5 Infrared spectrophotometry

KEYPOINTS

Principles

- Electromagnetic radiation ranging between 500 cm^{-1} and 4000 cm^{-1} (2500 and 20 000 nm) is passed through a sample and is absorbed by the bonds of the molecules in the sample causing them to stretch or bend. The wavelength of the radiation absorbed is characteristic of the bond absorbing it.

Applications

- A qualitative fingerprint check for the identity of raw material used in manufacture and for identifying drugs.
- Used in synthetic chemistry as a preliminary check for compound identity particularly for the presence or absence of a carbonyl group, which is difficult to check by any other method.
- Can be used to characterise samples in the solid and semi-solid states such as creams and tablets.
- Used as a fingerprint test for films, coatings and packaging plastics.
- Can be used to detect polymorphs of drugs (polymorphs are different crystal forms of a molecule that have different physical properties such as solubility and melting point which may be important in the manufacturing process).

Strengths

- Provides a complex fingerprint which is unique to the compound being examined.

- Computer control of instruments means that matching of the spectrum of a compound to its standard fingerprint can now be readily carried out.

Limitations

- Rarely used as a quantitative technique because of relative difficulty in sample preparation and the complexity of spectra.
- Usually can only detect gross impurities in samples.
- Sample preparation requires a degree of skill, particularly when potassium bromide (KBr) discs are being prepared.
- The technique is lacking in robustness since sample handling can have an effect on the spectrum obtained and thus care has to be taken in sample processing.

Introduction

The infra region can be divided up as shown in Table 5.1.

Table 5.1 Infrared ranges

Ranges	Far infrared	Middle infrared	Near infrared
Wavelength range	50–1000 μm	2.5–50 μm	0.8–2.5 μm
Wave number range	200–10 cm^{-1}	4000–200 cm^{-1}	12 500–4000 cm^{-1}
Energy range	0.025 eV–0.0012 eV	0.5 eV–0.025 eV	1.55 eV–0.5 eV

The middle infrared region is commonly used for structural confirmation but near infrared spectrophotometry, which has been used for very many years to control the products such as flour and animal feed, is finding increasing applications in quality control in the pharmaceutical industry. For the purposes of explaining infrared spectroscopy, a molecule is viewed as being joined by bonds which behave like springs. If the simple molecule HCl is examined in the gas phase it can be seen that it has an absorbance maximum at *ca* 2900 cm^{-1}, which results from the transition between the bottom vibrational state V_0 and the first excited state V_1 (Fig. 5.1). The spacing of the lower vibrational levels in IR spectrophotometry is equal so that even if the V_1–V_2 transition occurred the energy absorption would be the same as for V_0–V_1. Quantum mechanics does not allow a V_0–V_2 transition, although these types of transition over 2 or 3 levels occur weakly and give rise to near-infrared spectra.

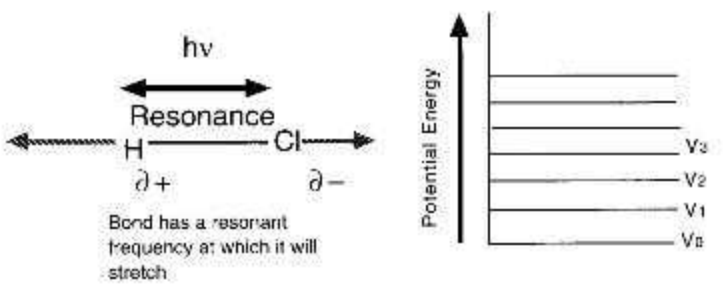


Fig. 5.1 Absorbance in IR radiation by a simple molecule HCl.

In order for the electrical component in electromagnetic radiation to interact with a bond, the bond must have a dipole. Thus symmetrical bonds such as those in O_2 or N_2 do not absorb infrared radiation. However, the majority of organic molecules have plenty of asymmetry. In even small organic molecules the modes of vibration are complex. This is illustrated by the vibrational modes which can occur in a

methylene group shown in Figure 5.2. The large number of bonds in polyatomic molecules means that the data obtained by IR analysis is extremely complex and provides a unique 'fingerprint' identity for the molecule. Quite a lot of structural information can be obtained from an IR spectrum but even with modern instrumentation it is not possible to completely 'unscramble' the complex absorbance patterns present in IR spectra.

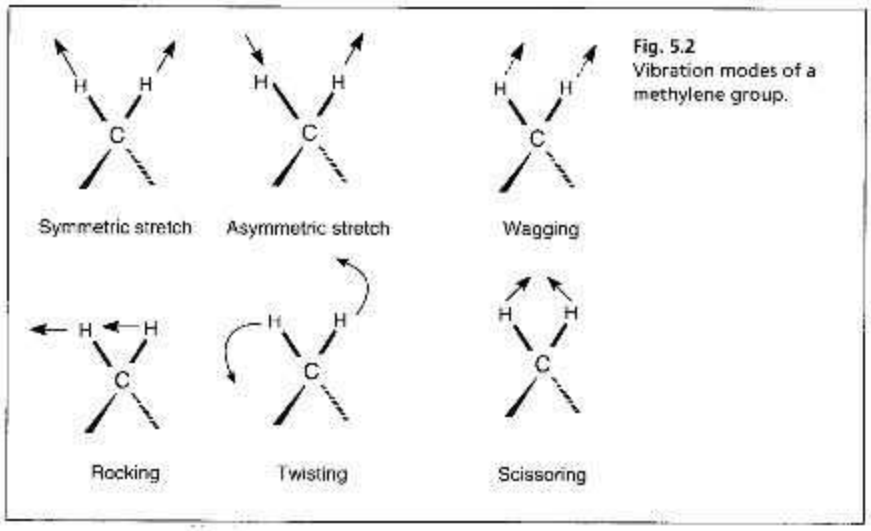


Fig. 5.2
Vibration modes of a methylene group.

Factors determining intensity and energy level of absorption in IR spectra

Intensity of absorption

The intensity with which a bond absorbs radiation depends on its dipole moment. Thus the order of intensity of absorption for the following C–X bonds is:

$$C{-}O \;>\; C{-}Cl \;>\; C{-}N \;>\; C{-}C{-}OH \;>\; C{-}C{-}H$$

Similarly:

$$OH \;>\; NH \;>\; CH$$

The intensity depends on the relative electronegativity of the atoms involved in the bond.

Self-test 5.1

Predict the order of intensity of absorption of the following bonds:

(i)	(ii)	(iii)	(iv)	(v)	(vi)
C–OH	C=NH	C=C–H	C=C–OH	C–F	C=S

Answers: (v) > (i) > (iv) > (ii) > (i) > (vi)

The intensity of the stretching of carbon–carbon double bonds is increased when they are conjugated to a polar double bond and such bonds in the A ring of the corticosteroids are quite prominent (e.g. see Fig. 5.12).

The order of intensity is as follows:

$$C{=}C{-}C{=}O > C{=}C{-}C{=}C > C{=}C{-}C{-}$$

Energy level of absorption

The equation which determines the energy level of vibration of a bond is shown below:

$$Evib \propto \sqrt{\frac{k}{\mu}}$$

k is a constant related to the strength of the bond, e.g. double bonds are stronger than single bonds and therefore absorb at a higher energy than single bonds. μ is related to the ratio of the masses of the atoms joined by the bond.

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

e.g. for O–H bonds $\mu = \frac{16 \times 1}{17} = 0.94$ for C–O bonds $\mu = \frac{12 \times 16}{17} = 11.3$

where m_1 and m_2 are the masses of the atoms involved in the bond.

According to the μ term the highest energy bonds are the X–H (OH, NH, CH). The order of energy absorption for some common bonds is as follows, which reflects μ and the strength of the bonds:

$$O{-}H > N{-}H > C{-}H > C{=}N > C{\equiv}C > C{=}O > C{=}C > C{-}O > C{-}C > C{-}F > C{-}Cl$$

Instrumentation

Two types of instrument are commonly used for obtaining IR spectra: dispersive instruments which use a monochromator to select each wavenumber in turn in order to monitor its intensity after the radiation has passed through the sample and Fourier transform instruments that use an interferometer. The latter generates a radiation source in which individual wavenumbers can be monitored within a *ca* 1 s pulse of radiation without dispersion being required. In recent years, Fourier transform instruments have become very common. A simple diagram of the layout of a continuous wave instrument is shown in Figure 5.3. The actual arrangement of the optics is much more complicated than this but the diagram shows the essential component parts for a dispersive IR instrument. The filament used is made of metal oxides, e.g. zirconium, yttrium and thorium oxides and is heated to incandescence in air. The sample is contained in various ways within discs or cells made of alkali metal halides. Once the light has passed through the sample it is dispersed so that an individual wavenumber or small number of wavenumbers can be monitored in turn by the detector across the range of the spectrum.

In a Fourier transform IR instrument the principles are the same except that the monochromator is replaced by an interferometer. An interferometer uses a moving mirror to displace part of the radiation produced by a source (Fig. 5.4) thus producing an interferogram which can be transformed using an equation called the 'Fourier transform' in order to extract the spectrum from a series of overlapping frequencies. The advantage of this technique is that a full spectral scan can be acquired in about 1 s compared to the 2–3 min required for a dispersive instrument

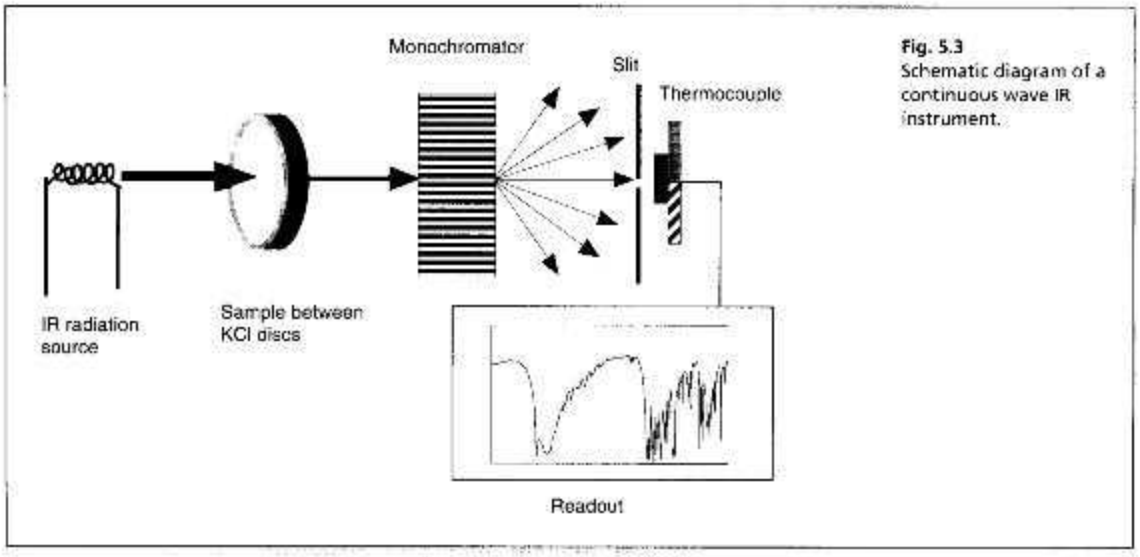


Fig. 5.3
Schematic diagram of a continuous wave IR instrument.

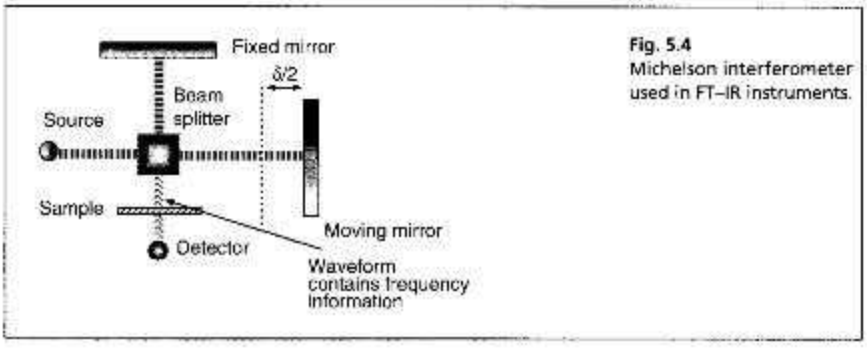


Fig. 5.4
Michelson interferometer used in FT–IR instruments.

to acquire a spectrum. Also, because the instrument is attached to a computer several spectral scans can be taken and averaged in order to improve the signal:noise ratio for the spectrum.

Instrument calibration

In order to ensure that instruments conform with BP specifications, the wavelength scale of the instrument is checked by obtaining an IR spectrum of polystyrene film (shown in Figure 5.5). Some of the bands used to check the accuracy of the wavelength scale of an IR spectrophotometer are shown in Figure 5.5. The permitted tolerances for variation in the wavelengths of absorption are mainly ± 0.3 nm. Two of the bands at 907 cm^{-1}, 1028 cm^{-1}, 1495 cm^{-1} or 1601 cm^{-1} (usually 1028 and 1601 cm^{-1}) are overlayed onto standard BP spectra to indicate that the spectra have been obtained on a correctly calibrated instrument. In addition to specifying tolerances for the wavelength scale, the BP specifies the degree of resolution which the instrument must be capable of achieving, e.g. the maxima at 2851 cm^{-1} and the minimum at 2870 cm^{-1} should have a valley between them of > 18% transmittance. In Figure 5.5 the valley between the minimum and maximum at these two wavelengths is *ca* 25% transmittance.

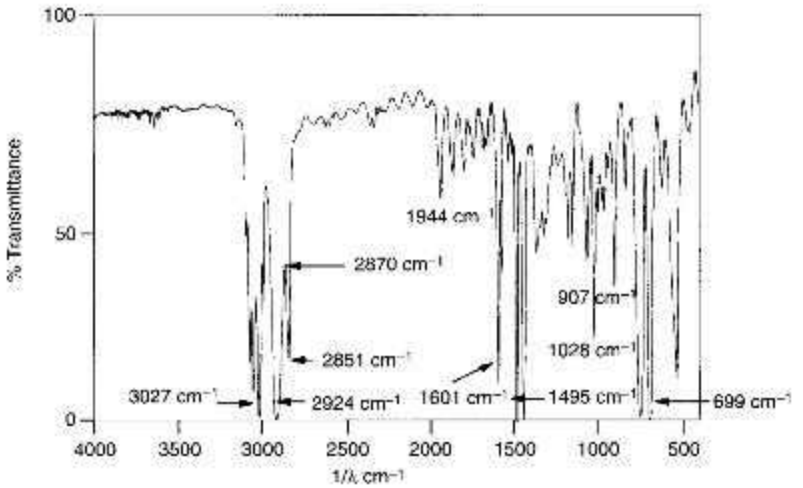


Fig. 5.5
IR spectrum of polystyrene film used to check instrument calibration.

Sample preparation

Traditionally three modes of sample preparation have been used prior to IR analysis:

(i) The sample is run as a film sandwiched between two NaCl or potassium chloride (KCl) discs. For this method the sample must be a liquid, in which case it can be run without preparation, or must be ground to a paste in a liquid matrix, usually liquid paraffin (Fig. 5.6). In this case the liquid paraffin (nujol) contributes some peaks to the spectrum at *ca* 3000 cm^{-1} and *ca* 1400–1500 cm^{-1}. However, sample preparation is relatively simple and this procedure is used where a chemist just wants a quick identification of certain structural features in a molecule. This procedure is also used to identify different crystal forms (polymorphs) of a drug because the pressures used to prepare KBr discs can cause polymorph interconversion.

(ii) The sample is ground to a powder with KBr or KCl. KBr is usually used unless a hydrochloride salt is being analysed in which KCl is used to avoid halogen exchange. On a weight-for-weight basis the weight of the sample used is about 1% of the weight of KBr used. About 200 mg of the finely ground powder are transferred to a die block and the sample is then compressed into a disc under vacuum by subjecting it to a pressure of 800 KPa (Fig. 5.6). This is the procedure used in pharmacopoeial methods to prepare a drug for analysis by IR.

(iii) IR spectra of liquids or solutions in an organic solvent, commonly chloroform, may be obtained by putting the liquid into a short pathlength cell with a width of *ca* 1 mm. Cells are constructed from sodium or potassium chlorides and obviously aqueous samples cannot be used.

(iv) A more recent development in sample preparation is the use of diffuse reflectance (Fig. 5.7). Diffuse reflectance is a readily observed phenomenon. When light is reflected off a matt surface the light observed is of the same intensity no matter what the angle of observation. Samples for diffuse reflectance are treated in the same way as those prepared for KBr disc formation except that instead of being compressed the fine powder is loaded into a small metal cup, which is placed in the path of the sample beam. The incident radiation is reflected from the base of the cup and during its passage through the powdered sample and back absorption of radiation takes

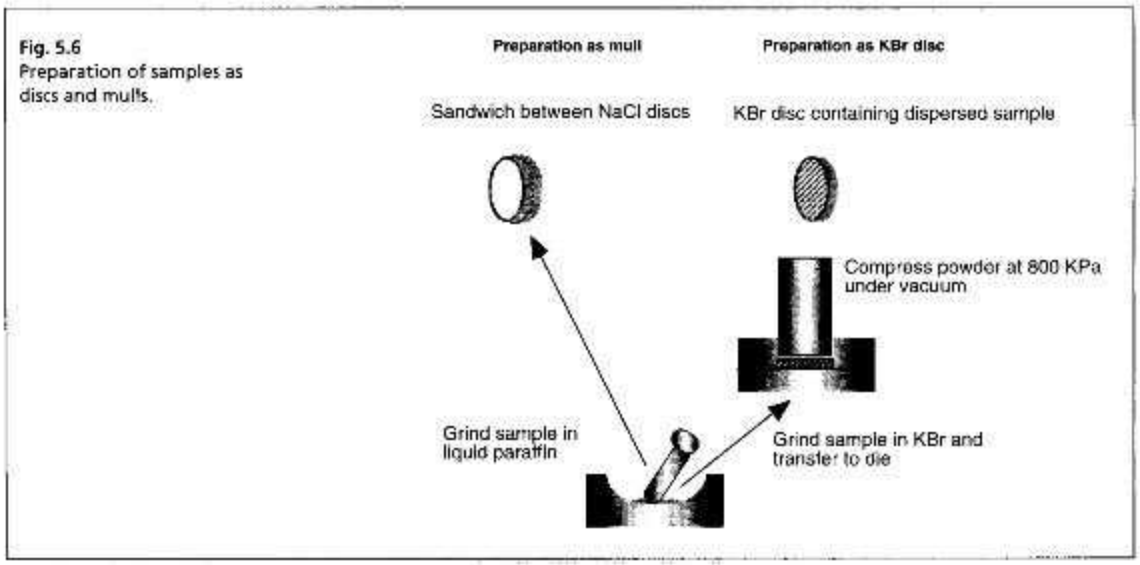


Fig. 5.6
Preparation of samples as discs and mulls.

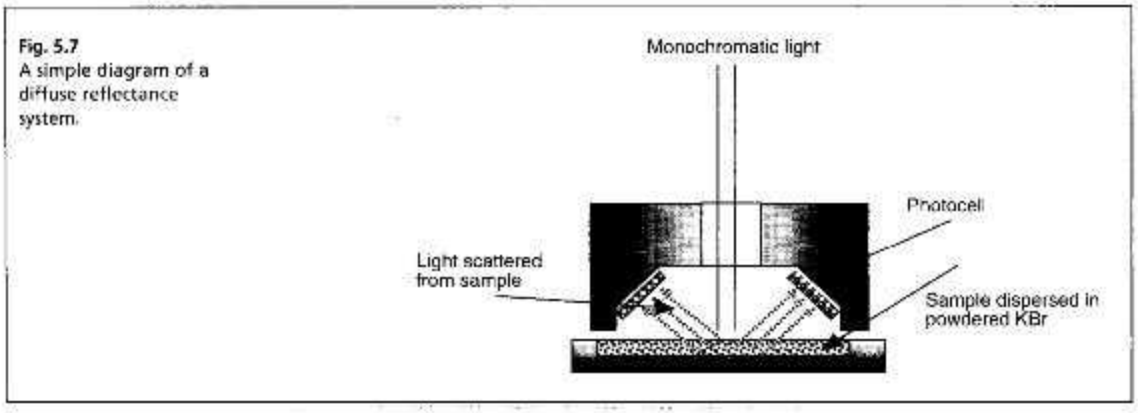


Fig. 5.7
A simple diagram of a diffuse reflectance system.

place–yielding an infrared spectrum which is very similar to that obtained from the KBr disc method. In fact the spectrum produced is an absorbance spectrum rather than a transmittance spectrum but it can be readily converted into a transmittance spectrum if the instrument is attached to a computer. The diffuse reflectance technique is widely used in near-infrared spectrophotometry and it can also be used to examine films and coatings if they are put onto a reflective background. It is also a useful technique for examining polymorphs since the sample can be prepared for analysis with minimal grinding and compression, which can cause interconversion of polymorphs.

(v) Attenuated total reflectance (ATR) is another recent development in sample handling (Fig. 5.8). In this case the sample may be run in a gel or cream and this method may be used to characterise both formulation matrices and their interactions with the drugs present in them. If the active ingredient is relatively concentrated and if a blank of the matrix is run using the same technique it may be subtracted from the sample to yield a spectrum of the active ingredient. ATR also provides another technique which can be used for the characterisation of polymorphs.

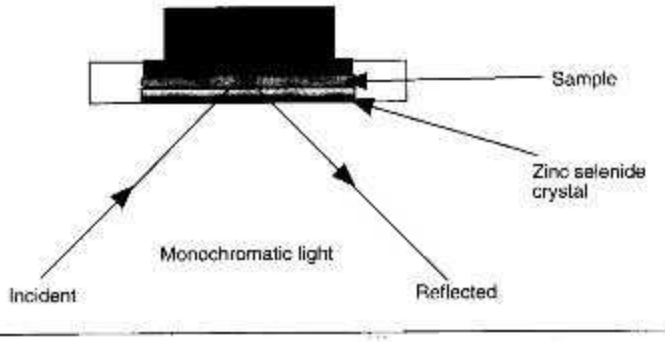


Fig. 5.8
Attachment for analysis of a sample by ATR.

Self-test 5.2

Suggest methods for analysis of the following samples by IR spectrophotometry:

(i) Pethidine hydrochloride.
(ii) Pethidine free base (liquid).
(iii) A cream containing 2% w/w salicylic acid.
(iv) A polymorphic form of a drug.
(v) A plastic to be used in packaging.

Answers: (i) KCl disc or diffuse reflectance infrared Fourier transform (DRIFT) in KCl powder; (ii) Analysed as a liquid film between two NaCl discs; (iii) Analysis by ATR; (iv) Preparation as a nujol mull to avoid interconversion of polymorphs or using DRIFT; (v) A sample of the film is inserted into the IR instrument.

Application of IR spectrophotometry in structure elucidation

As indicated earlier, the extent to which IR spectrophotometry can be used to elucidate structures is limited. The information given in Figure 5.9 is confined to the more easily recognisable bands in the IR spectra of molecules; this is to discourage the notion that IR is a technique used for extensive structure elucidation—in pharmaceutical analysis it is a fingerprint technique. The most readily assigned absorptions are usually at > 1500 cm^{-1}. The bands < 1500 cm^{-1} are in the fingerprint region of the spectrum where the absorption is very complex and it is difficult to be confident in the assignment of absorptions to particular functional groups. Fuller tables of the bands in the fingerprint region are given elsewhere[1] and the present treatment is focused largely on the bands > 1500 cm^{-1}.

Examples of IR spectra of drug molecules

Some examples of interpretations are given in Figures 5.10–5.14 and Tables 5.3–5.6. In the examples only limited interpretation of the fingerprint region is attempted since often assignments in this region are not certain. Even above 1500 cm^{-1} it is sometimes difficult to assign bands thus IR is not a primary structure elucidation technique.

Fig. 5.9
The major absorptions which can be observed in drug molecules.
stg. = strong absorption; md. = medium absorption; wk. = weak absorption; brd. = broad; shp. = sharp; conj. = conjugated; v.brd. = very broad; A–F additional notes below.

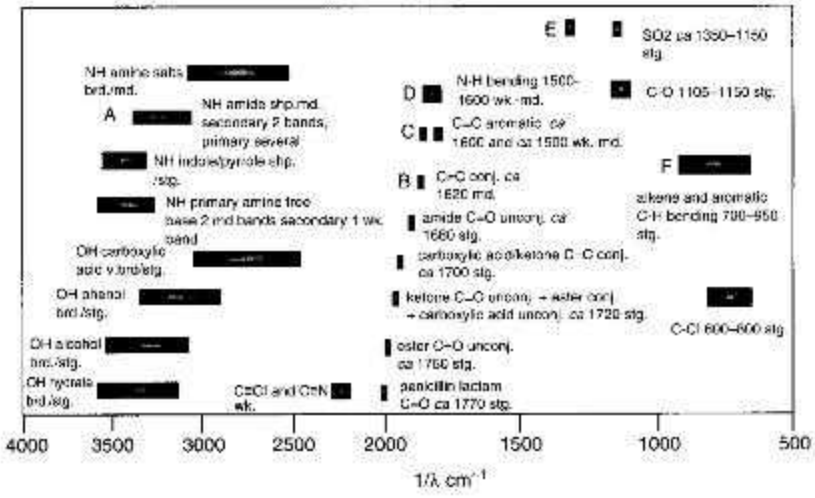


Table 5.2 Additional comments on IR bands in Figure 5.9

Band	Comment
A	Restricted rotation about N–CO bond produces diastereomers giving two bands in the case of secondary amides; see spectrum of phenoxymethylpenicillin (Fig. 5.14)
B	C=C unconjugated gives a very weak absorption but when conjugated the C=C bond gives a much stronger absorption found typically in many steroids
C	C=C aromatic: the band at 1600 cm^{-1} may be weak unless the aromatic ring is substituted with polar substituents, e.g. a phenol, aromatic ether or aromatic amine free base
D	N–H bend is often obscured by stronger aromatic C=C stretching bands
E	SO_2 bands: although this absorption is in the fingerprint region, these bands are quite prominent in sulphonamides
F	C–H bending in many cases is not very distinctive in drug molecules because of the complexity of the fingerprint region

Fig. 5.10
Infrared spectrum of paracetamol as a KBr disc.

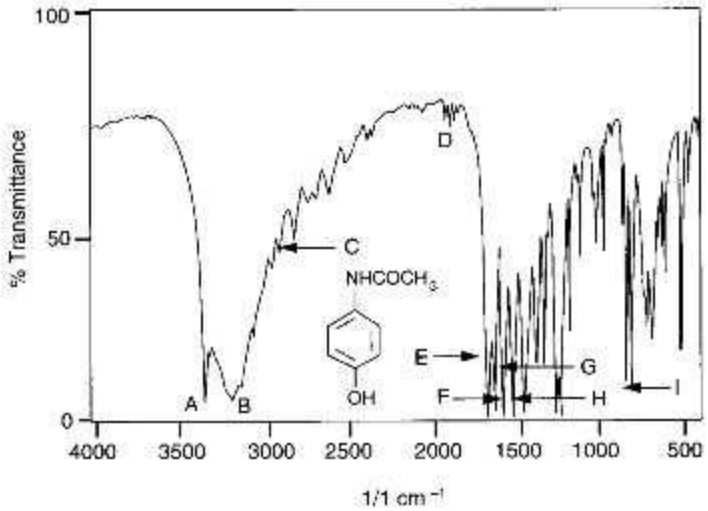


Table 5.3 Interpretation of the IR spectrum of paracetamol

Wavenumber	Assignment	Comments
A 3360 cm^{-1}	N–H amide stretch	This band can be seen quite clearly although it is on top of the broad OH stretch
B 3000 cm^{-1}–3500 cm^{-1}	Phenolic OH stretch	Very broad due to strong hydrogen bonding and thus obscures other bands in this region
C ca 3000 cm^{-1}	C-H stretching	Not clear due to underlying OH absorption
D 1840–1940 cm^{-1}	Aromatic overtone region	Quite clear fingerprint but does not reflect 2 band pattern proposed for *p* disubstitution.[4]
E 1650 cm^{-1}	C=O amide stretch	C=O stretching in amides occurs at a low wavenumber compared to other unconjugated C=O groups
F 1608 cm^{-1}	Aromatic C=C stretch	This band is strong since the aromatic ring has polar substituents which increase the dipole moment of the C=C bonds in the ring
G 1568 cm^{-1}	N–H amide bending	Strong absorption in this case but this is not always so
H 1510 cm^{-1}	Aromatic C=C stretch	Evidence of a doublet due to interaction with ring substituents
I 810 cm^{-1}	=C–H bending	Possibly aromatic C–H bending but the fingerprint region is too complex to be completely confident of the assignment

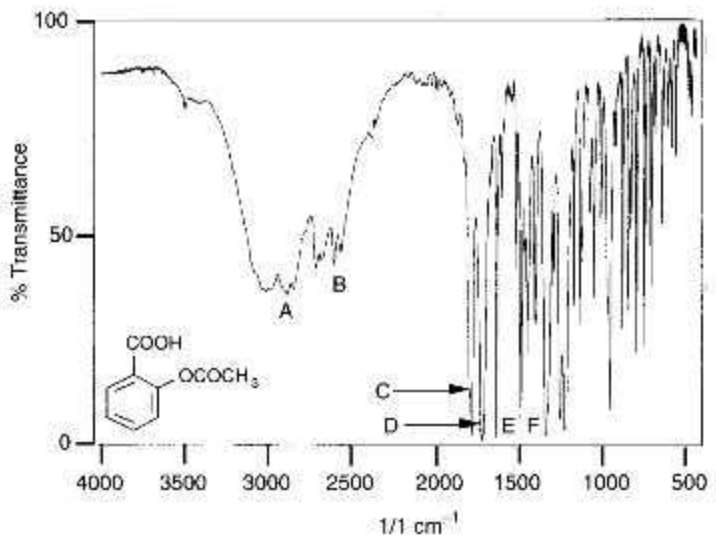


Fig. 5.11
The infrared spectrum of aspirin as a KBr disc.

Table 5.4 Interpretation of the IR spectrum of aspirin

Wavenumber	Assignment	Comments
A 2400–3300 cm^{-1}	Carboxylic OH stretch	Very broad and complex due to strong hydrogen bonding. The broad band obscures other bands in this region
B *ca* 3000 cm^{-1}	C–H stretching	Not clear due to underlying OH absorption
C 1757 cm^{-1}	C=O ester stretch	Due the acetyl group which is an unconjugated aliphatic ester
D 1690 cm^{-1}	C=O conjugated carboxylic acid stretch	C=O of the acid is conjugated to the aromatic ring
E 1608 cm^{-1}	Aromatic C=C stretch	These bands are intense since the ring is substituted with polar groups
F 1460 cm^{-1}	Aromatic C=C stretch	

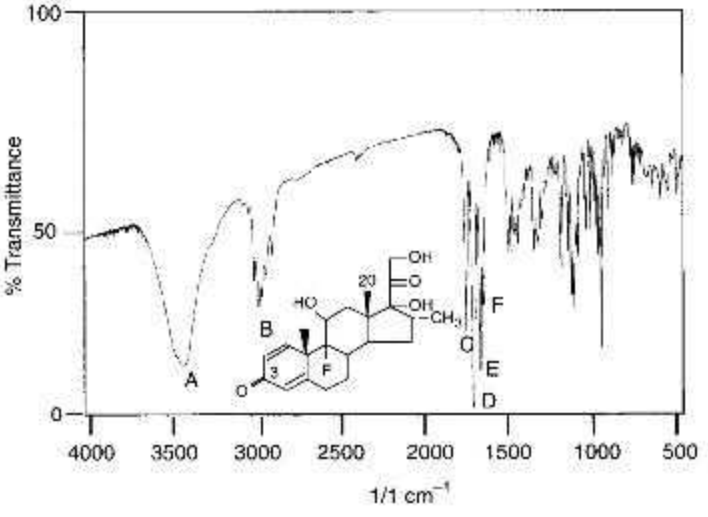


Fig. 5.12
The infrared spectrum of dexamethasone obtained as a KBr disc. Corticosteroids provide some of the best examples for the assignment of IR bands because of the prominence of the bands in their spectra above 1500 cm^{-1}.

Table 5.5 Interpretation of the IR spectrum of dexamethasone

Wavenumber	Assignment	Comments
A 3140–3600 cm^{-1}	Alcoholic OH stretch	Broad due to hydrogen bonding
B 2750–3122 cm^{-1}	C–H stretch	Complex region due to the large hydrocarbon skeleton of steroid
C 1705 cm^{-1}	C=O unconjugated ketone stretch	Ketone at 20-position C=O stretch, generally lower than an ester C=O stretch
D 1655 cm^{-1}	C=O conjugated ketone stretch	Ketone at 3-position
E 1615 cm^{-1}	C=C conjugated	Strengthened by being conjugated to a C=O group. Trisubstituted C=C absorbs at a higher wavenumber than disubstituted
F 1600 cm^{-1}	C=C conjugated	Strengthened by being conjugated to a C=O group. Disubstituted C=C absorbs at lower wavenumber than trisubstituted

The spectrum of dexamethasone obtained by the DRIFT technique is shown in Figure 5.13 and is very similar to that obtained using a KBr disc. However, the proportion of dexamethasone powdered with KBr and used to obtain the DRIFT spectrum was 10 times that used to prepare the KBr disc, which yielded the spectrum shown in Figure 5.12. As discussed earlier in this chapter, the use of DRIFT has some advantages over the preparation of a KBr disc.

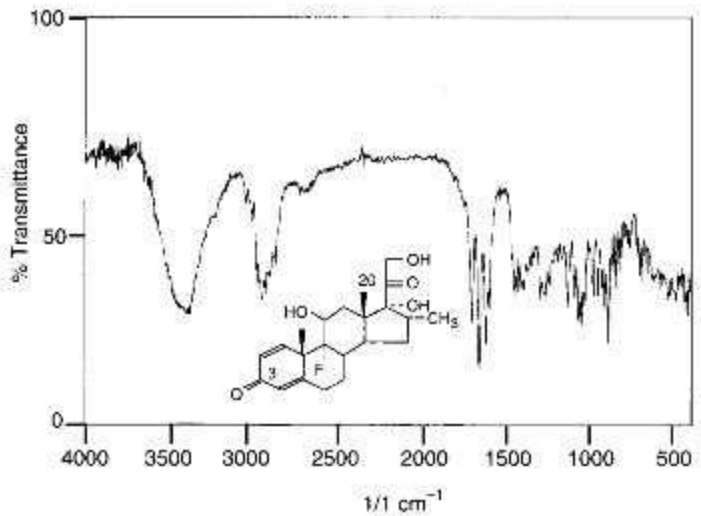


Fig. 5.13
IR spectrum of dexamethasone obtained using the DRIFT technique.

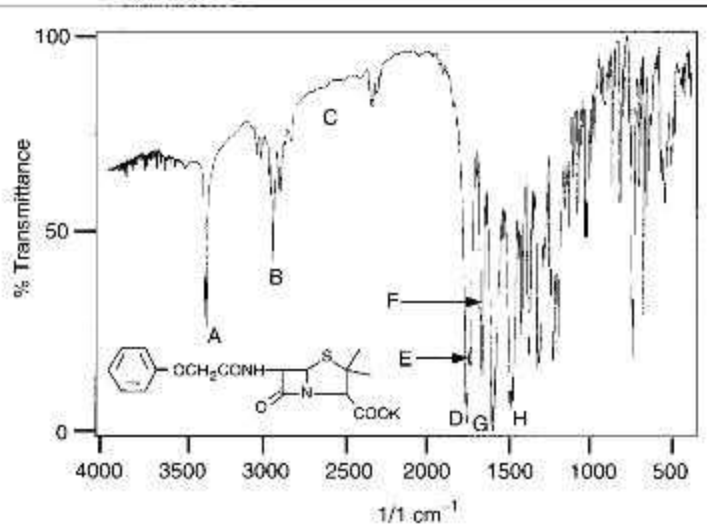


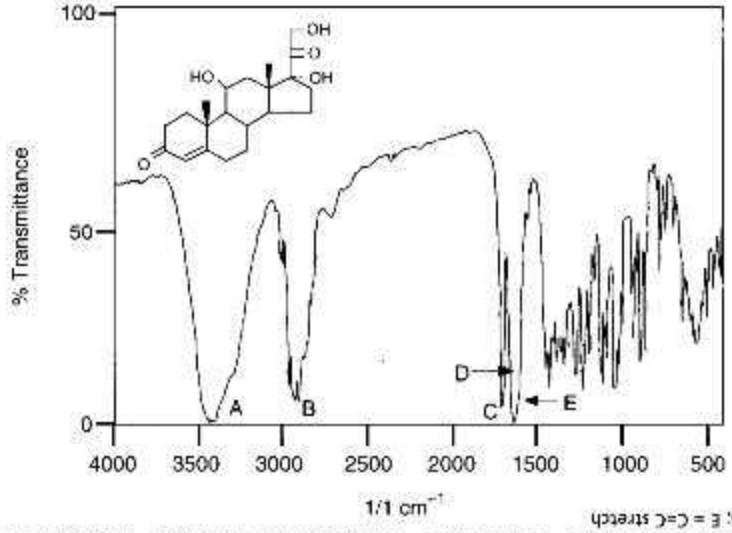
Fig. 5.14
IR spectrum of phenoxymethyl penicillin potassium. Obtained as a KBr disc.

Table 5.6 Interpretation of IR spectrum of phenoxymethyl penicillin potassium

Wavenumber	Assignment	Comments
A 3360 cm^{-1}	N–H amide stretch	Two bands indicating restricted rotation about the N–CO bond resulting in stereoisomers
B 2900–3100 cm^{-1}	C–H stretch	Aliphatic and aromatic C–H stretching
C 2400–*ca* 3000 cm^{-1}		OH stretch absent since the carboxylic acid is in the form of its potassium salt
D 1765 cm^{-1}	C=O lactam ring stretch	High energy C=O stretch typical of a lactam ring
E 1744 cm^{-1}	C=O carboxylic acid stretch	Salt thus absence of H bonding means the stretch is of higher energy than in an acid. Compare with esters
F 1690 cm^{-1}	C=O amide stretch	
G 1610 cm^{-1}	C=C stretch	Aromatic ring stretch, broad band possibly obscuring amide N–H bend
H 1505 cm^{-1} and 1495 cm^{-1}	C=C stretch	Aromatic ring

Self-test 5.3

Assign the bands A–E indicated in the spectrum of hydrocortisone.



Answer: A = OH stretch; B = C–H stretch; C = unconjugated C=O stretch; D = conjugated C=O stretch; E = C=C stretch

IR spectrophotometry as a fingerprint technique

Preparation of samples for fingerprint determination

The majority of samples prepared for fingerprint determination in order to determine their degree of conformity with BP standards are prepared as KBr or KCl discs. The instructions with regard to sample preparation stipulate that 1–2 mg of the substance being investigated should be ground with 0.3–0.4 g of KBr or KCl. The KBr or KCl should be free from moisture. The mixture should be compressed at 800 KPa and discs should be discarded if they do not appear uniform. Any disc having a transmittance < 75% at 2000 cm^{-1} in the absence of a specific absorption band should be discarded. The instrument used to measure the IR spectrum should be calibrated using a polystyrene film. Formulations are usually extracted with a specified solvent and it is stipulated that adequate spectra will be obtained only if excipients in the formulation are adequately removed. For pure substances, if difficulty is encountered with obtaining a fingerprint match to the BP spectrum of a reference standard for the substance being examined, the analysis should be repeated where the substance being investigated and the reference standard have been recrystallised from the same solvent. As can be seen in Figure 5.15, even closely related compounds give different IR spectra in the fingerprint region. Dexamethasone and betamethasone only differ in their stereochemistry at the 16 position on the steroid skeleton. However, this small difference is great enough to result in a different fingerprint spectrum for the two compounds. There are even slight differences in the absorptions of the bands due to C=C stretching at 1620 cm^{-1}.

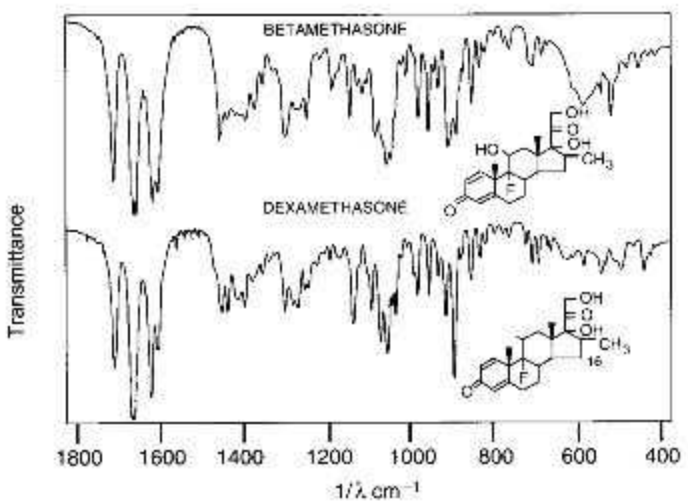
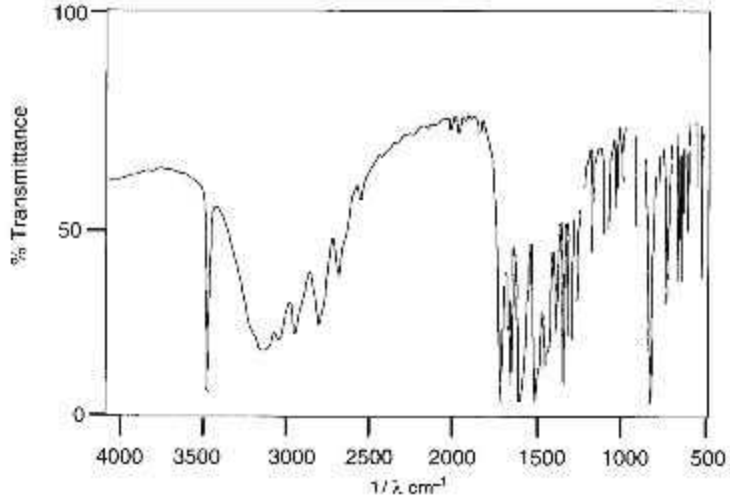
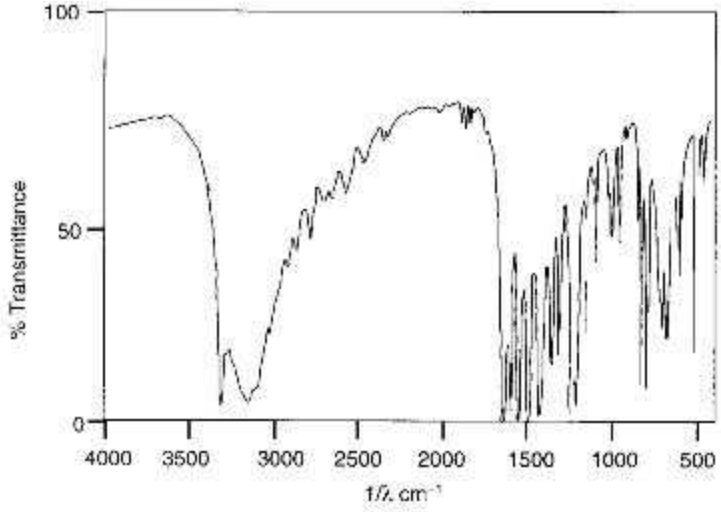


Fig. 5.15
Comparison of the fingerprint regions of dexamethasone and betamethasone.

Self-test 5.4

Compare the fingerprint regions of the following spectra with the spectrum of paracetamol given in Figure 5.10. Which spectrum is due to paracetamol? Suggest what the structure of the unknown compound might be.





Answer: Spectrum B corresponds to paracetamol. Spectrum A is an isomer of paracetamol o-hydroxyacetanilide

Infrared spectrophotometry as a method for identifying polymorphs

IR spectrophotometry along with differential scanning calorimetry and X-ray powder diffraction provides a method for characterising polymorphic forms of drugs. The existence of polymorphs, different crystalline forms of a substance, has an important bearing on drug bioavailability, the chemical processing of the material during manufacture and on patent lifetime. Until recently the standard method of sample preparation for characterising polymorphs by IR was by using a nujol mull to prepare the sample. However, the DRIFT technique has an advantage since it does

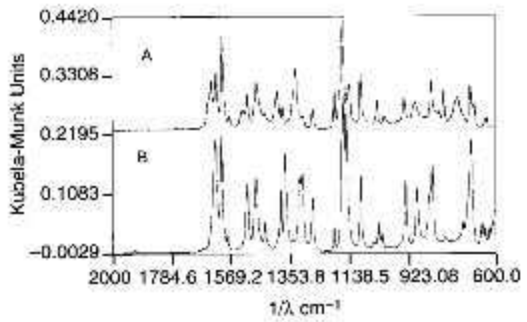


Fig. 5.16
Fingerprint regions of two polymorphs (A & B) of sulphamethoxazole. Spectra obtained by DRIFT. Reproduced with permission from Int. J. Pharmaceutics (see Reference 3).

not introduce the interfering peaks which are present in nujol and which may obscure areas of interest in the fingerprint region of the spectrum. In addition, low polarity samples may be soluble in nujol thus causing their polymorphs to break down. Figure 5.16 shows the spectra of the fingerprint region of two polymorphs of sulphamethoxazole prepared by powdering the samples with KBr and then analysing using DRIFT.[3] The units on the Y axis are Kubela Munk units, which are an expression of the data obtained by DRIFT. These can be mathematically converted into transmittance or absorbance if required.

Near-infrared analysis (NIRA)

KEYPOINTS

Principles
- Electromagnetic radiation in between 1000 and 2500 nm is weakly absorbed by the X-H bonds of molecules causing them to stretch. The wavelength of the radiation absorbed is characteristic of the bond absorbing it.

Applications
- Quantitative analysis of multiple components in a sample and in pack quantification of drugs in formulations
- Fingerprint check for the identity of a drug and quality control of complex excipients such as lactose and cellulose used in formulation
- Determination of physico-chemical properties of drugs and excipients such as particle size, water content and polymorphism
- Determination of the physical properties of formulations such as blend uniformity and particle size.

Strengths
- NIR radiation has good penetration properties and thus minimal sample preparation is required and thick sample layers can be used to compensate for the weakness of NIR absorption
- Intense radiation sources can be used since they can be protected by quartz envelopes unlike middle IR sources
- Has the potential to replace chromatography as a method for more rapid analysis of multicomponent samples.

Limitations

- Extensive method development is required before the technique can be used as a truly rapid analysis technique. Development of a method requires a specialist operator with computing knowledge
- Instruments are expensive compared with middle-IR instruments.

Introduction

The near-infrared region of the spectrum is generally defined as the wavelength range from 700 nm to about 2500 nm. The absorption bands in this region of the spectrum are due to overtones and combinations of fundamental mid-IR vibration bands. Quantum mechanical selection rules forbid transitions over more than one energy level. However, molecules do not behave as ideal oscillators and anharmonic vibration enables overtone bands to occur at two, three, four times, etc. the energy level of the fundamental bands of the mid-IR region. Such overtone bands are *ca* 1000 times weaker than the bands seen in the mid-infrared region. Most of the useful bands in this region are overtones of X–H stretching. The NIRA technique was developed in the 1950s but the paucity of structural information which could be obtained from it caused it to be neglected until the 1980s when applications for it were found in the agricultural and textile industries. The strength of NIRA lies in the quantitative information which it can yield and its ability to identify constituents in multicomponent samples. The applications in quantitative analyses arrived with the ready availability of advanced computing facilities and this is the weakness of the technique, i.e. extensive software development has to take place before the spectral measurements yield useful information. However, it might be anticipated that increasingly sophisticated software will become available. NIRA has the potential to produce great savings in sample preparation and analysis and lends itself very well to process control. The technique is largely used in the DRIFT mode.

Examples of NIRA applications

Extensive use has been made of NIRA in agriculture where it has been used to determine the protein, fibre, water and triglyceride contents of feedstuffs and the quality of crops. By training the computer to recognise the near-infrared (NIR) spectra of the major components making up a crop, the individual components can be monitored in the crop itself. The components that can be measured by NIRA often cannot be measured by the usual spectroscopic methods. The fundamental work done in the quality control of agricultural products can be readily extended to the quality control of pharmaceutical formulations.

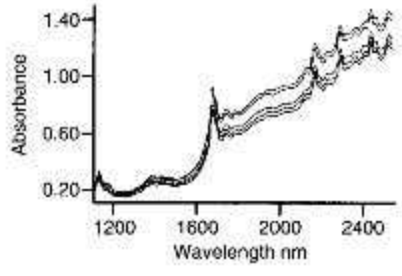


Fig. 5.17
NIRA of USP aspirin samples with decreasing particle size. Reproduced with permission from Applied Spectroscopy Reviews (see Reference 4).

Determination of particle size in United States Pharmacopoeia (USP) grade aspirin

It has been found that there is a linear relationship between NIR absorption and particle size. NIRA can provide a rapid means for determining particle size. Figure 5.17 shows the effect of particle size on the NIR spectra of USP grade aspirin;[4] the absorbance of the sample increases with decreasing particle size. Particle size is an important factor to be controlled in formulation and manufacture and NIRA provides a rapid means for its determination. In order to validate such a technique it would have to be calibrated against one of the existing methods for particle size determination.

Determination of blend uniformity

NIRA provides an excellent method for the direct monitoring of the uniformity of blends when drugs are being formulated. Figure 5.18 shows the effect of blending time on the uniformity of a sample containing hydrochlorothiazide, lactose, magnesium stearate and croscarmellose sodium.[5] The most notable variations in absorbance intensity in the spectrum of the blend occur at 2030 nm and 2240 nm. Absorbance at these wavelengths can be attributed to hydrochlorothiazide and lactose, respectively. The more complete the blend, the less the standard deviations of the absorbances at these wavelengths obtained when several batches sampled at the same time point are compared. As would be expected the standard deviations shown in Figure 5.17 decreases with blend time but the decrease is less marked after 10 min. In this study it was found that blending for more than 20 min caused a loss in uniformity due to an alteration in the flow properties of the powder resulting from a change in the distribution of the magnesium stearate. NIR probes can be inserted directly into blenders to monitor mixing.

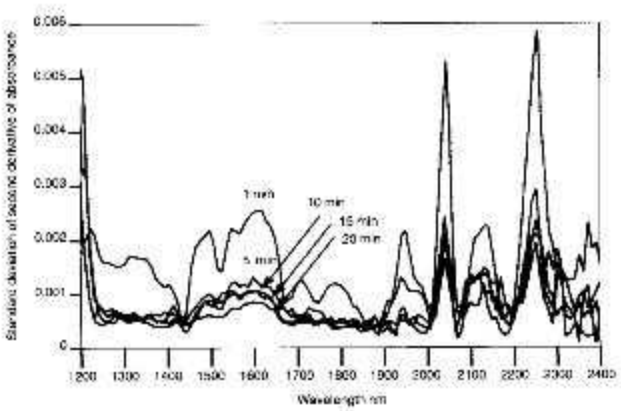


Fig. 5.18
The effect on differences in blend time on the uniformity of a formulation containing lactose and hydrochlorothiazide. Reproduced with permission from J. Pharm. Biomed. Anal. (see Reference 5).

Determination of active ingredients in multicomponent dosage forms

NIRA has been used to analyse multicomponent tablets, e.g. aspirin/caffeine/butalbarbital, and can examine such tablets in a pass/fail manner.[6] The tablets fail

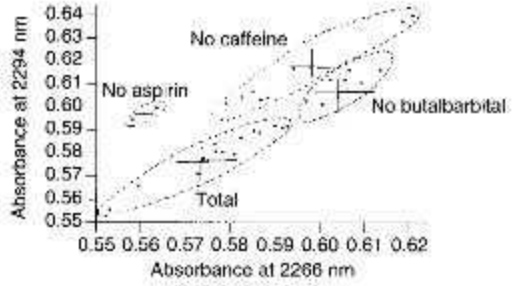
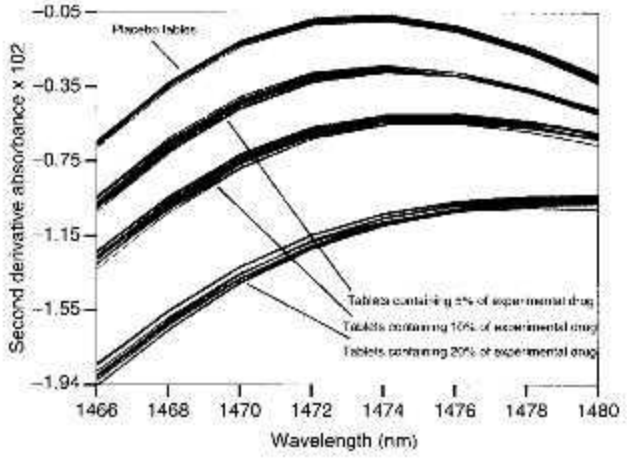


Fig. 5.19
Application of NIRA to control of the ingredients in tablets containing three components. Reproduced with permission from Spectroscopy (see Reference 6).

Fig. 5.20
Determination of the amount of active ingredient in tablets by direct use of NIRA. Reproduced with permission from J. Pharm. Biomed. Anal. (see Reference 7).



when the ingredients fall outside the specified range as shown in Figure 5.19, which is derived by the monitoring of two wavelengths in the NIR spectrum of the formulation. This might appear simple but a great deal of development work was carried out in order to determine which wavelengths to monitor in order give the best discrimination.

In-pack determination of active ingredients

In clinical trials of a new drug it is important to ensure that the tablets have been packed and coded correctly. Figure 5.20 shows the absorbance of tablets monitored at a wavelength which can be correlated with the content of active ingredient.[7] It was possible to distinguish between tablets containing 0, 5, 10, 15 and 20% of the active ingredient. It was also possible to adapt the method to determination of the active ingredient of the tablets 'in pack' using a fibreoptic probe, although the precision was not quite as good as that obtained from the unpackaged tablets.

Determination of polymorphs

NIRA provides a non-destructive alternative to differential scanning calorimetry for the determination of polymorphic forms of drugs, e.g. the polymorphic forms of caffeine.[4] NIRA has also been used to determine optical purity. While the pure opposite enantiomers of a substance have identical NIR spectra, mixing two

enantiomers together causes a change in the spectrum. Thus there is potential for determining the percentage of each enantiomer in an enantiomeric mixture and hence for the control of enantiomeric impurities.[4]

Moisture determination

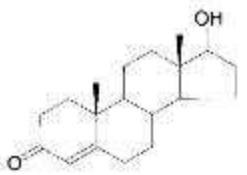
Use can be made of a strong absorption band for water at 1940 nm in the NIR region in order to quantify the water in pharmaceuticals; good agreement has been found with Karl Fischer determinations. Recently a study was carried out in order to determine water content in freeze-dried sterile product in glass ampoules.[5] The method developed was only partially successful due to variations in the hydrogen bonding of water with the product but it should be possible to optimise the wavelengths used for monitoring such bound water.

Additional problems

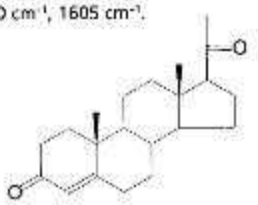
1. Four steroids (I), (ii), (iii) and (iv) correspond to the structures below (Fig. 5.21). The steroids are analysed by IR as KBr discs. The principal bands in their spectra between 1500 cm^{-1} and 4000 cm^{-1} are given below. Determine which of the structures given below correspond to (i), (ii), (iii) and (iv).

   (i) Steroid ca 3000 cm^{-1}, 1710 cm^{-1}, 1670 cm^{-1}, 1620 cm^{-1}.
   (ii) Steroid 3460 cm^{-1} (broad band), ca 3000 cm^{-1}, 1710 cm^{-1}, 1660 cm^{-1}, 1620 cm^{-1}, 1610 cm^{-1}.
   (iii) Steroid 2900–3500 cm^{-1} (very broad band obscuring other bands in this region), 1605 cm^{-1}, 1580 cm^{-1}, 1500 cm^{-1}.
   (iv) Steroid 3400 cm^{-1} (broad band), ca 3000 cm^{-1}, 1670 cm^{-1}, 1605 cm^{-1}.

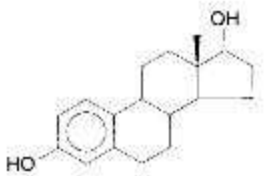
Fig. 5.21



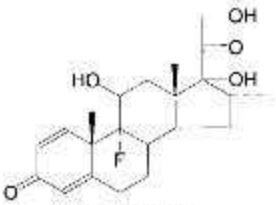
Testosterone



Progesterone



Oestradiol



Dexamethasone

Answer: (i) progesterone; (ii) dexamethasone; (iii) oestradiol; (iv) testosterone

2. The principal bands between 1500 cm^{-1} and 4000 cm^{-1} are given for the molecules shown below (Fig. 5.22). Associate each set of data with one of the molecules.

   (i) ca 3000 cm^{-1}, 2300–2900 cm^{-1} (very broad), 1600 cm^{-1} (weak), 1500 cm^{-1}.
   (ii) 3300–3500 cm^{-1} (broad), ca 3000 cm^{-1}, 1750 cm^{-1}, 1720 cm^{-1}, 1650 cm^{-1}, 1612 cm^{-1}, 1600 cm^{-1}.
   (iii) 3370 cm^{-1} (sharp), 2300–3200 cm^{-1} (broad band obscuring other bands in this region), 1780 cm^{-1} (with slight shoulder at 1750 cm^{-1}), 1690 cm^{-1}, 1605 cm^{-1}, 1580 cm^{-1}, 1500 cm^{-1}.
   (iv) 3380 cm^{-1}, 3320 cm^{-1}, ca 3000 cm^{-1}, 2300–2900 cm^{-1} (very broad), 1690 cm^{-1}, 1620 cm^{-1}, 1600 cm^{-1}, 1500 cm^{-1}.

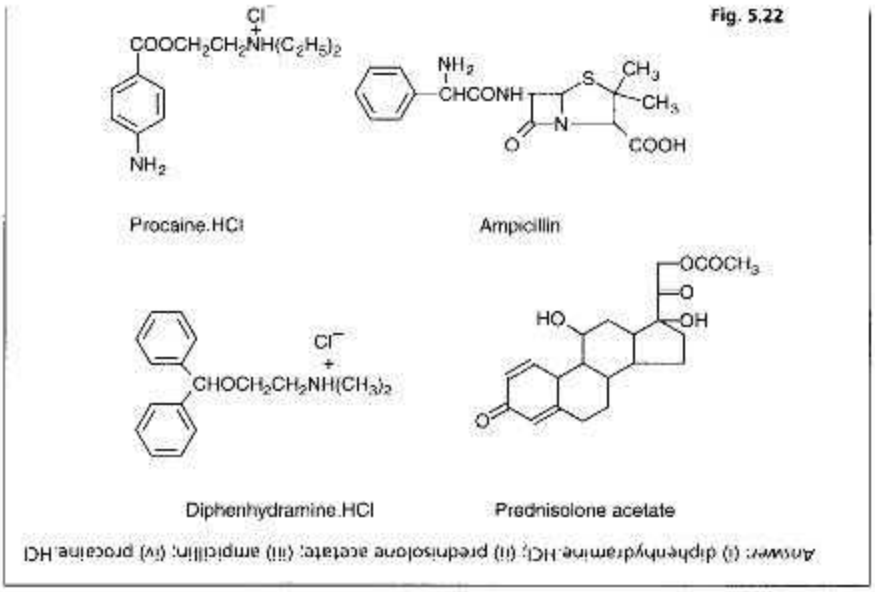


Fig. 5.22

Answer: (i) diphenhydramine.HCl; (ii) prednisolone acetate; (iii) ampicillin; (iv) procaine.HCl

Process control of components in a shampoo

NIRA was studied as a technique for process control in the manufacture of shampoo.[9] The formulation contained detergent, solids, water and glycerol. In order to carry out the process control samples of shampoo were taken at various points in the production process. NIR reflectance spectra were obtained for 75 samples over the range 1100–2500 nm. A multiple step-up linear regression analysis was performed at nine wavelengths. This type of statistical test consists in multiple correlations of absorbances at different wavelengths with the concentration of the ingredients of the shampoo determined by classical methods. Correlation coefficients of 0.99 were obtained for water, solids and detergent with a rather lower correlation for glycerol, which at 1% in the matrix was close to the limits of detection. The technique was deemed suitable for flow through monitoring. The computer monitoring of the the process by NIRA could be used to control actuators and valves within the chemical processing plant.

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