrum is reported as a plot of percentage transmittance (T%) against wavenumber $\tilde{\nu}$. The IR transmission spectrum of a polystyrene film used to calibrate the wavenumber scale is given in Fig. 22.2.

Transmission spectrum of a polystyrene film.

The absorption of light in Fig. 22.2 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. 22.3).

Transmission of light by a sample.

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

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:

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

The stretches of symmetrical bonds, such as H–H (hydrogen gas), –C–C– (ethane), O–O (oxygen) and N–N (nitrogen), do not have a transition dipole moment and therefore ε→0 and these stretches are not observed and are said to be forbidden. Similar vibrations in complex molecules do absorb, but extremely weakly. On the other hand, gases such as NO, NO2, CO, CO2, SO2, CH4 and H2O (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 1 m or more). Water vapour and CO2 present in the normal atmosphere produce absorption effects in an IR spectrometer in the absence or presence of a sample. High–precision work therefore benefits from flushing with dry air or nitrogen. The water vibrations centred at 3782 cm−1 and 1587 cm−1 show a fine structure associated with the rotations of the water molecule.

The actual movement of charge during a vibration is in practice very small and a value of ε of approximately 200 is a practical upper limit. In tables of IR data, 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). For older designs of instrument the measurement of IR intensity was unreliable, which made 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 is detected only for concentrated solutions with an absorption that may be too strong for spectral regions in which ε is larger. The molar extinction coefficient, ε, is largely responsible for setting the sensitivity of an analysis.

Width of an infra–red absorption band

In ultraviolet (UV) and visible 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 also to excite associated vibrational and rotational states, and the absorption profile is broad. The UV–visible spectrum is characterised by only a few broad features. A typical half–height width of a UV–visible absorption is between 2000 and 5000 cm−1.

The IR spectrometer sees the excitation of bond vibrations and associated rotational motions. The IR spectrum comprises many relatively sharp features that provide an excellent fingerprint for identification. A typical half–height width of an IR absorption band is 10 to 20 cm−1.

Instrumentation

Dispersive spectrometers

Conventional spectrometers start with an appropriate light source 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 Io and the transmitted intensity It need to be measured at every wavelength.

Single–beam dispersive spectrometers

With a single–beam instrument, the Io spectrum is measured first with air (or solvent) as a reference in the light beam. In a separate measurement the It spectrum of the sample is recorded. 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 Io does not drift.

Nowadays, single–beam dispersive IR spectrometers are likely to be found only in monitoring processes (e.g. 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 Io and It in separate measurements. The layout of a typical double–beam spectrometer is illustrated in Fig. 22.4. Dispersive instruments in the IR have the sample located next to the light source before the monochromator, unlike their UV–visible counterparts.

Double-beam dispersive IR spectrometer.

IR light from the source, typically an electrically conducting element such as a Globar maintained at about 1000 K,

illuminates equally two mirrors M1 and M2. The light from mirror M1 acts as the reference beam; the light from M2 is the sample beam. Mirrors M3 and M4 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 that passes through the system. In the reference–beam chopper period the detector registers Io and in the sample–beam chopper period It is measured. The alternating signals are amplified and their ratio calculated to give It/Io = T or log(Io/It) = A. The preferred detector, certainly by the 1980s, was deuterated triglycine sulfate (TGS). This is a pyroelectric detector with an electrical resistance 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 (e.g. from solvents). The measurement cell pathlengths in the reference and sample beams must be identical. A computer interfaced with the spectrometer enables post–measurement subtraction of a reference spectrum from the sample spectrum.

The amount of polychromatic IR light that hits the detector from the natural, black–body, room–temperature radiation of the cell compartment walls can be 10 fold or more than the desired monochromatic IR radiation from the reference or the sample. This background radiation is stray light that severely affects the accuracy of the Io/It 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.

Interferometric spectrophotometers

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

The FTIR spectrometer incorporates an interferometer in place of a monochromator (Fig. 22.5). The way a FTIR spectrometer operates is presented here with reference to Fig. 22.5:

Layout of an IR interferometer (FTIR spectrometer).

• If the mirror M1 is set to oscillate along the optic axis, the distance travelled by beam (B + D) varies, while the distance travelled by (C + E) remains unchanged.
• Identical (B + D) and (C + E) distances means that D and E are in–phase and recombination is fully constructive. As mirror M1 moves towards the beam splitter, the beam D arrives 'ahead' of beam E; recombination is not fully constructive and the intensity of beam F is reduced and its phase changes. Eventually the mirror movement of M1 leads to beam D being a half wave ahead of beam E. The recombination is now fully destructive and the intensity of beam F becomes zero. At this point, the movement of M1 is reversed past 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.
• As the mirror moves back and forth through a single mirror oscillation period, the intensity of a single wavelength of IR light varies considerably. In practice, all wavelengths are passing through the system simultaneously. Therefore, the total IR light intensity registered as falling on the detector during a single mirror oscillation period is very complicated and takes the form illustrated in Fig. 22.6.
• The signal illustrated in Fig. 22.6 is now subjected to a mathematical procedure called a Fourier transform. This extracts the light intensity versus wavelength (wavenumber) information.
• With no reference or sample in the beam, this measurement is the background or Io spectrum, which is stored in the computer to be used as the Io for all subsequent transmittance (It/Io) or absorbance [log(Io/It)] measurements during the working session.
• 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 a sample, light–intensity throughput spectrum.
• Subsequent data manipulation in the computer produces the transmission or absorption spectrum of the sample.

Interferogram produced by a single mirror oscillation.

Technical details

• The IR source is a Globar or similar proprietary ‘hot’ element that operates at approximately 1300 K. The detector needs to have a fast response and have low inherent noise. The TGS 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 approximately 32°). 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).
• FTIR spectrometers have advantages over dispersive instruments. The interferometer offers greater light collection and throughput. More light means less noise and greater sensitivity. The 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 for the dispersive instrument can be spent 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 min. Taking 10 min over an FTIR measurement allows the averaging of very many scans. The signal–to–noise (S/N) ratio in an IR spectrometer is proportional to √(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 or 32 fold, respectively.
• The level of stray light associated with FTIR spectroscopy is low, typically less than 0.02% because the technique is devoid of imperfect gratings and the signals selected are associated only 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.
• Resolution is generally excellent over the whole spectrum, with an effective measurement spectral bandwidth (SBW) of 1 cm−1 being achieved readily.

Data processing

All modern IR spectrometers are computer controlled, with measured data stored digitally. Computer control simplifies the process of running instruments and allows the easy implementation of standard operating procedures (SOPs). Although software is usually manufacturer specific, companies such as Galactic Industries among others have produced software (GRAMS/AI from Thermo Galactic, 395 Main Street, Salem, NH 03079, USA) that operates many instruments and certainly accepts data from effectively all spectrometers on the market.

Computers readily allow changes between spectral units. In the early days, the IR spectrum of a compound was reported as percentage transmittance as a function of wavelength in microns; by the 1970s 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 and an improvement in sensitivity. For example, there is little difference between the spectra of carbon disulfide and of benzocaine in carbon disulfide (Fig. 22.7), but with spectrum manipulation, a good spectrum of benzocaine is obtained readily (Fig. 22.8). The amount of benzocaine in the cell was approximately 4 µg but only about one quarter of this was in the IR beam. Each spectrum was recorded in less than 30 s.

Figure 22.7. IR spectrum of A, benzocaine in carbon disulfide; B, carbon disulfide; C, the difference spectrum (A – B) (courtesy of Perkin-Elmer Ltd).

Figure 22.8. The difference spectrum of benzocaine shown in Fig. 22.7 smoothed, corrected for baseline contribution and with percentage transmittance scale expanded 200 times (courtesy of Perkin-Elmer Ltd).

Digitised spectra can be corrected easily for solvent absorption or the presence of impurities. Various mathematical procedures can be applied, which include baseline corrections and 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 are assisted greatly 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.

Six factors need to be considered when calibrating an IR spectrometer:

- wavelength (wavenumber) scale
- absorbance scale
- stray light
- spectral resolution
- data resolution
- time scale of measurement (time constant).

Wavelength (wavenumber) scale

A card carrying an accredited transparent film of polystyrene (0.04 mm in thickness) is used to calibrate the wavelength (wavenumber) scale.

Absorbance scale

Unfortunately, there are no internationally recognised standards for IR spectra. The absorbance scale is set in the factory with the aid of complicated optics. Filters are available in the 4000 to 2000 cm−1 range. The high background blackbody radiation that falls on the detector from sources other than the sample means the ideal absorbance for good signal–to–noise is approximately 0.4.

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. All the relevant light at this wavelength (wavenumber) has been effectively absorbed.

Spectral resolution (spectral bandwidth)

In dispersive instruments, the SBW 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 1 cm−1, 2 cm−1 and 4 cm−1 available in the spectrometer control software. The narrower the SBW the more faithfully are sharp absorption bands registered; however, the noisier the spectrum (less light), the more time (accumulations) is required to keep the noise level down.

The British Pharmacopoeia (British Pharmacopoeia Commission 2002) recommends the following to ensure good spectral resolution:

Record the spectrum of a polystyrene film 0.04 mm in thickness. The difference x between the percentage transmittance at the transmission maximum A at 2870 cm−1 and that at the transmission minimum B at 2849.5 cm−1 should be greater than 18. The difference y between the percentage transmittance at the transmission maximum C at 1589 cm−1 and that at the transmission minimum D at 1583 cm−1 should be greater than 12.

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

Data resolution

In a computer, spectra are stored as lists of wavenumber and transmittance (absorbance) data pairs. These X,Y data pairs are plotted on demand. A sufficient number of data points are required to give an undistorted picture. Too many points may be unnecessary and require excessive memory allocations.

The terms spectral resolution and data resolution are often confused. Some instruments, in modifying the Fourier transform mathematics to reduce the SBW, also produce a change in the data resolution of the spectra computed. The 1154 cm−1 band of polystyrene is reproduced measured with different SBW and data resolution. In general, for typical drug molecules, a SBW of 4 cm−1 is probably sufficient to describe faithfully the natural bandwidth of a vibrational absorption band. However, a data resolution of 1 cm−1 is preferred to ensure that the measured data are a faithful representation of the capability of the spectrometer. Increasing SBW, reducing data resolution, or both, can reduce noise.

Time scale of measurement (time constant, averaged scans)

In analogue dispersive instruments, the scan speed refers to the rate of rotation of the monochromator diffraction

grating, which in turn controls the rate–of–change of the wavelength (wavenumber) that comes from the monochromator exit slit. The instrument has an inherent controllable response time (time constant). Scanning too fast means that measured peaks are 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 and yet the longer the time required to measure spectra. The choice of time response versus scan speed is sample dependent and needs 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).

Sample preparation

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). By their nature, these samples are often not chemically pure and IR spectroscopy can 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 IR 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 FTIR 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) spectrometry 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 spectroscopy 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. When the starting material is a residue from the evaporation of a solvent extract of urine, blood, tissue or other material, the most suitable method of purification is some form of chromatography.

Thin–layer chromatography

Suitable systems for thin–layer chromatography (TLC) are described. 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 IR 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 UV light, it could be applied to the thin–layer plate twice and only a portion of the chromatogram sprayed, which thus allows the unsprayed portion to be eluted.

The use of aqueous acid or alkali to elute the compound from the thin–layer plate, 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 onto a wall of potassium bromide (KBr) built around the tip of the spot. The KBr is then pressed into a disk. This technique is only suitable for well–resolved spots. Elution of the spot sideways reduces contamination from compounds that are not resolved as well. The recovery of material from chromatograms varies from nil to over 70%. Compounds that contain 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 KBr. 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 10 µg of compound is required to produce a satisfactory spectrum. The advantage of this technique is that the lower part of the KBr 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 KBr 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 (10 mg) of dry KBr 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 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:

- Use the minimum amount of the purest adsorbent available.
- Elute with less than 1 mL of a solvent that contains <0.0001% (1 ppm) of non–volatile residue.
- Keep sample handling to a minimum.
- Clean all glassware with an efficient detergent in an ultrasonic bath.
- Avoid contact of materials and samples with plastics.

Gas chromatography

Gas chromatography can provide a very convenient method of obtaining pure samples for IR spectroscopy. However, the sample can still be contaminated with impurities eluted from the stationary phase. The effluent from the chromatograph is a hot vapour and the problem is to obtain small quantities in a form suitable to present 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 IR 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 IR 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 to trap a compound depends 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 to present to the spectrometer are given below. 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.

Cooled tubes

Most techniques to collect the effluent employ cooled tubes of glass or metal, but it is difficult to obtain good recoveries of a few micrograms of compounds of different volatilities by any one technique. Drugs such as the 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 the amfetamines, need to be cooled in liquid nitrogen or

solid carbon dioxide (Curry et al. 1968; De Leenheer 1972).

Alkali halide tubes

A straight tube that contains 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 that are solid at room temperature.

High performance liquid chromatography

High performance liquid chromatography (Chapter 29) provides a very convenient method of purification, particularly if gas chromatography is either inapplicable or 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 to present to the spectrometer depends upon whether the compound is a solid or a liquid and, if the latter, on its volatility and the quantity present. All the common solvents absorb in the IR region. However, with the data–processing facilities of modern IR 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. In many cases, however, the amount of material is too small to enable the compound to be collected and transferred to standard cells.

Alternatively, the compound can be recovered by evaporation of the solvent. However, evaporation also concentrates any non–volatile impurities in the solvent, so 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. Drugs may be sublimed from an evaporated solvent extract in the tube onto the cold finger of the apparatus, and the sublimate transferred by grinding the KBr powder gently with the cold finger.

Sample presentation

IR spectra can be measured in the gas, liquid or solid phase. However, most compounds of interest are solids at room temperature. In principle, an IR spectrum can be obtained from as little as 1 µg of a compound. From a practical point of view, quantities of the order of 200 to 1000 µg 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 microcell 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 with bonds that do not have vibrations. Crystal lattice vibrations cause absorption in the far IR. Barium fluoride (and calcium fluoride) are excellent for aqueous media, although window absorption prevents measurements below 1000 cm−1. Silver chloride windows are water resistant, will allow transmission down to 400 cm−1, but are friable. The most popular IR window material is sodium chloride, which is water soluble but transparent down to 600 cm−1.

Gases

In normal laboratory experiments, IR spectroscopy is used only rarely 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 sodium chloride 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

Liquids have a very high molarity. Thus chloroform, with a relative molecular mass of 119.4 and density 1.48 g/mL, can be said to be 124 M. According to Beer's law, to detect the C–H stretch ($\varepsilon \approx 10$) with absorption A = 0.4, the pathlength required, given by $l = A/(\varepsilon c)$, is approximately $3 \times 10–4$ 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 (<0.1 mm) measurement pathlength. Volatile liquids may need proper sealed liquid cells.

Liquid cells usually consist of two parallel transparent windows (0.1 to 0.01 mm apart) separated by a precise gasket made of Teflon or lead and fitted with inlet and outlet ports. Cells with variable pathlengths 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 0.01 mm 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. The measurement of the IR absorption of a solute is only possible in a spectral range for which the solvent is relatively transparent. Carbon tetrachloride and carbon disulfide, which lack hydrogen and contain a minimal number of bond types, are often suggested as the most useful solvents because they have relatively few absorption bands in the IR 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. Chloroform–deuterochloroform, acetonitrile, water–deuterated water, toluene and 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 caused by intermolecular hydrogen bonding. Interactions between the compound and the solvent can occur, which 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 and require very short pathlengths. This combination can lead to solvent evaporation, which produces large 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 placed after the interferometer. An example is presented here of the determination of the amount of dimeticone in a cream formulation.

Dimeticone 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 1260 cm−1 in a 0.1 mm sodium chloride liquid cell.

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 more than 1/20th 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 less than 1 µm. The Christansen effect, which results from severe refractive index changes at the sample surface, leads to distorted band shapes. Peaks take on an S-shape with an apparent reduced (or even negative) absorption at the longer wavenumber edge. As a result, asymmetric bands may be observed that 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 μm) and they must be dry.

The polymorphic form of a solid sample can affect the IR spectrum. This is an important issue in the pharmaceutical industry, as the rate of dissolution of a solid drug can depend upon its crystal morphology. Success has been achieved by simply crushing the sample between two diamond windows in a device known as a Diamond anvil.

Mulls

Solid compounds are dispersed in a liquid, such as 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 KBr, and another plate placed on top, taking care to exclude air bubbles. The plates are pressed together strongly. A disadvantage of this method is that the spectrum of the mulling agent is 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 hexachlorobutadiene, 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, KBr was used and the technique is still often referred to as the ‘KBr technique’. However, potassium chloride (KCl) is superior to KBr because it is less hygroscopic. Storage of the alkali halide in an 80° oven helps to ensure anhydrous conditions.

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

If only small quantities of the compound are available (about 200 μg), 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 0.5 mm can be prepared by using metal (lead or stainless steel) disks

of 13 mm diameter with a hole of the appropriate size in the centre. The hole is filled with KBr (about 1 mg) that contains 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, as this causes 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 KBr particles is picked up on the end of the needle by a trace of chloroform expressed from the needle. The solvent is evaporated gently and the rest of the solution is fed into the KBr from the syringe as it evaporates. A disk is then made from the powder. It is important that the end of the needle be 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.

KBr is hygroscopic which means it is sometimes difficult to remove the last trace of water, and so silver chloride may be used instead. An indentation about 0.8 mm deep and slightly wider is made in the centre of a small piece of silver chloride sheet, and a solution (about 0.1 µL) that contains as little as 500 ng 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 80° for 30 to 60 min with an IR 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:

- Formation of anhydrides from carboxylic acids.
- Ketals and cyanohydrins revert to the parent ketone.
- Loss of water from secondary alcohols.

Several disadvantages are 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 that contain 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 that chemical changes will occur during the preparation of the disk. For example, double decomposition can occur:

Hence, preferably hydrochlorides should be examined in KCl. Bromide may be oxidised to bromine by some compounds, particularly strong oxidising agents, which may result in 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 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 that are made of thallium bromide and thallium iodide as they appear to react with the plates.

Despite these disadvantages, the technique is still very useful 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 if it is necessary to obtain spectra free from the dispersing media. The film can be prepared either by melting the solid and pouring it onto a suitable plate or by evaporation of a solution on an IR transparent plate.

Measurement of strongly absorbing or strongly light scattering samples

IR light incident on solid, powders or other materials such as creams is transmitted only poorly, if at all, because of the 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 and these techniques can be used to examine intact pharmaceutical preparations.

Light scattering

IR light falling on a powder can be reflected in two ways. The light can be reflected truly 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 are 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 IR Fourier transform spectroscopy). The sample is placed on a sample tray located beneath two ellipsoidal mirrors M3 and M4. Heterogeneous powders and fibres often benefit from being ground and 'diluted' with KBr. Pure KBr can 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 approximately 150 µg over a 35 mm2 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–Munk transformation can be applied (Kubelka and Munk 1931).

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

Attenuated total reflectance

Light that arrives at an appropriate angle to the boundary between two media (or materials) with appropriate refractive indices n1 and n2 can be reflected back into the first medium. 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 approximately 10 µm. The light in this fine slice of the second medium is referred to as an evanescent wave. If the second medium has absorption properties this is sensed by the evanescent wave and the reflected beam has a reduced (attenuated) intensity, the attenuated total reflectance (ATR). The detected beam now provides intensity versus wavenumber characteristics that are effectively the absorption spectrum of the second medium.

Several proprietary attachments on the market are based upon a single rhomboid prism. Suitable optical materials for medium 1 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 50 mm × 1 or 2 mm, which gives 15 to 45 reflections, depending on rhomb angles. In this case, 5 to 10 µm sections of medium 2 are sampled seven to 22 times, which results in an effective optical pathlength of the order 35 to 220 µm. 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 that forms a good optical contact with the prism can, in principle, be measured (solutions, oils, waxes, creams, pastes, powders and films).

More sophisticated devices exist, 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 M2. 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 and no sample pre–treatment. This device produces excellent results; baselines are flat as the technique is not dependent on light scattering.

Interpretation 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. 22.21) with the formula C8H9NO2 has (3 × 20 − 5) = 55 fundamental (normal modes of vibrating). Assigning 55 peaks in an IR spectrum is 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.

Figure 22.21. The IR spectra of aspirin, Nujol and paracetamol. The drug spectra were measured as Nujol mulls.

There are three aspects to identifying a chemical entity. In the first instance, the properties (biological, chemical and spectroscopic) of a drug are assessed and the drug classified according to its type (e.g. 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, confirms the identity of the compound. This may be possible through computer matching of the spectra. The molecular structure of a new chemical entity will most likely need to be determined by NMR spectroscopy, perhaps in combination with MS. 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.

Infra–red spectra matching and fingerprint identification

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

When the spectrum of a substance being examined is compared with a reference spectrum, such as those in the British Pharmacopoeia, the positions and relative intensities of the absorption bands of the spectrum of the substance being examined should conform to those of the reference spectrum. When the two spectra are compared, care should be taken to allow for the possibility of differences in resolving power between the instrument on which the reference spectrum was prepared and the instrument used to examine the substance. It is good practice to run a spectrum of a polystyrene film on the same instrument to compare it with that recorded on the reference spectrum. The greatest variations through differences in resolving power are likely to occur in the region between 4000 and 2000 cm−1 (British Pharmacopoeia Commission 2002, p. A129).

When a chemical reference substance is available, the substance being examined and the chemical reference substance should be prepared by the same procedure before recording the spectra (see later under Polymorphism). The transmission minima in the spectrum obtained with the substance being examined should correspond in position and relative size to those in the spectrum obtained with the reference substance (British Pharmacopoeia Commission 2002, p. A128).

In recent years, IR spectral databases have been created and stored electronically in databases and/or libraries. 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 lists 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 for which IR spectra have been measured is now 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’ by 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 ensures 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, 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 that give the next–best fit.
• Different forms of the same compound give different IR spectra (different polymorphs, racemate and/or enantiomer, ionisation status, cations and anions).
• If the spectra have been recorded on different instruments, 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 IR peaks in Part 3 of this book 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 were recorded under similar conditions on the same type of instrument, they should be very similar in appearance. Some examples of the identification of drugs are given below.

Infra–red spectra of amfetamines

The IR spectra of amfetamine base and the hydrochloride have many similarities, but the hydrochloride spectrum shows much finer detail. The IR spectra of the hydrochloride and mandelate salts show differences because of the absorption of the mandelic acid. However, the spectra of the hydrochloride and sulfate salts are very similar since they both have inorganic anions. The only major difference is the absorption band caused by the sulfate at 1110 cm−1.

Infra–red 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 sulfur to form thiobarbiturates.

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 IR spectrum of a barbiturate therefore depends on the class of compound, the nature of the substituents and whether it is the free acid or the sodium salt.

With the exception of phenobarbital and barbituric acid, the free barbiturates do not absorb appreciably above 3300 cm−1 (e.g. barbital), a feature that distinguishes them from the ureides; a weak band of unknown origin sometimes occurs between 3500 and 3400 cm−1. All the barbiturates have two bands, which occur near 3200 and 3100 cm−1 and are caused by N–H stretching vibrations. In the 5,5–disubstituted compounds, the relative intensities of the two bands are 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 3200 cm−1, e.g. metharbital. Methylphenobarbital appears to be an exception in that the band at 3100 cm−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.

A series of up to four medium–to–intense bands occurs in the region 3000 to 2800 cm−1, and is caused by 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 that occurs at 3000 to 2950 cm−1 is usually increased in intensity, compared to that of the free acid, and becomes the strongest band. Compare, for example, the spectra of barbital and barbital sodium.

The barbiturates have up to three strong bands in the region 1765 to 1670 cm−1, which result from C=O stretching vibrations. Knowledge of the origin of these bands helps 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, 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 1650 cm−1. In addition, a broad strong band occurs between 1600 and 1550 cm−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 1680 cm−1. They do, however, exhibit the broad, strong band that occurs between 1650 and 1600 cm−1. Therefore, the number, position and intensity of the bands between 1800 and 1500 cm−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 1250 cm−1, and some of these result from C–H deformation and C–N stretching vibrations. The sodium salts of the thiobarbiturates have a broad strong band between 1500 and 1480 cm−1, which is believed to be caused by C–N stretching vibrations of the carbon atom attached to sulfur. This band is not present in the ordinary barbiturates and therefore provides another way to distinguish those that contain sulfur. Many barbiturates exhibit a few weak–to–medium intensity bands in the region 1150 to 900 cm−1. The 1–substituted barbiturates exhibit a greater number of sharp bands of medium intensity. Those compounds that contain an allyl group exhibit bands at about 1000 to 960 cm−1, which probably result from C–H deformation vibrations. The sodium salts of the thiobarbiturates show a band of medium intensity between 1020 and 1000 cm−1. Finally, many barbiturates, but not the thiobarbiturates, exhibit a broad band of medium–to–strong intensity between 900 and 800 cm−1.

Infra–red spectra of aspirin, Nujol and paracetamol

The spectra of aspirin, Nujol and paracetamol, which illustrates the differentiation of N–H, O–H, ester, carboxylic acid and amide groups. In particular, the effect of Nujol on the drug spectra is apparent.

Polymorphism

Many drugs exist in polymorphic forms and have different IR spectra for each different crystalline form. IR spectroscopy can therefore be used to distinguish between different polymorphic forms, to identify them and also to measure quantitatively the proportions of each in a mixture.

If a test compound gives a different spectrum to the corresponding chemical reference substance, and polymorphism is suspected, both should be treated in the same manner so that they crystallise or are produced in the same form. This can often be achieved by dissolving them in a suitable solvent and evaporating to dryness.

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.

Interferences

Spurious bands can occur readily in IR 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 IR bands. A useful list has been compiled by Szymanski (1971).

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, which allows the identity of the test spectrum to be established. This type of work requires specialised database reference sets and computer programs. Much of it is proprietary and related to the software of the spectrometer being used.

However, it has been shown (Curry et al. 1969; Ingle and Mathieson 1976) that an IR 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 that consist of six major absorption bands have been selected from the recorded spectrum over the range 2000 to 650 cm^{-1} (5 to 15 µm) and 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 those in the region where Nujol absorbs (1490 to 1320 cm^{-1}, 6.7 to 7.6 µm) 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 principal peaks are also listed in descending order of amplitude in Part 3 of this book. Details of how to use the data are given in the Indexes to Analytical Data.

Quantitative analysis

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–visible 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 estimated readily from the relative intensities of two specific absorption bands:

where $A_{1\lambda1}$, $A_{2\lambda2}$, $\varepsilon_{1\lambda1}$ and $\varepsilon_{2\lambda2}$ 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, and the pathlength is l.

Collections of data

General collections

Compilations of IR spectral data are available in two forms, either as pictures or as digital absorbance/wavenumber in electronic 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. Electronic 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 SP8 4XT, UK, or their web site http://www.sigmaaldrich.com.
- Thermo Nicolet OMNIC FTIR available from Thermo Nicolet Corporation, 5225 Verona Road, Madison, WI 53711–4495, USA.

Other instrument manufactures can provide suitable software.

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 specialised groupings:

- polymers and related compounds
- pure organic compounds
- industrial compounds
- forensic sciences
- environmental applications
- inorganics
- organometallics.