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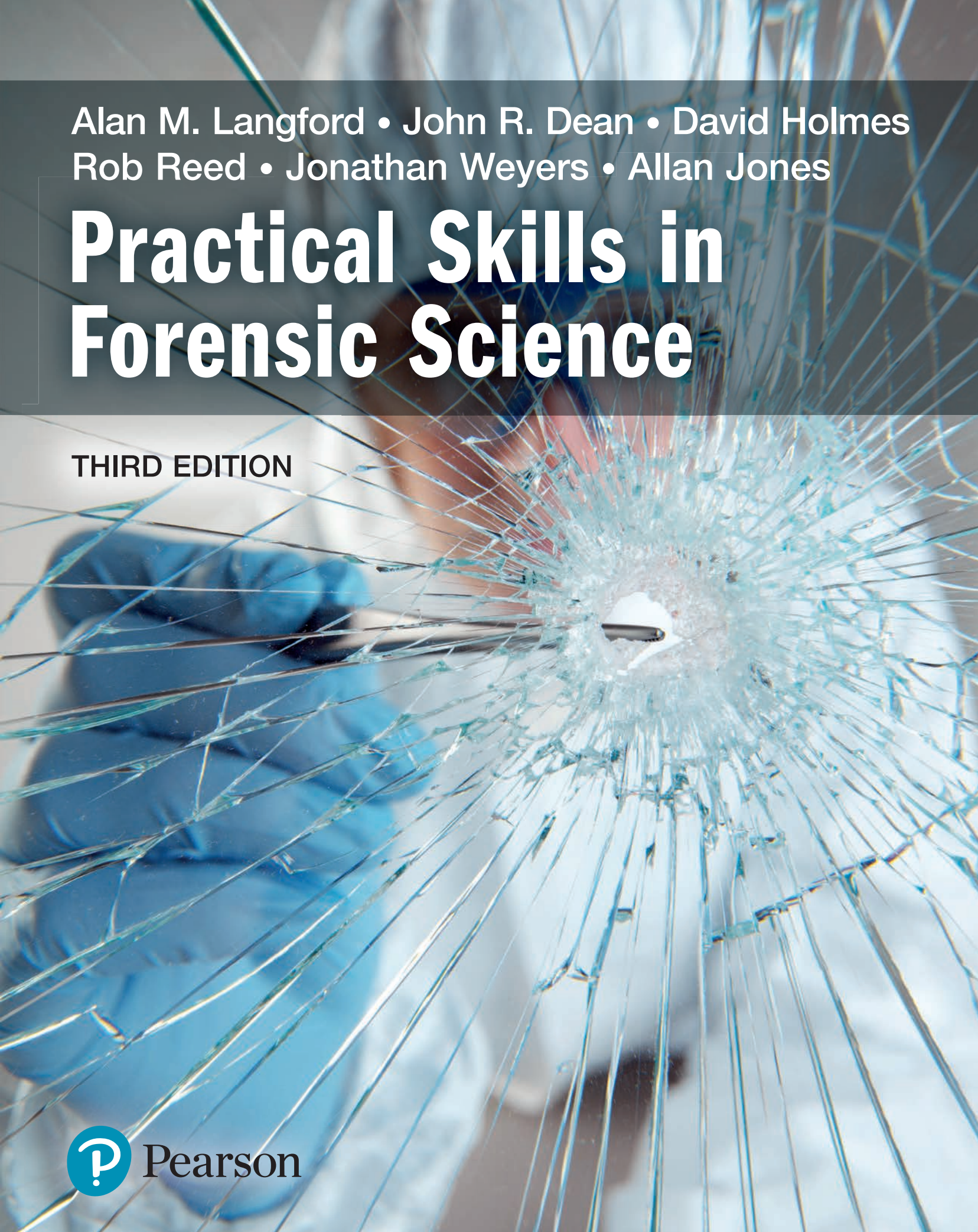
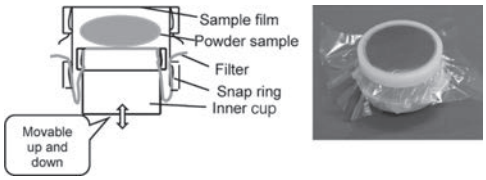
Practical Skills in Forensic Science

THIRD EDITION



Pearson

Practical Skills in Forensic Science



(a) Structure of cup (b) Cup with sample

Fig. 18.8 Cup arrangement.

(a) Structure of cup (b) Cup with sample

Fig. 18.9 Alternative cup arrangement.

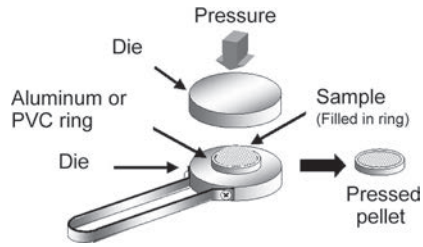


Fig. 18.10 Sample preparation with flat type dies.

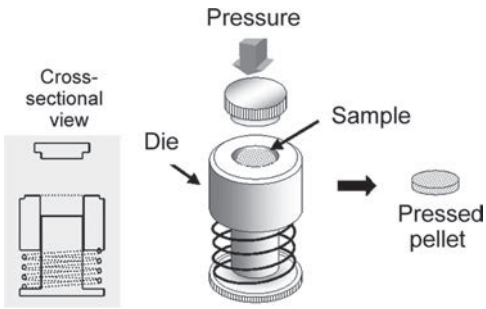


Fig. 18.11 Sample preparation with cylinder type dies.

support. Alternatively, the sample can be presented as a thin film by applying the liquid sample to the surface of a support material (e.g. filter paper or the sample cup itself). The liquid sample can then be analysed directly, wet in the case of the filter paper, or dry by evaporating the sample directly in the cup. A variation on this approach is to use an ion-exchange resin to pre-concentrate the elements from the liquid sample, filter the resin (now containing the elements) and analyse this directly.

Sample preparation for solid samples

Solid objects can be placed directly into the sample chamber of the XRF spectrometer. However, several factors can influence the analysis of solid samples – surface roughness, particle shape and size, homogeneity, particle distribution and mineralisation.

Loose powders can be analysed by simply filling a sample cup to approximately three-quarters without any need for additional sample preparation. However, while this approach will give results, it has significant shortcomings due in part to the heterogeneity of most samples and to variation in particle size of most loose powders. Figure 18.8 shows a sample cup where the loose powder is supported between a sample film attached to the sample cup and a semi-permeable (microporous) filter. An alternative approach is shown in Fig. 18.9. In each case the sample cups are reusable when cleaned.

When pelletising samples, there is a possibility that contamination can occur due to previously pelletised sample remaining on the die surface. Therefore, it is recommended to clean the die surface every time before pelletisation and to prepare samples starting with lower concentrations. Use of the film is effective not only to minimise contamination but also for samples such as iron or titanium oxides, which cannot be easily pelletised since a significant amount of powder can stick to the die surface. Sample films such as polypropylene or polyester can be used as a film for pelletisation. If a sample needs to be repelletised due to breakage, contamination due to ring or cup material can occur.

Pressed pellets can be prepared by pressing powders filled in a ring or a cup using a set of dies and a press machine. There are two types of dies – namely flat disc (Fig. 18.10) and cylinder types (Fig. 18.11). The type used depends on the characteristic of the powder sample. Ease of pelletisation depends on sample characteristics and grain size, and can be improved by sufficient pulverisation.

Box 18.2 How to prepare a loose powder sample for XRF analysis

1. **Dry and grind the loose powder sample** to a particle size corresponding to 300–400 mesh (15–35 μm) or better.
2. **Mix a portion of the dried powder with a binder** – for example, paraffin or cellulose (10:2 w/w). The binder helps to hold the finished pellet together.
3. **Clean the die** – this can be done by wiping with methanol or another solvent.
4. **Insert the backing (i.e. an aluminium cap) into the die and accurately weigh the sample added** – for example, 4 g. It is important to keep the weight constant to allow consistent results.
5. **Place a polished pellet over the sample** – to produce a smooth finish.
6. **Insert the plunger and position the die in the press** – press the pellet at a pressure of 5–30 tonnes maintained for approximately 60 s.
7. **Remove the pellet from the die** – taking care not to crack it in the process. The pellet is then ready for analysis.

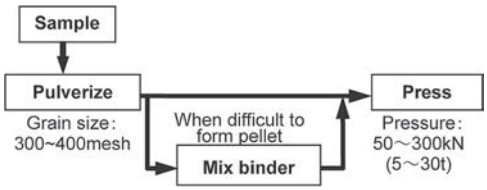


Fig. 18.12 Process for making a pressed pellet.

Forensic environmental application

A forensic environmental sample was subjected to EDXRF. The soil sample was prepared by air-drying it for 48 h prior to grinding and sieving. The sample was prepared as indicated in Box 18.2. The pelletised soil sample was then analysed. The results, shown in Fig. 18.15 as a plot of energy against signal, identify the range of elements present in the sample. By calibration of the EDXRF, the elements identified can be converted into quantitative data.

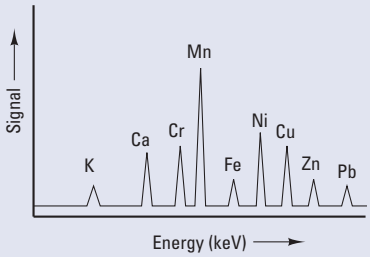
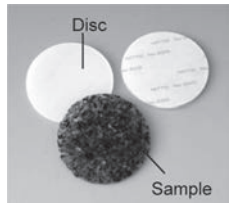


Fig. 18.15 Representative EDXRF trace of a forensic sample.

Mixing the powder sample with a forming agent ('binder') is another solution if pelletisation is difficult (Fig. 18.12). Without a binder, fine powder particles may fall off or scatter from the pellet surface and cause contamination of the spectrometer's sample chamber in vacuum mode. Powders in which particles are spherically shaped, such as SiO_2 or burned ash, are difficult to pelletise. Mixing ratio of sample to binder is typically 10 (sample):1(binder) or 10:2. It is also necessary to determine the purity of the binder as the binder choice should not include the elements to be analysed. Binders typically used are wax types – for example, SpectroBlend®, polystyrene-based powders, or boric acid and cellulose powders. Accurate weighing and complete mixing is essential to minimise analysis errors. An example of pressed pellets is shown in Fig. 18.13.

Manual press machines are available capable of delivering a 300 kN maximum load (Fig. 18.14). The press can be used for pelletisation with flat and cylinder type dies. The procedure for preparation of a pressed pellet is shown in Box 18.2.



Pressed samples

Fig. 18.13 Pressed samples.

Fig. 18.14 A manual press.

Sources for further study

Jenkins, R. (1999) *X-ray Fluorescence Spectrometry,* 2nd edn. Wiley, Chichester.

Potts, P.J. and West, M. (eds) (2008) *Portable X-ray Fluorescence Spectrometry: Capabilities for in situ Analysis.* Royal Society of Chemistry, Cambridge.

Ritz, K, Dawson, L. and Miller, D. (eds) (2009) *Criminal an Environmental Soil Forensics.* Springer, Netherland

Vandecasteele, C. and Block, C.B. (1993) *Modern Methods for Trace Element Determination.* Wiley, Chichester.

Study exercises

18.1 Explain the principle of X-ray fluorescence spectroscopy – in a manner that would be understandable in court. Ask a colleague to listen to your explanation and provide feedback.

18.2 Test your knowledge. What are the main types of interferences associated with XRF and explain how they occur and how to avoid them.

18.3 Analysing a sample of soil by XRF. After reading this chapter, prepare a detailed stepwise protocol explaining how to prepare the soil sample and analysing it by XRF. Ask another student to evaluate your protocol and provide you with written feedback – either simply by reading through your protocol, or by trying it out as part of a class exercise (check with a member of staff before you attempt this in a laboratory).

19 Infrared and Raman spectroscopy

Identifying compounds *– the combination of techniques described in this and the following chapters can often provide sufficient information to identity a compound with a low probability of error.*

Definitions

Spectrometry – any technique involving the measurement of a spectrum, e.g. of electromagnetic radiation, molecular masses.

Spectroscopy – any technique involving the production and subsequent recording of a spectrum of electromagnetic radiation, usually in terms of wavelength or energy.

Wavenumber – the reciprocal of the wavelength (expressed as cm^{-1}): a term used widely in IR spectroscopy, but rarely in other types of analysis.

Interpreting spectra *– the spectrum produced in UV–vis, IR and NMR spectroscopy is a plot of wavelength or frequency or energy (x-axis) against absorption of energy (y-axis). Convention puts high frequency (high energy, short wavelength) at the left-hand side of the spectrum.*

Understanding the origins of IR and Raman spectra – *the IR spectrum is due to changes in charge displacement in bonds. The Raman spectrum is due to changes in polarisability in bonds.*

In addition to ultraviolet–visible (UV–vis) spectroscopy (p. 154), there are three other essential techniques that you will encounter during your laboratory course. They are:

1. ***Infrared (IR) and Raman spectroscopy*** – this is concerned with the energy changes involved in the stretching and bending of covalent bonds in molecules.
2. ***Nuclear magnetic resonance (NMR) spectroscopy*** – this involves the absorption of energy by specific atomic nuclei in magnetic fields and is probably the most powerful tool available for the structural determination of molecules (Chapter 20).
3. ***Mass spectrometry (MS)*** – this is based on the fragmentation of compounds into smaller units. The resulting positive ions are then separated according to their mass-to-charge ratio (m/z) (Chapter 15).

As with UV–vis spectroscopy, IR and NMR spectroscopy are based on the interaction of electromagnetic radiation with molecules, whereas MS is different in that it relies on high-energy particles (electrons or ions) to break up the molecules. The relationship between the various types of spectroscopy and the electromagnetic spectrum is shown in Table 19.1.

Infrared and Raman spectroscopy

Both of these techniques involve the measurement of frequencies produced by the vibration of chemical bonds (bending and stretching). The IR/Raman region is generally considered to be from 800 to 2,500 nm (for near-IR) and up to 16,000 nm (for mid-IR). Near-IR spectroscopy involves recording the spectrum in that region in a manner analogous to UV/visible spectroscopy, and quantitative analysis is possible. However, the most widely used technique is mid-IR spectroscopy, which allows identification of groups or atoms in a sample compound, but is inappropriate for quantitative measurement. A peak at a particular frequency can be identified by reference to libraries or computer databases of IR spectra – e.g. a peak at a wavenumber of 1,730–1,750 cm^{-1} corresponds to a carbonyl group that is present in fatty acids and proteins. The 1,400–600 cm^{-1} region is known as the 'fingerprint' region (see p. 187) because no two compounds give identical spectra.

A covalent bond between two atoms can be crudely modelled as a spring connecting two masses and the frequency of vibration of the spring is defined by Hooke's law (eqn [19.1]), which relates the frequency of the vibration (ν) to the strength of the spring, expressed as the force constant (k), and to the masses (m_1 and m_2) on the ends of the spring (defined as the reduced mass $\mu = (m_1 \times m_2) \div (m_1 + m_2)$).

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \qquad [19.1]$$

In simple terms, this means that:

- the stretching vibration of a bond between two atoms will increase in frequency (energy) on changing from a single bond to a double bond and then to a triple bond between the same two atoms (masses), i.e. the spring gets stronger. For example,

$$\nu \text{ for } C{\equiv}C > \nu \text{ for } C{=}C > \nu \text{ for } C{-}C$$

Table 19.1 The electromagnetic spectrum and types of spectroscopy

Type of radiation	Origin	Wavelength	Type of spectroscopy
γ-rays	Atomic nuclei	$<$0.1 nm	γ-ray spectroscopy
X-rays	Inner shell electrons	0.01–2.0 nm	X-ray fluorescence (XRF)
Ultraviolet (UV)	Ionisation	2.0–200 nm	Vacuum UV spectroscopy
UV/visible	Valency electrons	200–800 nm	UV/visible spectroscopy
Infrared	Molecular vibrations	0.8–300 mm	IR and Raman spectroscopy
Microwaves	Molecular rotations	1 mm to 30 cm	Microwave spectroscopy
	Electron spin		Electron spin resonance (ESR)
Radio waves	Nuclear spin	0.6–10 m	Nuclear magnetic resonance (NMR)

- as the masses of the atoms on a bond increases, the frequency of the vibration decreases, i.e. the effect of reducing the magnitude of μ. For example,

$$\nu \text{ for C—H} > \nu \text{ for C—C} > \nu \text{ for C—H} > \nu \text{ for C—D;}$$

$$\nu \text{ for O—H} > \nu \text{ for S—H}$$

Bonds can also bend, but this movement requires less energy than stretching and thus the bending frequency of a bond is always *lower* than the corresponding stretching frequency. When IR radiation of the same frequency as the bond interacts with the bond, it is absorbed and increases the amplitude of vibration of the bond. This absorption is detected by the IR spectrometer and results in a peak in the spectrum. For a vibration to be detected in the IR region, the bond must undergo a change in dipole moment when the vibration occurs. Bonds with the greatest change in dipole moment during vibration show the most intense absorption, for example C=O and C—O.

IR absorption bands *– since the frequency of vibration of a bond has a specific value, you would expect to see line spectra on the chart. However, each vibration is associated with several rotational motions and bands (peaks) are seen in the spectrum.*

Since bonds between specific atoms have particular frequencies of vibration, IR spectroscopy provides a means of identifying the type of bonds in a molecule – for example all alcohols will have an O—H stretching frequency and all compounds containing a carbonyl group will have a C=O stretching frequency. This property, which does not rely on chemical tests, is extremely useful in diagnosing the functional groups within a covalent molecule.

IR spectra

A typical IR spectrum is shown in Fig. 19.1 and you should note the following points:

- **The *x*-axis, the wavelength of the radiation,** is given in wavenumbers ($\bar{\nu}$) and expressed in reciprocal centimetres (cm^{-1}). You may still see some spectra from old instruments using microns (m, equivalent to the SI unit 'micrometres', mm, at 1×10^{-6} m) for wavelength – the conversion is given by eqn [19.2]:

$$\text{wavenumber } (cm^{-1}) = \frac{1}{\text{wavelength (cm)}} = \frac{10{,}000}{\text{wavelength } (\mu m)} \qquad [19.2]$$

- **The *y*-axis, expressing the amount of radiation absorbed by the molecule,** is usually shown as% transmittance (Fig. 19.1). When no radiation is absorbed (all is transmitted through the sample) we have 100% transmittance, and 0 % transmittance implies that all radiation is absorbed at a particular wavenumber. Since the *y*-axis scale goes from 0 to 100%

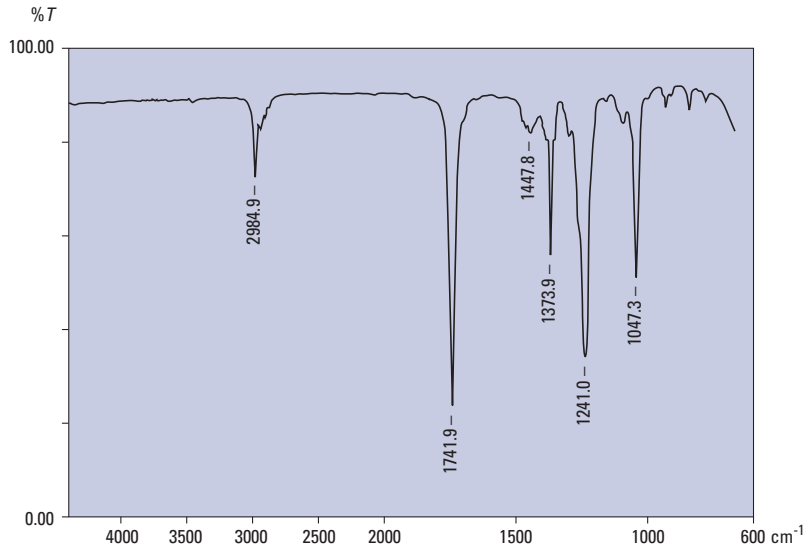


Fig. 19.1 IR spectrum of ethyl ethanoate $CH_3COOCH_2CH_3$ as a liquid film.

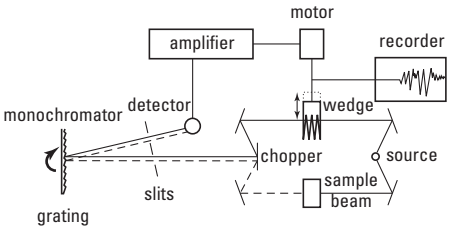


Fig. 19.2 Schematic diagram of a double-beam IR spectrometer.

The use of wavenumber *– this is a long-established convention, since high wavenumber = high frequency = high energy = short wavelength. Expression of the IR range, 4,000 cm^{-1} to 650 cm^{-1}, is in 'easy' numbers and the high energy is found on the left-hand side of the spectrum. Note that IR spectroscopists often refer to wavenumbers as 'frequencies', e.g. 'the peak of the C = O stretching 'frequency' is at 1,720 cm^{-1}'.*

Using double-beam instruments *– you can identify the sample beam by quickly placing your hand in the beam. If the pen records a peak, this is the sample beam, but if the pen moves up, then this is the reference beam.*

Using the 100% control *– if you use this control to set the base line for the sample, you must turn down the 100% control when you remove the sample, otherwise the pen-drive mechanism may be damaged in trying to drive off the top of the chart.*

transmittance, the absorption peaks are displayed *down* from the 100% line; this is *opposite* to most other common spectra.

- **The cells holding the sample** usually display imperfections and are not completely transparent to IR radiation, even when empty. Therefore, the base line of the spectrum is rarely set on 100% transmittance and quantitative applications of IR spectroscopy are more complex than for UV–vis (p. 154).

IR spectrometers

There are two general types:

1. **Double-beam or dispersive instruments** – in which the IR radiation from a single source is split into two identical beams. One beam passes through the sample, and the other is used as a reference and passes through air or the pure solvent used to dissolve the sample. The difference in intensity of the two beams is detected and recorded as a peak. The principal components of this type of instrument are shown in Fig. 19.2. The important controls on the spectrometer are:
   (a) **scan speed** – this is the rate at which the chart moves; slower for greater accuracy and sharp peaks;
   (b) **wavelength range** – the full spectrum or a part of the IR range may be selected;
   (c) **100% control** – this is used to set the pen at the 100% transmittance line when no sample is present (base line). It is usual practice to set the pen at 90% transmittance at 4,000 cm^{-1} when the sample is present, to give peaks of the maximum deflection.

   You should remember that this is an electromechanical instrument and you should always make sure that you align the chart against the calibration marks on the chart holder. In the more advanced instruments, an on-board computer stores a library of standard spectra, which can be compared with your experimental spectrum.
2. **Fourier transform infrared (FTIR) spectrometer** – the value of IR spectroscopy is greatly enhanced by Fourier transformation, named after the mathematician J.B. Fourier. The FT is a procedure for interconverting frequency functions and time or distance functions. In FTIR, information is

Box 19.1 How to run an infrared spectrum of a liquid or solid film, mull or KBr disk

A. Double-beam spectrometer

1. **Ensure that the instrument is switched on** and that it has had a few minutes to warm up.
2. **Make sure that the chart is aligned with the calibration marks on the chart bed or chart drum.** Most spectrometers scan from 4,000 cm^{-1} to 650 cm^{-1} and the pen should be at the 4,000 cm^{-1} mark.
3. **Adjust the 100% transmittance control to about 90%,** if necessary.
4. **Place the sample cell in the sample beam and adjust the 100% transmittance control to 90%,** or the highest value possible.
5. **Select the scan speed.** You must balance the definition required in the spectrum with the time available for the experiment. For most qualitative applications, the fastest setting is satisfactory.
6. **Press the 'scan' or 'start' button to run the spectrum.** The spectrum will be recorded and the spectrometer will automatically align itself at the end of the run. *Do not press* any other buttons while the spectrum is running or the instrument may not realign itself at the end of the run.
7. **Adjust the 100% transmittance control to about 50%,** remove the sample cell from the spectrometer and turn the 100% transmittance control to about 90%.
8. **Enter all of the following data on the spectrum** – name, date, compound and phase (liquid film, Nujol® mull, KBr disk, etc.).

B. FTIR spectrometer

1. **Make sure that the sample compartment is empty** and close the lid.
2. **Select the number of scans** – usually four is adequate for routine work.
3. **Select 'background' on the on-screen menu,** and scan the background. *Do not press* any other buttons or icons while the spectrum is running.
4. **Place the sample cell in the beam,** close the lid, select 'sample' and scan the sample. *Do not press* any other buttons or icons while the spectrum is running.
5. **Select 'customise',** or a similar function, and enter all the data – name, date, compound, phase (liquid film, Nujol mull, KBr disk, etc.) – on the spectrum.
6. **Select 'print'** to produce the spectrum from the printer.

Problems with IR spectra (and solutions)

These are usually caused by poor sample preparation and the more common faults are:

1. **The large peaks have tips below the bottom of the chart or the large peaks have 'squared tips' near the bottom of the chart** – the sample is too thick; remove some sample from the cell and rerun the spectrum.
2. **The spectrum is 'weak', i.e. few peaks** – the sample is too thin; add more sample or remake the KBr disk.
3. **The base line cannot be adjusted to 90% transmittance** – the NaCl plates or KBr disk are 'fogged', scratched or dirty; replace or remake the KBr disk.
4. **The pen tries to 'go off' the top of the spectrum** – obviously due to some absorption at 4,000 cm^{-1} when you were setting the base line. Repeat baseline set-up but at 80% transmittance; and bear in mind that dirty plates, above, can be the cause.

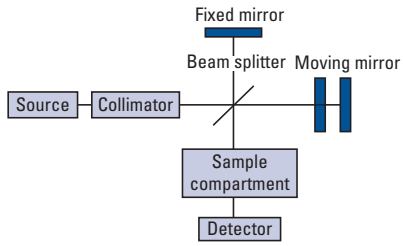


Fig. 19.3 Schematic diagram of an FT-IR spectrometer.

obtained from an interferometer, which splits the incident beam so that it passes through both the sample and a reference. When the beam is recombined, interference patterns arise because the two path lengths are different. The interference pattern has the same relationship to a normal spectrum as a hologram has to a picture, and integral computers use FT to convert the pattern into a spectrum in under a minute. A simple schematic diagram of an FT-IR spectrometer is shown in Fig. 19.3. The overall result is a greatly enhanced signal:noise ratio. The advantages of FTIR are:

(a) **rapid scanning speed** – typically four scans can be made per minute, allowing addition of the separate scans to enhance the signal:noise ratio and improve the resolution of the spectrum;