29 Techniques of analysis

In this topic we look at various methods used to separate mixtures of compounds and identify their components. We also explain the principles and applications of three major analytical techniques used to investigate the structures of molecules: mass spectrometry, infrared spectroscopy and nuclear magnetic resonance spectroscopy.

Learning outcomes

By the end of this topic you should be able to:

22.1a) explain and use the terms R_f value in thin layer chromatography and retention time in gas–liquid chromatography from chromatograms
22.1b) interpret gas–liquid chromatograms in terms of the percentage composition of a mixture
22.2a) analyse an infrared spectrum of a simple molecule to identify functional groups
22.3a) deduce the molecular mass of an organic molecule from the molecular ion peak in a mass spectrum
22.3b) deduce the number of carbon atoms in a compound using the M+1 peak
22.3c) deduce the presence of bromine and chlorine atoms in a compound using the M+2 peak
22.3d) suggest the identity of molecules formed by simple fragmentation in a given mass spectrum
22.4a) analyse a carbon-13 NMR spectrum of a simple molecule to deduce the different environments of the carbon atoms present, and the possible structures for the molecule
22.4b) predict the number of peaks in a carbon-13 NMR spectrum for a given molecule
22.5a) analyse and interpret a proton NMR spectrum of a simple molecule to deduce the different types of proton present using chemical shift values, the relative numbers of each type of proton present from relative peak areas, the number of non-equivalent protons adjacent to a given proton from the splitting pattern, using the $n + 1$ rule, and the possible structures for the molecule
22.5b) predict the chemical shifts and splitting patterns of the protons in a given molecule
22.5c) describe the use of tetramethylsilane, TMS, as the standard for chemical shift measurements
22.5d) state the need for deuterated solvents, e.g. $CDCl_3$, when obtaining an NMR spectrum
22.5e) describe the identification of O—H and N—H protons by proton exchange using D_2O.

29.1 Chromatography

The name 'chromatography', coming from two Greek words meaning 'colour image', was invented by the Russian scientist Mikhail Tsvet. He developed the technique around the start of the twentieth century to separate the carotenes, chlorophyll and xanthophylls that are the coloured pigments in plants. Today, however, most chromatography is carried out to separate colourless compounds, which can then be identified by a variety of methods.

We shall look at three types of chromatography:

- paper chromatography (PC)
- thin layer chromatography (TLC)
- gas chromatography (GC), otherwise known as gas–liquid chromatography (GLC).

Each type of chromatography relies on the same overall principle for separating the components of a mixture: the compounds are distributed between a *mobile* (or *moving*) *phase* (a liquid or a gas) and a *stationary phase*. The stationary phase may be a solid which adsorbs the mixture solutes, or a thin film of liquid on the surface of an inert solid.

Paper chromatography (PC)

Although it may appear dry to the touch, chromatography paper (which is like smooth filter paper but with an accurate and constant thickness) contains water molecules hydrogen-bonded to the OH groups on its cellulose molecules. This layer of water molecules is the stationary phase. The moving phase is chosen to be less polar than water. It is usually an organic solvent, or a mixture of solvents: ethanol or an ethanol–water mixture is often used.

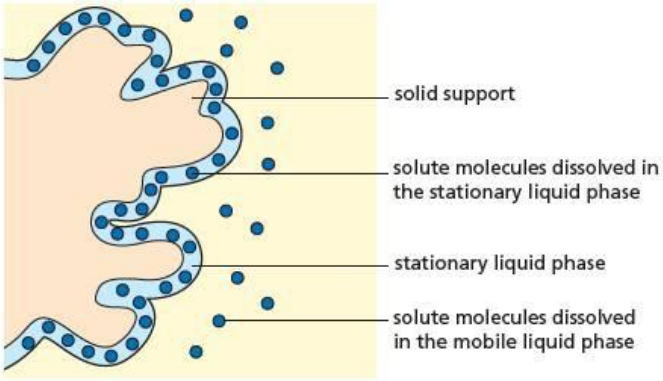


Figure 29.1 Separation by partition

A spot of the solution of the mixture to be analysed is placed about 1 cm from the edge of a rectangular sheet of chromatography paper (often about 20 cm × 20 cm), along with other spots of 'reference' compounds at the same distance from the edge. After the mixture solvent has evaporated, the edge of the sheet is placed in a chromatography 'tank' and immersed in the moving phase solvent, making sure the spots are above the surface of the solvent. The solvent is drawn up the sheet by capillary action. As it passes the point where the spots have been applied, the compounds in the mixture are partitioned between the stationary water layer and the moving solvent, depending on their polarity (Figure 29.1). Although the process is continually changing and never reaches equilibrium because fresh solvent is constantly sweeping up the paper, how quickly the spots move up the paper is determined by the values of their partition coefficients between the solvent and water. The more polar a compound is, the smaller is its partition coefficient. This means it will spend more time dissolved in the water layer on the cellulose than in the moving solvent, and it will progress more slowly up the paper.

When the solvent has almost reached the end of the sheet of paper, the paper is taken out of the tank and allowed to dry. As the solvent evaporates, the solutes will stay at the places they had reached on the paper. If they are not already coloured, they can be made visible by spraying the paper with a specific developing agent, which reacts with the compounds in the spots to form a coloured product. Specific agents have been developed for particular classes of compound: ninhydrin for amino

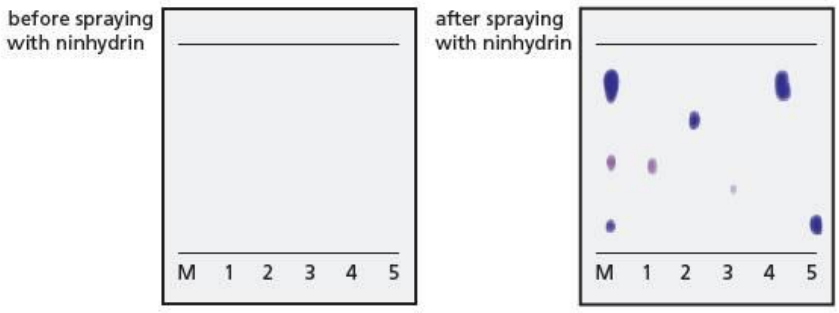
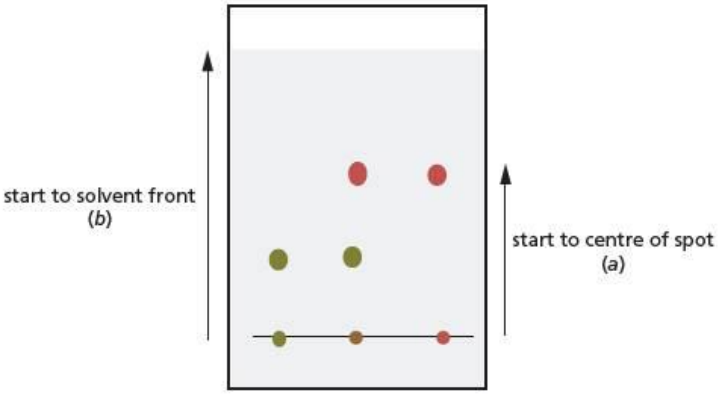


Figure 29.2 A paper chromatogram of an amino acid mixture with five 'references' spotted alongside, before and after development with ninhydrin

acids (Figure 29.2); Tollens' or Fehling's reagents for reducing sugars. A general visualising agent for most organic compounds is iodine: when the paper is placed in a tank containing iodine vapour, the iodine is absorbed preferentially by the organic compounds in the spots, turning them brown.

The usual way of identifying the compounds that make up the various spots on a chromatography sheet is to measure their **retardation factor** R_f values (Figure 29.3). These are compared with the R_f values of known 'reference' compounds, spots of which were applied to the sheet at the same time as the mixture.

Figure 29.3 The retardation factor, R_f



$$R_f = \frac{a}{b} = \frac{22\text{ mm}}{38\text{ mm}} = 0.58$$

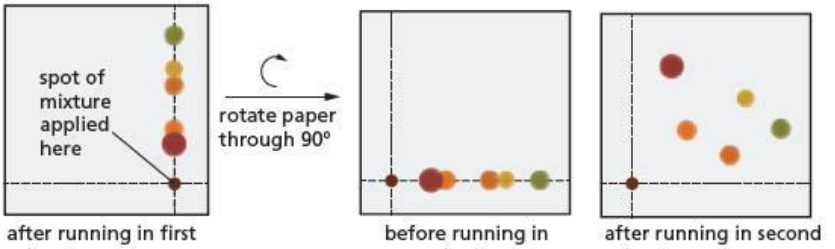
The retardation factor, R_f, is defined by the equation:

$$R_f = \frac{a}{b} = \frac{\text{distance moved by solute}}{\text{distance moved by solvent}}$$

Each compound has a characteristic R_f value for a given solvent, but occasionally different compounds can have very similar R_f values in a particular solvent, and so it is not easy to separate them. If a new solvent – one with a different polarity – is used, the compounds are likely to have different R_f values to each other, so they can now be separated.

This is applied in **two-dimensional chromatography**. This technique uses two moving phase solvents of different polarities. In this technique a spot of the mixture of compounds is placed at a corner of a square sheet of chromatography paper. The sheet is placed in a tank containing the first solvent, and is left until the solvent front reaches the far edge of the sheet. The sheet is removed from the tank, and allowed to dry thoroughly. It is then turned through 90° and placed in a different tank containing the second solvent, so that the spots that have been partially separated by the first solvent lie along the bottom of the sheet, just above the level of the second solvent in the tank. When the second solvent front has reached the end of the sheet, it is removed, dried and sprayed with the developing agent (Figure 29.4). The R_f values in each solvent can be measured, and compared with those of reference compounds. This type of chromatography has been used to identify the amino acids obtained from the hydrolysis of proteins.

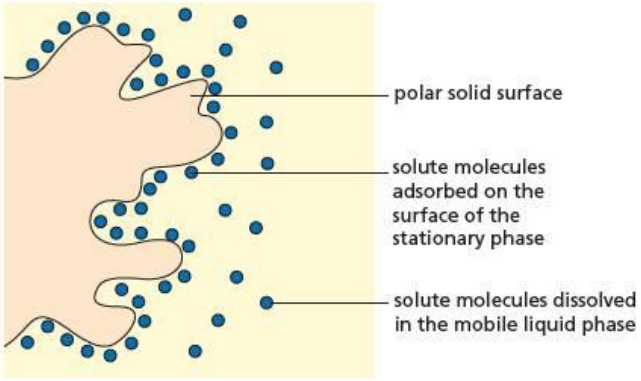
Figure 29.4 Two-dimensional chromatography



Thin layer chromatography (TLC)

The technique of thin layer chromatography is similar to that of paper chromatography, but the theory behind it is rather different. TLC relies on the fact that the attractive forces that cause different compounds to be adsorbed onto a solid surface differ from one compound to another (Figure 29.5). The solid used is usually powdered silica or alumina, with the small size of the particles providing a large surface area per gram, and it is coated onto a glass, plastic or thin aluminium plate.

Figure 29.5 Separation by adsorption



The method used is almost identical to that of paper chromatography. Spots of the mixture to be analysed, along with any reference compounds, are applied close to the lower edge of the TLC plate and allowed to dry. The plate is then placed in a tank containing the solvent, making sure that the solvent level is below the spot.

As the solvent ascends the plate by capillary action, and passes over the compounds which have been adsorbed onto the solid particles, the compounds will partially dissolve in the solvent. How readily this occurs depends on both how soluble a particular compound is in the solvent, and how strong the attraction is between the compound and the solid support. This is how separation is achieved. Once the solvent front is near the top of the plate, the plate is removed, allowed to dry, and the spots can then be made visible. The ways in which spots of colourless compounds on a TLC plate are made visible can be similar to those used for paper chromatography, but an additional technique is often used for compounds containing aromatic rings, or other systems that absorb ultraviolet (UV) radiation. The solid support is pre-impregnated with an insoluble compound that is fluorescent: it absorbs UV light and re-emits it as visible light. When placed under a lamp which shines only UV light, the plate emits a bright white light unless a particular spot contains a compound that absorbs UV light. These spots will show up as dark areas.

TLC has several advantages over paper chromatography: the plates are quicker to run; better separation of the components of a mixture can be achieved; and its results are usually more reproducible. This is because the plates can be made very precisely, with constant very small particle size and moisture content. It is the technique of choice for synthetic chemists wanting to observe the progress of their reactions in real time.

Gas chromatography (GC)

In gas chromatography (GC) the mobile phase is an inert gas such as helium or nitrogen. This is passed through a column made of glass or metal, which is between 1 m and 3 m in length. The column is packed with fine solid particles (brick dust is often used), coated with oil that has a high boiling point (Figure 29.6).

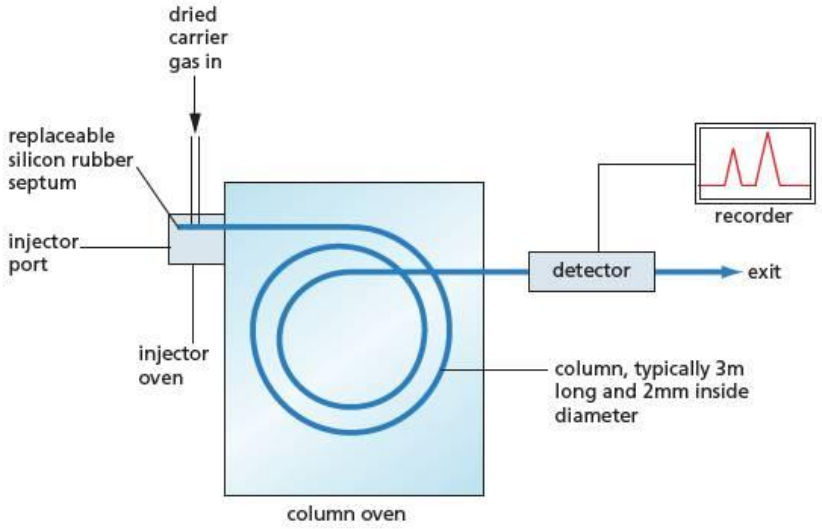


Figure 29.6 Diagram of gas chromatography apparatus

The components of mixtures that are separated by gas chromatography must have a reasonable vapour pressure at the temperature of the oven, which can be up to about 250 °C. The more volatile components spend more time in the vapour phase, and so travel through the column faster: they have shorter **retention times**. Less volatile compounds spend more of their time dissolved in the oil in the column, and so take longer to be carried through the column by the flowing gas: their retention times are longer.

The mixture to be analysed is first injected through a self-sealing disc (a rubber septum) into a small chamber. It is heated to maybe 50 °C above the temperature of the oven, where it is vaporised. The mixture then passes through the column, and separation occurs. The gas emerging from the column passes through a detector, which records the presence and amount of each component on a chart recorder or computer.

Use of gas chromatography in analysis

After a mixture has been separated by gas chromatography its components must be identified. If a sample is being analysed for the presence of known or suspected compounds, such as illegal recreational or performance-enhancing drugs, or the presence of known contaminants in foodstuffs, reference samples of these compounds will be used. If all variables are kept constant throughout the sampling of the mixture and the running of the reference compounds through the same machine, a simple comparison of retention times will allow an identification to be made.

The variables that must be kept the same are:

- the flow rate of the mobile solvent or the carrier gas
- the temperature of the oven
- the length and diameter of the column
- the chemical make-up of the solvent
- the polarity of the stationary phase.

This technique can routinely be coupled to an in-line mass spectrometer. The mass spectrum of each component in a sample can then be found, which enables further verification of the identity of the component. If, on the other hand, the sample is an unknown compound, the mass spectrum can allow its identification. Other physical methods of analysis that can be coupled to gas chromatography machines are infrared spectroscopy and ultraviolet-visible spectroscopy.

Examples of the use of this technique are:

- testing athletes for residues of performance-enhancing drugs in their blood or urine
- detection of explosive residues on skin or clothing
- comparing caffeine contents of various natural and decaffeinated coffees
- detection of pesticide residues in fresh and processed foodstuffs.

29.2 Mass spectrometry

In Topic 2 it was explained how a mass spectrometer can be used to determine the isotopic masses of individual atoms.

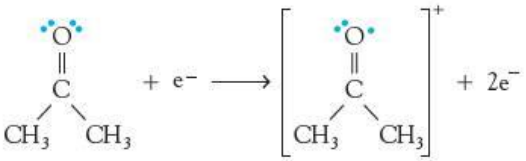
Mass spectrometry is used in four main ways to determine the structures of organic compounds:

1 finding the molecular formula of a compound by measuring the mass of its molecular ion to a high degree of accuracy
2 finding the number of carbon atoms in a molecule by measuring the abundance ratio of its molecular ion (M) peak and the M+1 peak
3 finding whether a compound contains chlorine or bromine atoms, and if so, how many of each, by measuring the abundance ratios of the M+2, M+4 and M+6 peaks.
4 working out the structure of a molecule by looking at the fragments produced when an ion decomposes inside a mass spectrometer.

Analysing the molecular ion

If we vaporise an organic molecule and subject it to the ionising conditions inside a mass spectrometer, the mass/charge ratio (m/e) for the molecular ion can be measured, and hence the relative molecular mass can be found.

For example, one of the non-bonding electrons on the oxygen atom of propanone can be removed by electron bombardment, to give an ionised molecule:



The m/e ratio for the resulting molecular ion is $(3 \times 12 + 6 \times 1 + 16) : 1$, which is 58.

Using **very high resolution mass spectrometry**, we can measure m/e ratios to an accuracy of five significant figures (1 part in 100 000). By this means, it is not only possible to measure the M_r value of a compound (its relative molecular mass), but also to determine its molecular formula. We can do this because the accurate atomic masses of individual atoms are not exact whole numbers.

Worked example

The three compounds in Table 29.1 all have an approximate M_r of 70.

Table 29.1 Three compounds with M_r of approximately 70

Name	Structure	Molecular formula
pentene	$CH_3CH_2CH_2CH{=}CH_2$	C_5H_{10}
2-aminopropanenitrile	$CH_3CH(NH_2)CN$	$C_3H_6N_2$
but-1-en-3-one	$CH_2{=}CHCOCH_3$	C_4H_6O

Use the following accurate relative atomic masses to calculate their accurate M_r values, and decide how sensitive the mass spectrometer needs to be in order to distinguish between them:

H = 1.0078
C = 12.000
N = 14.003
O = 15.995

Answer

The accurate M_r values are as follows:

C_5H_{10} $= 5 \times 12.000 + 10 \times 1.0078 = 70.078$
$C_3H_6N_2$ $= 3 \times 12.000 + 6 \times 1.0078 + 2 \times 14.003 = 70.053$
C_4H_6O $= 4 \times 12.000 + 6 \times 1.0078 + 15.995 = 70.042$

The last two are quite close together. They differ by 11 parts in 70 000, or about 0.16%. However, this is well within the capabilities of a high resolution mass spectrometer.

Now try this

A compound has an accurate M_r of 60.068. Use the accurate relative atomic masses given above to decide whether the compound is 1,2-diaminoethane, $H_2NCH_2CH_2NH_2$, or ethanoic acid, CH_3CO_2H.

The M+1 peak

There are two stable isotopes of carbon, ^{12}C and ^{13}C. Their relative abundances are 98.9% for ^{12}C and 1.1% for ^{13}C. This means that out of every 100 methane (CH_4) molecules, CH_4, about 99 molecules will be $^{12}CH_4$ and just one molecule will be $^{13}CH_4$. For ethane, C_2H_6, the chances of a molecule containing one ^{13}C atom will have increased to about 2 in 100, because each C atom has a chance of 1 in 100 to be ^{13}C, and there are two of them. By measuring the ratio of the M to M+1 peaks, we can thus work out the number of carbon atoms the molecule contains. The formula relating the (M+1)/M ratio to the number of carbon atoms is:

$$n = \frac{100}{1.1} \times \frac{A_{M+1}}{A_M}$$

where n = number of carbon atoms
A_{M+1} = the abundance of the M+1 peak, and
A_M = the abundance of the molecular ion peak.

The abundances of the M and M+1 peaks are sometimes quoted as percentages, and sometimes as the actual heights of the two peaks on a printout of the mass spectrum, in arbitrary units. The units do not matter, however, as it is only the *ratio* that is important.

Worked example

The molecular ion peak of a compound has an *m/e* value of 136, with a relative abundance of 17%, and an M+1 peak at *m/e* 137 where the relative abundance is 1.5%. How many carbon atoms are in the molecule?

Answer

$$n = \frac{100}{1.1} \times \frac{1.5}{17} = 8.02$$

A molecule of the compound therefore contains 8 carbon atoms.

Now try this

A compound contains C, H and O atoms. Its mass spectrum has a peak at *m/e* 132 with a relative abundance of 43.9 and a peak at *m/e* 133 with a relative abundance of 2.9.

Calculate the number of carbon atoms in each molecule, and suggest its molecular formula.

The M+2 and M+4 peaks

Although fluorine and iodine each have only one stable isotope, chlorine and bromine have two. Their natural percentage abundances are shown in Table 29.2.

Table 29.2 The abundances of the isotopes of chlorine and bromine

Element	Isotope	Natural abundance	Approximate ratio
chlorine	^{35}Cl	75.5	3 : 1
	^{37}Cl	24.5	
bromine	^{79}Br	50.5	1 : 1
	^{81}Br	49.5	

Any compound containing one chlorine atom, therefore, will have two 'molecular ion' peaks, one due to molecules containing ^{35}Cl and the other due to molecules containing ^{37}Cl. For example, the mass spectrum of chloromethane, CH_3Cl, will have peaks at *m/e* 50 (12 + 3 + 35 = 50) and at *m/e* 52 (12 + 3 + 37 = 52), corresponding to the species $CH_3{}^{35}Cl^+$ and $CH_3{}^{37}Cl^+$. The relative abundances of the two peaks will be in the ratio 3 : 1 which is the ratio of the two Cl isotopes.

A similar situation occurs with bromine, although in this case the two molecular ion peaks will be of equal heights, since the isotopic abundance ratio is near to 1 : 1.

Mass spectra are slightly more complicated when the molecule contains more than one halogen. The simplest situation is that for two bromine atoms. Take the molecule 1,2-dibromoethane, $C_2H_4Br_2$. Each carbon can be attached to either a ^{79}Br or a ^{81}Br atom, and there is a (roughly) equal chance of either. We therefore arrive at the possibilities in Table 29.3, each of which is equally likely.

Table 29.3

Formula	*m/e* value	Peak
$^{79}BrCH_2CH_2{}^{79}Br$	186	M
$^{79}BrCH_2CH_2{}^{81}Br$	188	M + 2
$^{81}BrCH_2CH_2{}^{79}Br$	188	M + 2
$^{81}BrCH_2CH_2{}^{81}Br$	190	M + 4

There will thus be three molecular ion peaks, with relative abundances of 1 : 2 : 1.

Worked example

Work out the *m/e* values and the relative abundances of the molecular ion peaks for dichloromethane, CH_2Cl_2.

Answer

Just as with dibromoethane above, there will be four possible formulae. Their *m/e* values are given in the following table.

Formula	Ion reference	*m/e* value
$CH_2{}^{35}Cl{}^{35}Cl$	***a***	84
$CH_2{}^{35}Cl{}^{37}Cl$	***b***	86
$CH_2{}^{37}Cl{}^{35}Cl$	***c***	86
$CH_2{}^{37}Cl{}^{37}Cl$	***d***	88

The abundance ratio of ion ***a***: ion ***b*** is 3 : 1 (because of the ^{35}Cl : ^{37}Cl ratio)
The abundance ratio of ion ***c***: ion ***d*** is also 3 : 1
The abundance ratio of ion ***a***: ion ***d*** is 9 : 1, because each of the two chlorine atoms has 3 times the probability of being ^{35}Cl rather than ^{37}Cl.
The overall probabilities are therefore:

Mass number	84	86	88
individual probabilities	3	1	
	3	1	
	3	1	
		3	1
total sum of probabilities	9	6	1

Analysing molecular fragments

If the ionising electron beam in a mass spectrometer has enough energy, the molecular ions formed by the loss of an electron can undergo bond fission, and molecular fragments are formed (see Figure 29.7). Some of these will carry the positive charge, and therefore appear as further peaks in the mass spectrum.

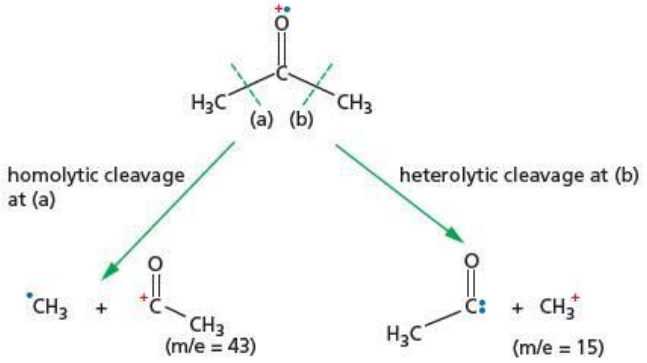


Figure 29.7 Ionic fragments formed from propanone

We therefore expect the mass spectrum of propanone to contain peaks at $m/e = 15$ and 43, as well as the molecular ion peak at 58 (see Figure 29.8).

Figure 29.8 Mass spectrum of propanone

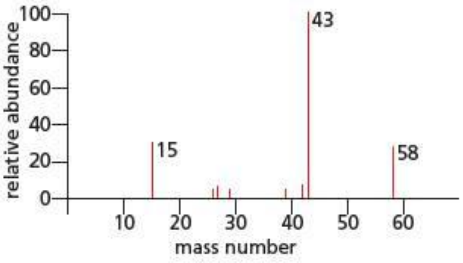
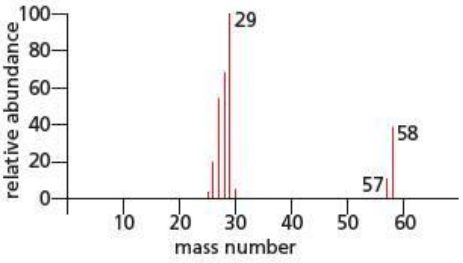
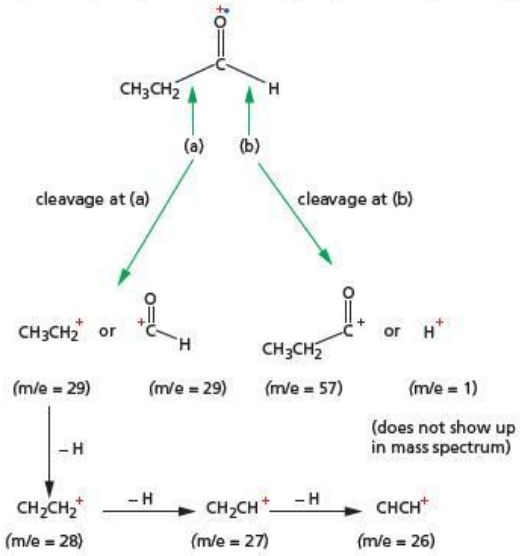


Figure 29.9 Mass spectrum of propanal



The fragmentation pattern can readily distinguish between isomers. Compare Figure 29.8 with Figure 29.9, which shows the mass spectrum of propanal. Here there is no peak at $m/e = 15$, nor one at $m/e = 43$. Instead, there is a peak at $m/e = 57$ and several from $m/e = 26$ to 29. This is readily explained by the fragmentations shown in Figure 29.10.

Figure 29.10 Ionic fragments formed from propanal



Worked example

Use the following atomic mass data to calculate the accurate M_r values for the two ionic fragments at *m/e* 29 in the mass spectrum of propanal. Would a mass spectrometer with a sensitivity of 1 part in 10000 be able to distinguish between them?

H = 1.0078 C = 12.000 O = 15.995

Answer

C_2H_5 is $2 \times 12.000 + 5 \times 1.0078$ = 29.039

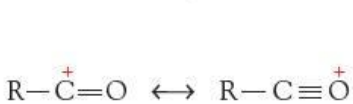
CHO is 12.000 + 1.0078 + 15.995 = 29.003

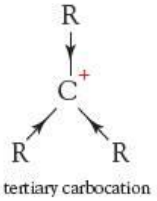
These masses differ by 36 in 29003 or 1 part in 8056, so this (fairly inaccurate) spectrometer would just be able to distinguish between them.

Now try this

Suggest the formulae of the ions at *m/e* values 26, 27 and 28 in the mass spectrum of propanal, and suggest an explanation of how they might arise.

Depending on what type of cleavage occurs at (a) and (b) in Figure 29.10, one or other or both of each pair of ion fragments may appear. The peaks of highest abundance in the mass spectra of organic compounds are associated with particularly stable cations, such as acylium and tertiary carbocations:





The interpretation of the fragmentation pattern in the mass spectra of organic compounds is therefore an important tool in the elucidation of their structures. A further example will show the power of the technique.

Worked example

Figure 29.11 shows the mass spectra of two compounds with the molecular formula $C_2H_4O_2$. One is methyl methanoate, and the other is ethanoic acid. Decide which is which by assigning structures to the major fragments whose *m/e* values are indicated.

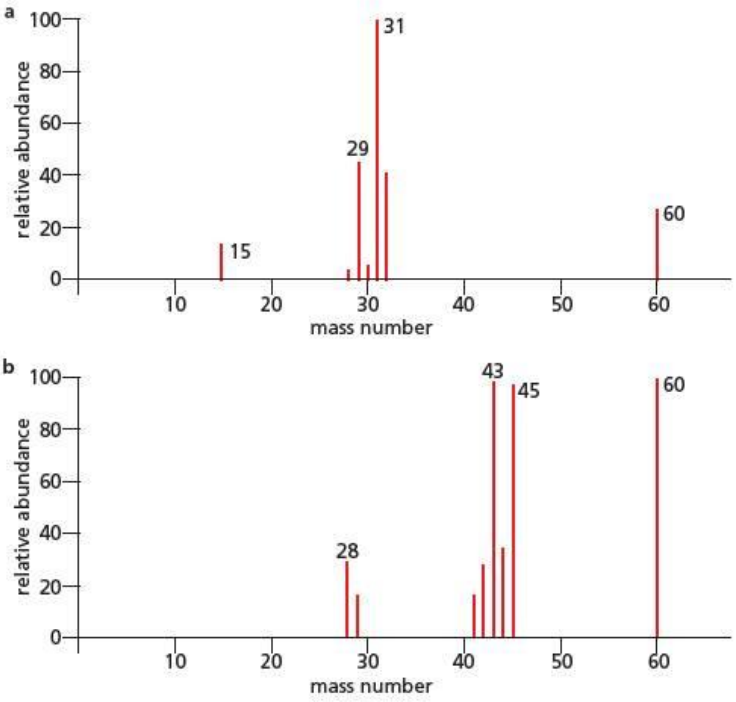


Figure 29.11 Mass spectra of methyl methanoate and ethanoic acid. Which is which?

Answer

Apart from the molecular ion at *m/e* = 60, the major peaks in spectrum **a** are at *m/e* values of 15, 29 and 31. These could be due to:

CH_3^+ (*m/e* = 15)

$C_2H_5^+$ or CHO^+ (*m/e* = 29)

CH_3O^+ (*m/e* = 31)

This fits with the structure of methyl methanoate (see Figure 29.12).

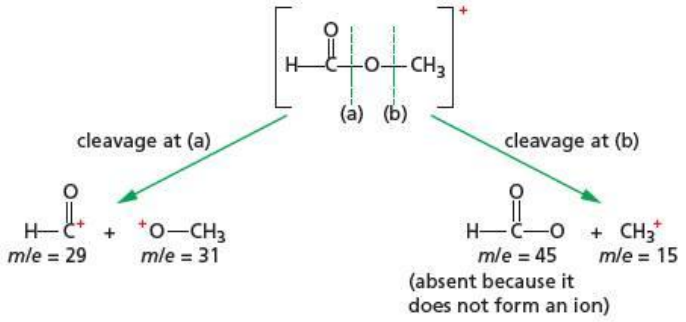


Figure 29.12 Ionic fragments formed from methyl methanoate

The peak at *m/e* = 29 can come *only* from methyl methanoate, and not from ethanoic acid. The major peaks in Figure 29.11b, apart from the molecular ion at *m/e* = 60, are at *m/e* values of 28, 43 and 45. These could be due to:

CO^+ (*m/e* = 28)
CH_3CO^+ (*m/e* = 43)
CO_2H^+ (*m/e* = 45)
These arise from the fragmentations shown in Figure 29.13.

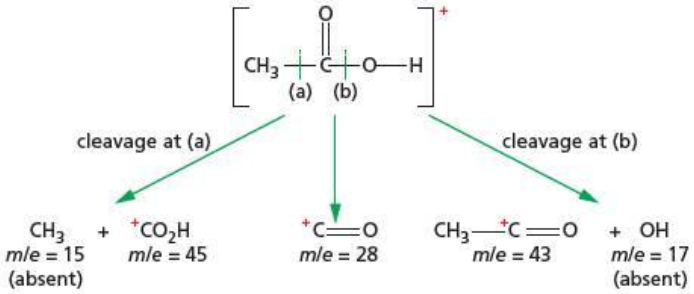


Figure 29.13 Ionic fragments formed from ethanoic acid

Now try this

A compound of molecular formula $C_3H_6O_2$ has major peaks at *m/e* = 27, 28, 29, 45, 57, 73 and 74. Suggest formulae for these fragments, and a structure for the compound.

The peak at *m/e* = 43 can come only from ethanoic acid, and not from methyl methanoate.

29.3 Infrared spectroscopy

Most organic molecules absorb infrared radiation. The frequencies that are absorbed depend on the stiffness of their bonds and the masses of the atoms at each end, and the intensity of an absorption depends on the change in dipole moment as the bond vibrates. So bonds to electronegative atoms such as oxygen, that are found in alcohols (O—H) and carbonyl compounds (C=O) show very strong absorptions. Although an infrared spectrum shows a series of absorptions (see Figure 29.14), these are always referred to as peaks rather than troughs.

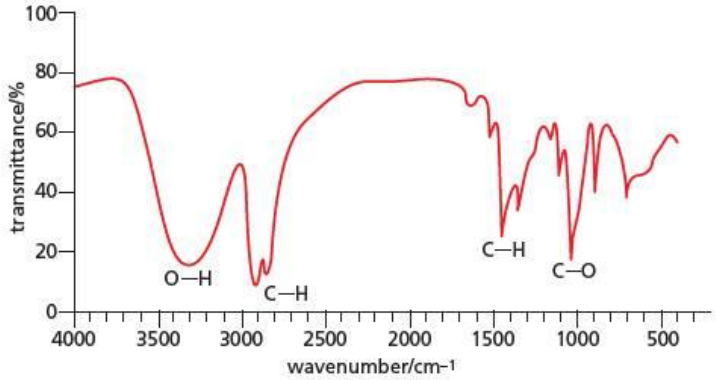


Figure 29.14 The infrared spectrum of hexan-1-ol

The process is important in that it is small molecules in the atmosphere (especially CO_2, CH_4, H_2O and CFCs) that are responsible for the greenhouse effect: they absorb infrared radiation that is emitted from the surface of the Earth, thus preventing it from being lost to space. In consequence, the amount of heat lost is less than that gained from solar radiation, and the Earth warms up (see the greenhouse effect, page 241).

The infrared spectrum of a compound can allow us to identify the functional groups it contains, as each functional group has a characteristic absorption frequency or range of frequencies. The data are listed in Table 29.4.

Table 29.4 Some infrared (IR) absorption frequencies for organic groups (The exact frequency of absorption of a group depends on its molecular environment, and can be 50 cm^{-1} or so higher or lower than the frequencies given here.)

Type of bond	Bond	Frequency of absorption (wavenumber)/cm^{-1}
bonds to hydrogen	O—H	3600
	O—H (hydrogen bonded)	3200–3500
	N—H	3400
	O—H in RCO_2H (strongly hydrogen bonded)	2500–3300
	C—H	2800–2900
triple bonds	–C≡C– or —C≡N	2200
double bonds to oxygen	C=O in RCOCl	1800
	C=O in RCO_2R	1740
	C=O in RCHO	1730
	C=O in RCO_2H	1720
	C=O in R_2CO	1715
C=C double bonds	C=C in alkenes	1650
	C=C in arenes	1600 and 1500
single bonds	C—O	1100–1250

Worked example

Compounds **T** and **U** are isomers with the molecular formula $C_3H_6O_2$. Suggest their structures based on the spectra shown in Figures 29.15 and 29.16.

Figure 29.15 Infrared (IR) spectrum of T

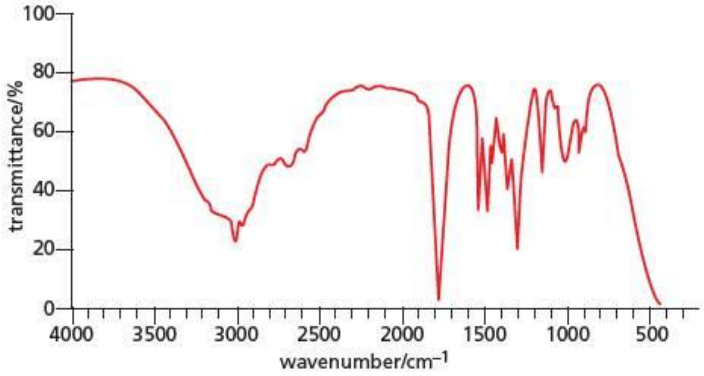
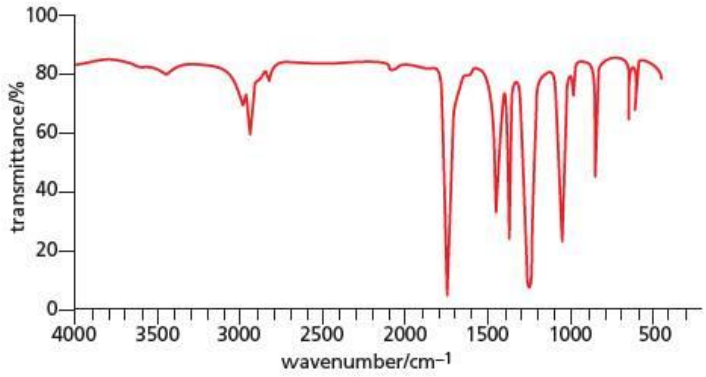


Figure 29.16 IR spectrum of U



Answer

Both **T** and **U** show a C=O absorption in their spectrum at about 1700–1800 cm^{-1}, and a C—O absorption at about 1250 cm^{-1}. **T** shows a broad hydrogen-bonded O—H band from 3300 to 2500 cm^{-1}, whilst **U** shows no O—H band at all.

So **T** is $CH_3CH_2CO_2H$ (propanoic acid) and **U** could be either the ester $CH_3CO_2CH_3$ (methyl ethanoate) or the ester $HCO_2CH_2CH_3$ (ethyl methanoate).

Now try this

Compound **V** (C_3H_6O) gives a silver mirror when warmed with Tollens' reagent. It can be converted to compound **W** by reagent **X**. Use the spectra in Figures 29.17 and 29.18 to identify the functional groups present in **V** and **W**, and suggest the identity of reagent **X**.

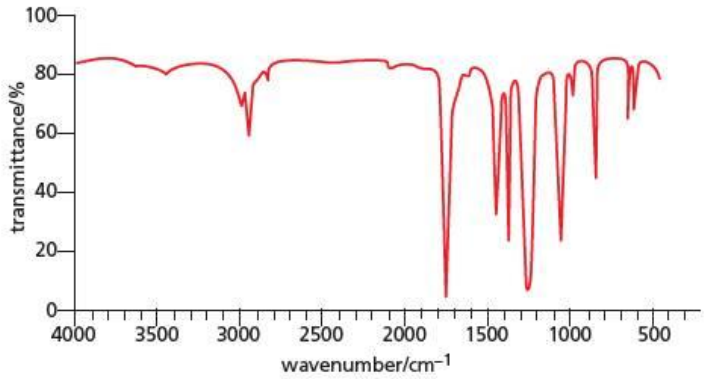


Figure 29.17 Infrared (IR) spectrum of **V**

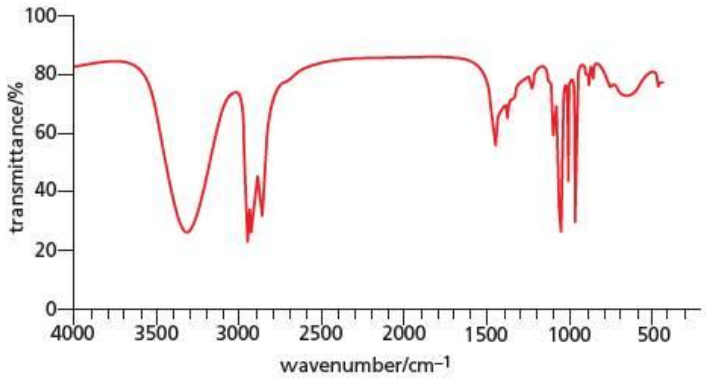


Figure 29.18 IR spectrum of **W**

29.4 Proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopy

The basis of NMR spectroscopy

We cannot see molecules with our naked eyes, but some of the most direct evidence for their structures and shapes comes to us from nuclear magnetic resonance (NMR) spectroscopy. Every aspect of an NMR spectrum demands an exact interpretation, and there is usually a unique molecular structure that gives rise to a particular spectrum. NMR is potentially the most powerful technique at the disposal of the structural organic chemist.

Like electrons, nucleons (protons and neutrons) have spin. If an atom has an even number of nucleons, the spins cancel out and there is no overall magnetic moment. If, however, an atom has an odd number of nucleons, there is an overall magnetic moment. As a result, the nucleus can take up one of two orientations. In the absence of a magnetic field, the energies of the two orientations are the same, but in the presence of an external magnetic field one orientation has a slightly higher energy than the other. This splitting into two energy levels forms the basis of NMR spectroscopy: nucleons can be persuaded to flip from the lower energy spin state to the higher spin state (i.e. to resonate) by irradiating a sample with electromagnetic

radiation of the right frequency. The absorption of this frequency is detected by the NMR spectrometer.

The extent of the splitting is proportional to the strength of the external field: to increase the splitting, very large external fields are used. The strength of the applied magnetic field is measured in **tesla**, T; one tesla is about 10 000 times as strong as the Earth's magnetic field. Many machines use a field strength of 9.4 T.

For a hydrogen atom, this creates an energy difference of 0.16 J. The Planck equation, $E=hf$, shows that this corresponds to a frequency of 400 MHz, which is in the UHF (ultra high frequency radiowaves) region of the electromagnetic spectrum. An NMR spectrometer therefore detects the absorption of UHF radiation by a sample, in a similar fashion to any other spectrometer. The principal difference is that the sample is also subjected to a strong magnetic field. Compounds containing hydrogen atoms therefore show a nuclear magnetic resonance absorption band at 400 MHz. The frequencies absorbed by other common atoms that have an odd number of nucleons are shown in Table 29.5.

Table 29.5 The absorption frequencies, in an external field of 9.4 T, for some common atoms studied with nuclear magnetic resonance (NMR). In this topic we are looking at ^{1}H and ^{13}C magnetic resonance.

Nucleus	Absorption frequency/MHz
^{1}H	400
^{13}C	101
^{19}F	377
^{31}P	162

Figure 29.19 This NMR spectrometer measures the absorbance of UHF radiofrequency radiation by hydrogen atoms (which have an odd number of nucleons – just one proton) in an external magnetic field. The absorption frequency varies slightly depending on the chemical environment of the hydrogen atom, allowing identification of hydrogen-containing compounds.

The absorption is very weak, because the populations of atoms at each of the two energy levels are almost the same. At room temperature for a hydrogen atom, the population of the upper level differs from the lower end by only 1 part in 30 000, so that absorption is nearly always cancelled out by re-emission. The situation is even worse for carbon-13, because carbon contains only 1% of this isotope. In order to make the absorbance as intense as possible:

- the sample is cooled, which increases the population difference
- as large a magnetic field as possible is used, to increase the splitting
- the absorption is measured many times and the results are averaged by computer.

NMR is often used to detect the hydrogen atoms in water, and the analysis of water in the human body forms the basis of magnetic resonance imaging (MRI) (see the panel on page 509).

Magnetic resonance imaging (MRI)

Because the human body is made up mostly of water, it responds to nuclear magnetic resonance. By suitable scanning, an image of the water distribution in the body can be built up, which is invaluable in the diagnosis of various illnesses, in particular brain disorders. The word 'nuclear' has been dropped from the name of the technique magnetic resonance imaging (MRI), to avoid suggesting to patients that nuclear radiation is involved.

The MRI scanner is large, because the magnetic field must pass through the human body. A fine beam of radiation is applied, giving the absorption pattern of a cross-section of the body about 1 cm thick. Within this cross-section, water molecules can be studied in different parts of the body, as follows:

- The magnetic field is not uniform but varies from one side to another. As the frequency of the radiation is changed, water at different depths inside the body responds to the signal. This enables a one-dimensional picture to be built up.
- The radiation beam is rotated through 360°. This enables a computer to produce a two-dimensional image – a 'slice'.

A typical brain scan containing 20–30 slices can be obtained in less than 10 minutes. More subtle analysis of the data makes it possible to distinguish between water held, for example, in grey or white tissue or in cancerous or normal cells. The technique is invaluable in the diagnosis of brain tumours or Alzheimer's disease. It has the great advantage of being non-invasive, and the UHF radiation is much safer than X-rays, which are used in alternative techniques.

A refinement of MRI is to detect phosphorus-31 rather than protons. Areas of the brain that are actively in use require ATP (adenosine triphosphate) for their biochemical reactions. It is therefore possible to locate the regions of the brain that are most actively involved when different mental processes (for example, sight, reasoning or spatial work) are being carried out.

Figure 29.20 A magnetic resonance imaging (MRI) scanner gives a three-dimensional picture of the inside of the body, allowing non-invasive diagnosis of many diseases.

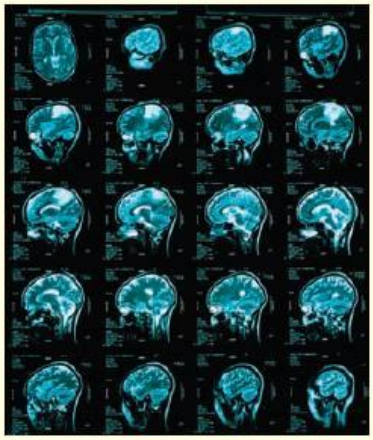


Figure 29.21 Images from an MRI scan of a human head

Analysing organic molecules

Both ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy are important in the analysis of organic compounds. We shall look first at ^{1}H NMR.

Magnetic resonance spectroscopy would be of little value in chemical analysis if all hydrogen atoms absorbed the same frequency of radiation. The electron cloud around the hydrogen atom partially screens the nucleus from the external magnetic field through the 'diamagnetic effect'.

The electrons within molecules – both bonded and non-bonded electrons – are usually 'paired' (that is, they occur as pairs of electrons spinning in opposite directions). When a molecule is placed in an external field, the electron pairs rotate in their orbits in such a way that they produce a magnetic field which opposes the external field. This phenomenon is called **diamagnetism** (see Figure 29.22). The effect is to shield nearby protons from the external field. This in turn reduces the frequency at which they absorb energy when they flip from their lower to their higher energy state.

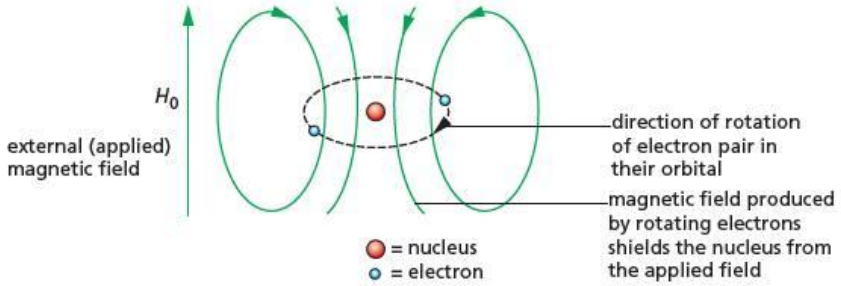


Figure 29.22 In an external magnetic field, the electron pairs rotate in such a way that they produce an opposing magnetic field.

When, however, a proton is near an electronegative atom (or group) within a molecule, the bonding electrons are drawn away from the proton to the electronegative atom. The proton is less shielded from the external magnetic field, and hence it absorbs radiation at a higher frequency. The effect is very pronounced if the proton is attached to a benzene ring. In this situation the mobile delocalised π electrons in the ring can create a strong diamagnetic effect, opposing the external field. This has the effect of strengthening the magnetic field in the vicinity of the protons (see Figure 29.23).

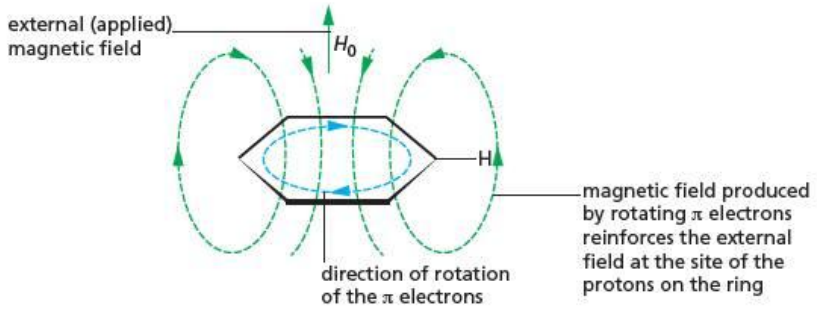


Figure 29.23 In benzene, the field created by the rotation of the π electrons reinforces the applied field.

The extent to which a proton is de-shielded from the external magnetic field is measured by its chemical shift.

Chemical shift

For convenience, the hydrogen atoms in the compound tetramethylsilane, $(CH_3)_4Si$ (known as TMS), are used as a reference. The sample being investigated is mixed with a drop of TMS and the frequency of the absorption, f, is measured relative to that of TMS, f_{TMS}. There are a number of reasons why this compound is used.

- All the hydrogen atoms in TMS are equivalent, so it gives a single absorption peak.
- Most other groups absorb at higher frequencies than TMS. This is because the protons in TMS are near the electropositive silicon atom, which does not draw the electrons away from the hydrogen atoms as much as carbon atoms.

The extent of the difference in frequency of absorption, is called the **chemical shift**, symbol δ (delta). It is defined as:

$$\delta = 10^6 \times \frac{(f - f_{TMS})}{f_{TMS}}$$

Values of δ are quoted in parts per million (ppm). Because chemical shifts are very small, the magnetic field must be identical throughout the sample. To achieve this, the superconducting magnets used in modern machines are very carefully constructed.

As mentioned above, the chemical shift increases as the electronegativity of the atom attached to the hydrogen increases. For example, in a halogenoalkane, a carbon atom attached to a fluorine atom is more electron-withdrawing than a carbon atom attached to an iodine atom. This is due to the inductive effect of the halogen atom, which in turn depends on its electronegativity (see section 3.10). The more electron-withdrawing carbon atom has a greater effect in drawing the electron cloud away from an attached hydrogen atom, reducing the screening and increasing the absorption frequency (see Table 29.6).

Table 29.6 Chemical shifts vary with the partial charge on the carbon atom attached to the hydrogen. A carbon atom attached to a highly electronegative atom such as fluorine has a larger partial charge than a carbon atom attached only to hydrogen atoms.

Group	Electronegativity of atom attached to carbon	δ/ppm
F — CH_3	4.0	4.5
Cl — CH_3	3.2	2.9
Br — CH_3	3.0	2.5
I — CH_3	2.7	2.0
H — CH_3	2.2	0.8

The values of some chemical shifts for hydrogen atoms within different organic groups are given in Table 29.7.

Table 29.7 Some chemical shifts. Protons associated with particular groups absorb in a *region* of the spectrum rather than at a definite frequency.

Proton type	Groups	δ/ppm	Range
$Si(CH_3)_4$	tetramethylsilane (TMS)	0.0	0
C—CH_3	end of alkyl chain	0.9	±0.5
C—CH_2—C	middle of alkyl chain	1.4	±0.5
=C—CH—	adjacent to C=C	1.9	±0.5
—C(O)—CH—	adjacent to C=O (ketones, esters, acids)	2.3	±0.5
C_6H_5 — CH —	adjacent to arene ring	2.5	±0.5
O—CH—	adjacent to oxygen (alcohols, ethers, esters)	3.3	±0.5
=C—H	alkenyl	5.5	±1
C_6H_5—H (benzene)	aryl	7.5	±1.5
—C(O)—H	aldehydic	9.0	±1.5
—O—H	alcohols	3	±2*
Ar—O—H	phenols	5.5	±1*
—CO—O—H	carboxylic acids	11.0	±2*

* These δ values are very dependent on the nature and acidity of the solvent and the extent of hydrogen bonding between molecules and the solvent.

Low and high resolution NMR spectroscopy: splitting patterns

When the ^1H NMR spectrum of ethyl ethanoate is scanned at low resolution (Figure 29.24), three peaks are observed, corresponding to the three different chemical environments of the protons in the molecule.

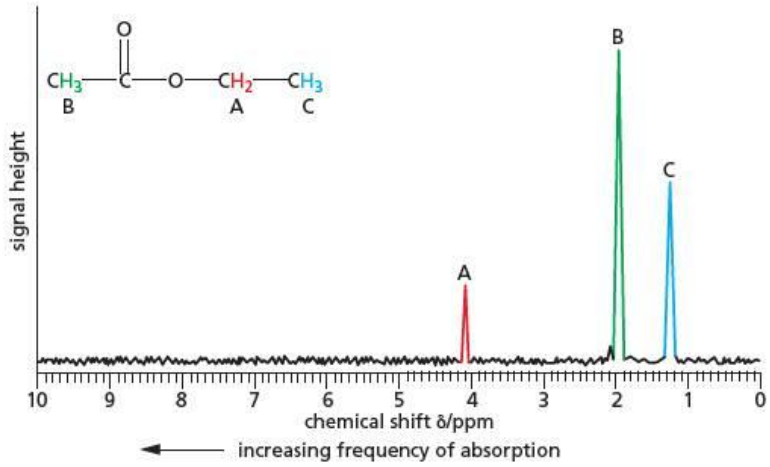


Figure 29.24 Nuclear magnetic resonance (NMR) spectrum of ethyl ethanoate at low resolution. Note that the δ scale, by convention, has its zero on the right of the *x*-axis.

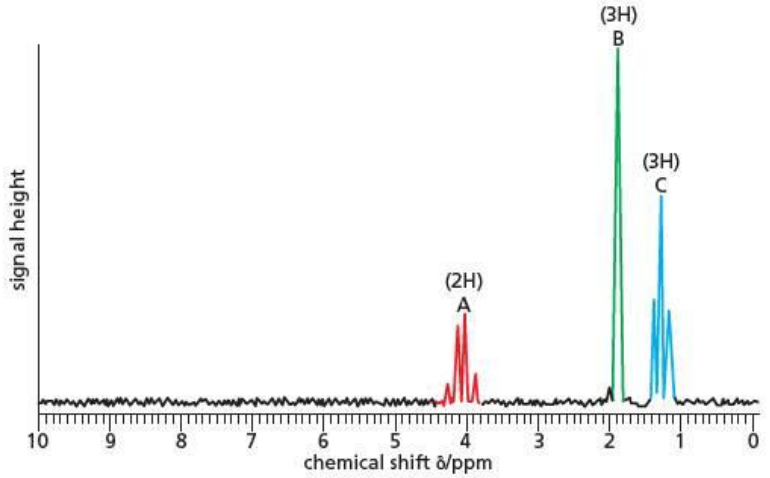


Figure 29.25 NMR spectrum of ethyl ethanoate at high resolution

The areas under the three peaks are in the ratio 2:3:3, being proportional to the number of protons at each chemical environment. On published NMR spectra, the relative area under each peak is sometimes shown by a number above the peak as can be seen in Figure 29.25. At higher resolution (see Figure 29.25), peaks A and C are seen to be multiple peaks, although B remains a single peak. This is because the nuclear spins of the protons in the ethyl group, responsible for peaks A and C, interact with each other. This is called **spin–spin coupling**, and it is a general phenomenon observed whenever protons on adjacent carbon atoms are in different chemical environments. The splitting of the peak arises because the magnetic field experienced by a proton is slightly altered due to the orientation of the magnetic moments (the spin states) of the protons on the adjacent carbon atom. Consider the protons in the CH_3 group of the ethyl chain. The field they experience will depend on the orientation of the magnetic moments of the $—CH_2—$ protons, as shown in Figure 29.26

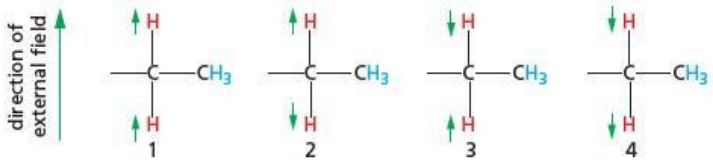


Figure 29.26 The directions of the magnetic moments of the $—CH_2—$ protons have an effect on the $—CH_3$ protons.

In situations 2 and 3, the magnetic moments of the two $—CH_2—$ protons cancel each other out, so the field experienced by the $—CH_3$ group protons will be the same as the applied field. In situations 1 and 4, however, the magnetic moments of the $—CH_2—$ protons reinforce each other. Consequently the field experienced by the protons of the $—CH_3$ group will be, respectively, higher and lower than the applied field. There should therefore be a total of three frequencies at which these $—CH_3$

protons absorb. What is more, the probabilities of the four states 1 to 4 are equal, so overall there is twice the chance of the $-CH_3$ protons experiencing no change in field (situations 2 and 3) as there is for the $-CH_3$ protons to experience either an enhanced field (situation 1) or a reduced field (situation 4). We therefore expect the intensities of the lines in the triplet of lines to be in the ratio 1:2:1.

A similar argument can be applied to the modification of the magnetic field experienced by the $-CH_2-$ group protons, by the protons in the adjacent $-CH_3$ group. In this case we expect a quartet of lines, in the ratio of 1:3:3:1.

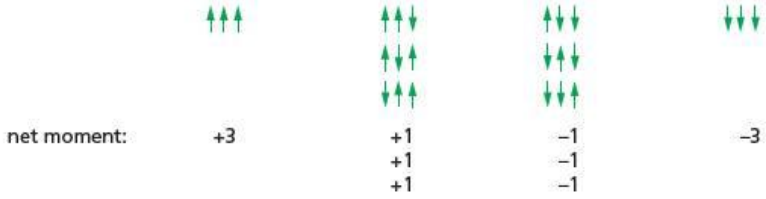
Worked example

By considering the different combinations of ↑ and ↓ magnetic moments of the $-CH_3$ protons, explain how the ratio 1:3:3:1 arises.

Answer

The possible combinations of the three $-CH_3$ protons are shown in Figure 29.27.

Figure 29.27



There are three times as many combinations giving a net magnetic moment of +1 or –1, compared with +3 or –3.

Now try this

Predict the splitting pattern (the number of lines and the relative intensities of the lines) for a proton adjacent to:

a one other proton
b four other protons.

The general rules concerning the splitting of the resonance peak of a proton by other protons are as follows.

- Protons in identical chemical environments do not split each other's peaks.
- The peak of a proton adjacent to n protons in a different environment is split into $(n+1)$ lines.
- The relative intensities of the $(n+1)$ lines are in the pattern shown in Table 29.8.
- The interaction between protons separated by more than three bonds is usually too weak to cause any splitting of each other's peaks.

Table 29.8 The splitting of a peak for a proton next to protons in a different chemical environment

Number of protons adjacent to resonating proton	Number of lines in split peaks	Relative intensities of lines
1	2	1:1
2	3	1:2:1
3	4	1:3:3:1
4	5	1:4:6:4:1

Peaks that are not split are referred to as *singlets* (*s*). If a peak is split into two lines it is a *doublet* (*d*); three lines is a *triplet* (*t*); four lines a *quartet* (*q*), etc. If there are so many lines that it is difficult to count them, the peak is referred to as a *multiplet* (*m*).

The use of 'heavy water', D_2O

Protons directly attached to oxygen or nitrogen atoms can appear almost anywhere in an NMR spectrum. The field strength at which they resonate depends on the acidity and hydrogen-bonding ability of the solution. Because of easy proton exchange with other O—H or N—H protons in the sample, these protons often do not cause the splitting of the peaks of adjacent protons. They can, however, be identified by **deuterium exchange**. If the compound containing them is dissolved in D_2O ('heavy water', D=^{2}H), the protons are exchanged with deuterium atoms in the water. The peaks due to the —OH or $-NH_2$ protons disappear (deuterium atoms, having an even number of nucleons, do not resonate):

$$CH_3CH_2{-}OH + D_2O \rightleftharpoons CH_3CH_2{-}OD + HDO$$

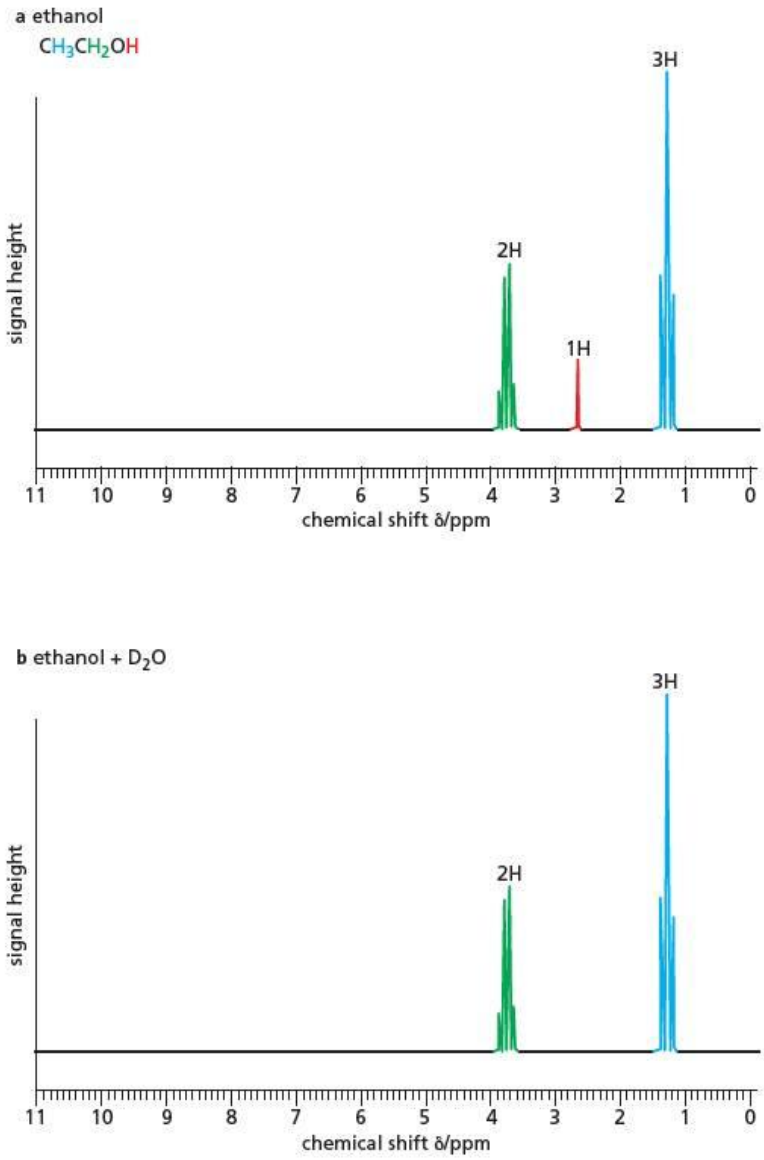
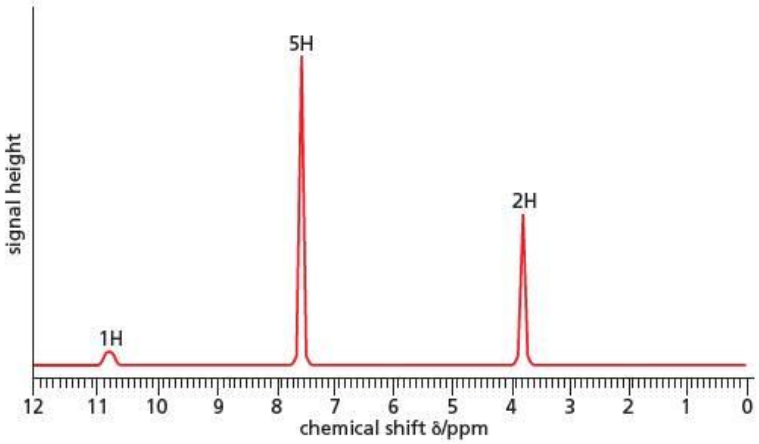


Figure 29.28 NMR spectra of ethanol: **a** showing the —OH peak, and **b** with D_2O added

Worked example

Figure 29.29 shows the nuclear magnetic resonance (NMR) spectrum of an acid with the molecular formula $C_8H_8O_2$. Work out its structure. (Use the δ values in Table 29.7, page 511 to help you.)

Figure 29.29



Answer

The high C : H ratio in the molecular formula suggests the presence of a benzene ring, and this is confirmed by the peak at $\delta = 7.6$. The broad peak at $\delta = 10.8$ is typical of the O—H hydrogen of a carboxylic acid. The two-proton single peak at $\delta = 3.7$ is a CH_2 group flanked by both an aryl ring and a CO_2H group, both of which would cause a high-field shift in resonance (by about 1 δ unit each).

The structure is therefore **C_6H_5—CH_2—CO_2H**.

Now try this

1 Apart from ethyl ethanoate (see Figure 29.25) there are at least 15 other isomers of $C_4H_8O_2$. The ^1H NMR spectra of two of them, **W** and **X**, are shown in Figure 29.30. Explain the splitting patterns seen in these spectra, and use the spectra to suggest the structures of **W** and **X**.

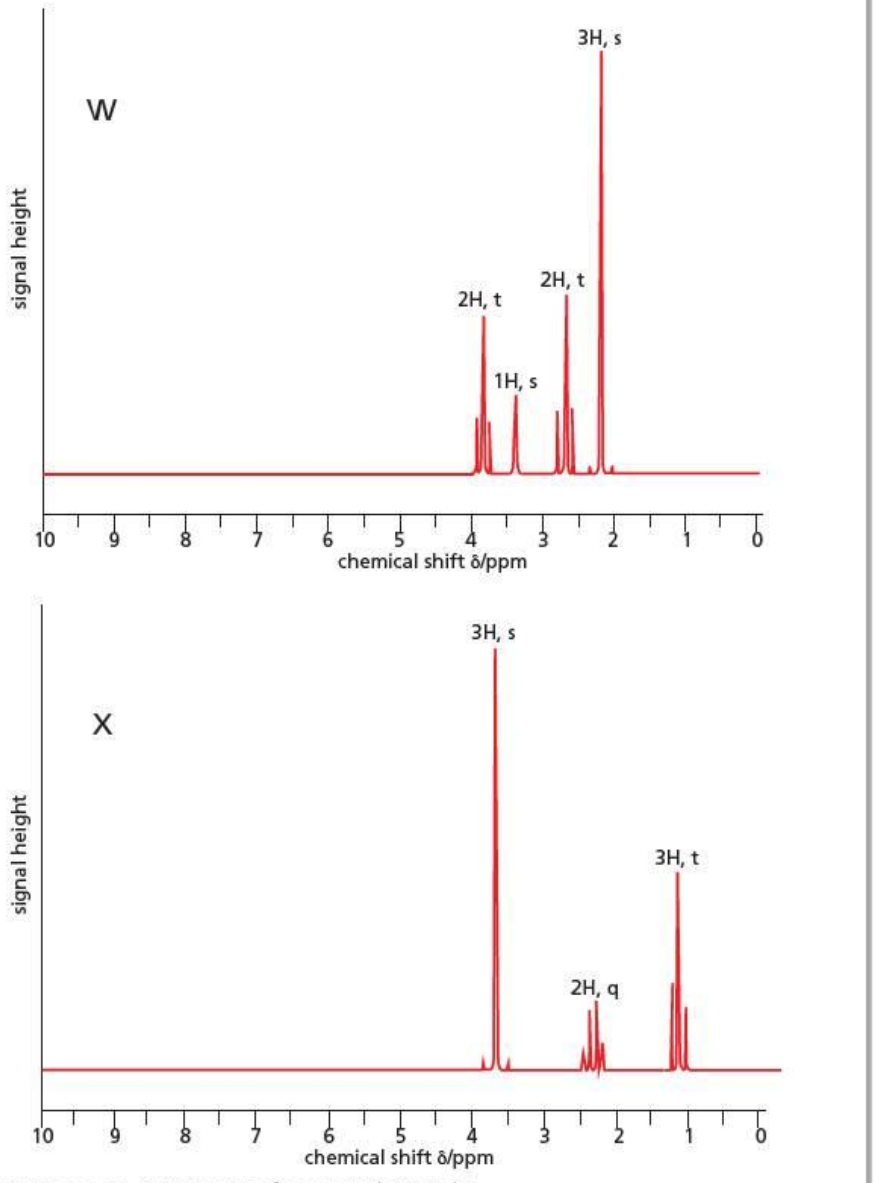


Figure 29.30 NMR spectra of compounds **W** and **X**

2 Figure 29.31 shows the NMR spectrum of compound **Y**, whose molecular formula is $C_3H_6O_2$. Suggest a possible structure for **Y**, with reasons.

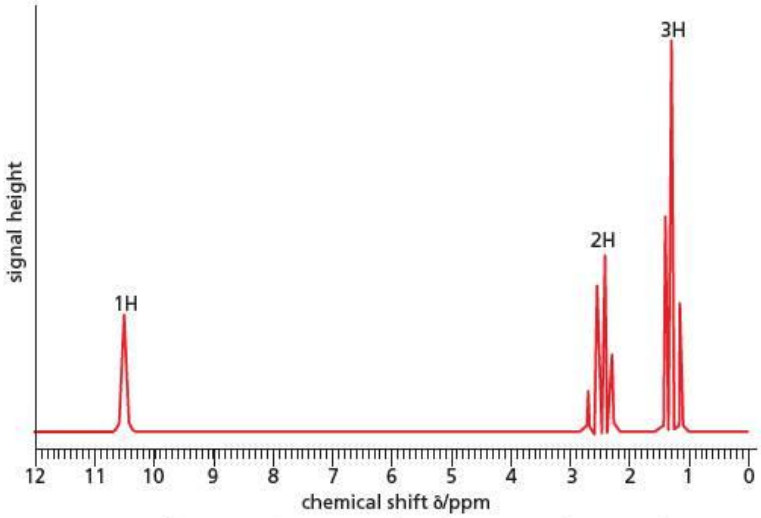


Figure 29.31 Nuclear magnetic resonance (NMR) spectrum of compound Y

3 Figure 29.32 shows the ^{1}H NMR spectrum of an alcohol **Z**.

Suggest the structure of **Z**, explain the splitting pattern, and predict which peak would disappear when D_2O is added.

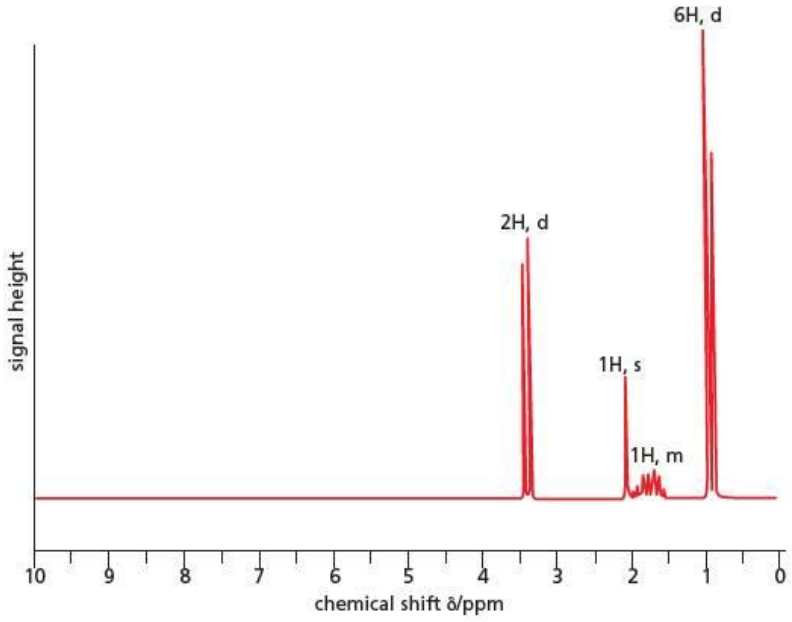


Figure 29.32 NMR spectrum of compound Z

29.5 ^{13}C Nuclear magnetic resonance (NMR) spectroscopy

A ^{13}C NMR spectrum is simpler than a ^{1}H spectrum. This is not only because there are usually fewer carbon atoms in a molecule than there are hydrogen atoms, but also because the absorbances in a ^{13}C spectrum usually appear as singlets – they are not split into multiplets by adjacent atoms as are many lines in a ^{1}H spectrum.

Because of the very small natural abundance of ^{13}C atoms (1.1%), the chances of two adjacent carbon atoms in a molecule both being ^{13}C atoms is only just over 1 in 100, and so the splitting of a peak due to adjacent ^{13}C atoms is very unlikely. Although the spin-interaction between ^{13}C atoms and ^{1}H atoms is very large, usually when a ^{13}C spectrum is run, the ^{1}H–^{13}C coupling is removed by irradiating the sample with broad-frequency 'white noise'. Although this greatly simplifies the spectrum, it has the disadvantage that the intensities of the peaks are not dependent on the number of carbon atoms, and so it is not possible to determine the number of carbon atoms associated with a particular absorbance.

Each carbon atom in a different chemical environment produces a single-peak absorbance at a different chemical shift. Table 29.9 shows some chemical shift values for ^{13}C in different chemical environments.

Table 29.9 Some ^{13}C chemical shifts

Environment of ^{13}C	Chemical shift range (ppm from TMS)
C (alkane)	0–30
C (alkene)	110–150
C—N	50–55
C—O	60–65
C (aryl)	110–160
C in —COX (X = O, N)	160–175
C=O	200–220

The ^{13}C NMR spectrum of ethyl ethanoate is illustrated in Figure 29.33.

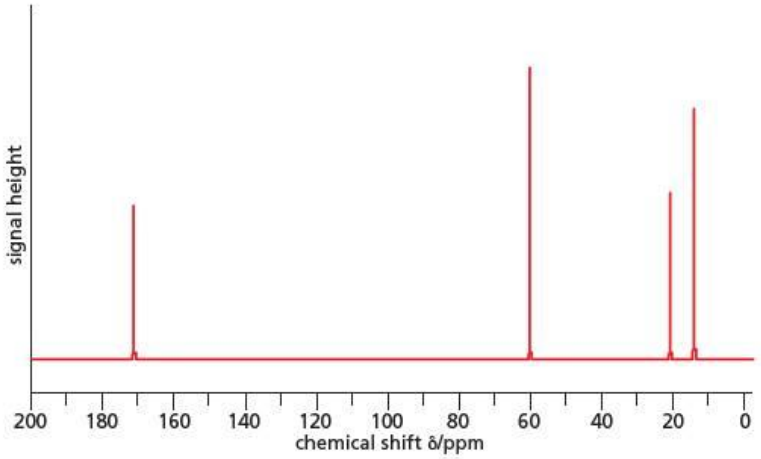


Figure 29.33 ^{13}C NMR spectrum of ethyl ethanoate, $CH_3CO_2CH_2CH_3$

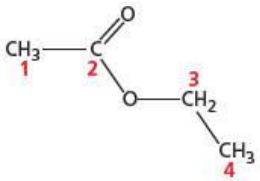


Figure 29.34 Ethyl ethanoate

The spectrum in Figure 29.33 shows four peaks, one for each of the carbon atoms in the molecule. From Table 29.9 we can see that the peak on the left of the spectrum, at 171 ppm, is for carbon **2** in Figure 29.34, and the peak at 60 ppm is for carbon **3** in Figure 29.34.

It is a little more difficult to assign the other two peaks, at 13 and 22 ppm, but because the CH_3 next to the C=O is likely to be more deshielded due to the electron-withdrawing effect of the C=O, we can identify carbon **1** with the peak at 22 ppm, leaving the peak at 13 ppm associated with carbon **4**.

Now try this

Two isomers of ethyl ethanoate are propyl methanoate and prop-2-yl methanoate. Their ^{13}C spectra are shown in Figure 29.35. Decide which compound gives which spectrum.

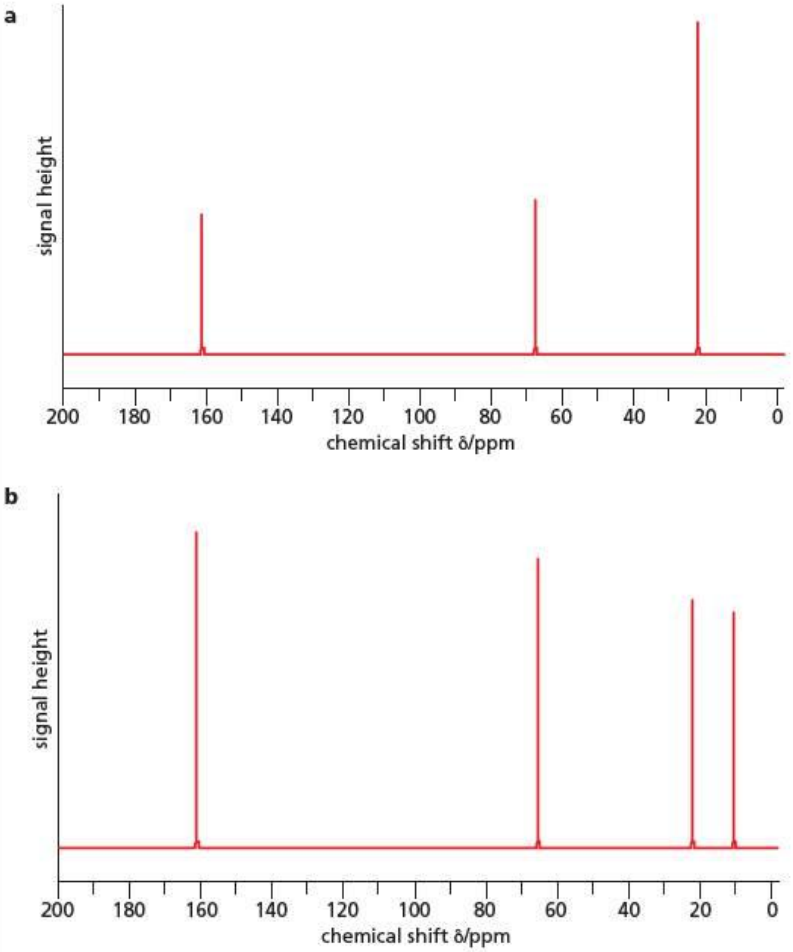


Figure 29.35 ^{13}C spectra of the two propyl methanoates, $HCO_2C_3H_7$

Summary

- **Paper chromatography (PC)** uses the principle of **partition** to separate components of a mixture.
- Components of a mixture are separated in **thin layer chromatography (TLC)** through the principle of **adsorption**.
- Components are separated in **gas chromatography (GC)** by their relative volatilities and attraction to the non-polar coating on the solid support.
- The routine uses of gas chromatography in analysis include the detection of alcohol, drugs, food additives and impurities and explosive residues.
- Measuring the accurate mass of the molecular ion peak in **mass spectrometry** allows us to work out the molecular formula of a compound.
- Use of the M+1 peak in mass spectrometry enables us to determine the number of carbon atoms in a molecule.
- Use of the M+2 peak in mass spectrometry enables us to determine the number of chlorine and/or bromine atoms in a molecule.
- The **fragmentation pattern** in mass spectrometry helps us to determine the structure of molecules.
- **Infrared (IR)** spectroscopy can identify the functional groups within a molecule.
- **Nuclear magnetic resonance (NMR)** spectroscopy can be carried out on compounds that contain atoms such as ^{13}C and ^{1}H, which have magnetic moments that take up orientations with different energies in an external magnetic field.
- Both the **chemical shift** (δ) values and the splitting patterns in a ^{1}H NMR spectrum allow us to determine the structures of molecules.
- The ^{13}C spectrum can allow us to determine the number and environment of carbon atoms within a molecule.

Examination practice questions

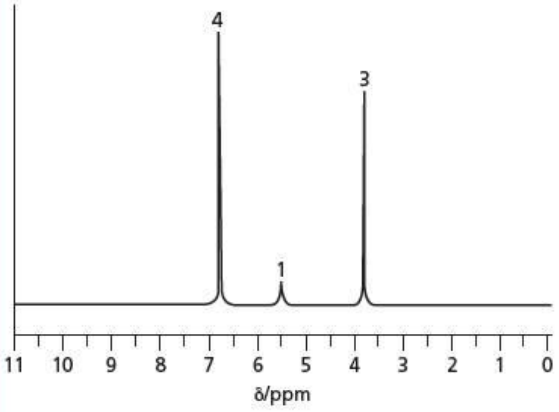
Please see the data section of the CD for any A_r values you may need.

1 This question is about the modern techniques of analysis which may be used to determine molecular structures.

a NMR spectroscopy, in contrast to X-ray crystallography, is frequently used to examine protons in organic molecules.

i What feature of protons enables their detection by NMR spectroscopy?

ii The NMR spectrum below was obtained from a compound **X**, $C_xH_yO_z$. In the mass spectrum of the compound, the M : M+1 ratio was found to be 25:2. Determine the values of x, y and z in the formula of X and deduce a possible structure for the compound, explaining how you arrive at your conclusion.



[6]

[Cambridge International AS & A Level Chemistry 9701, Paper 41 Q7 c November 2009]

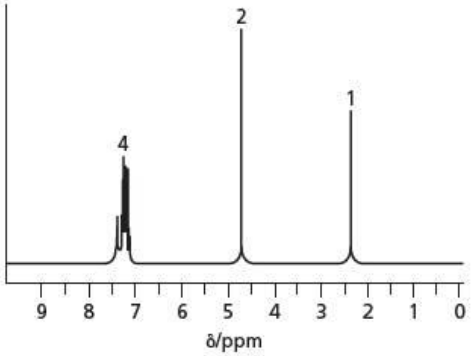
2 The techniques of mass spectrometry and NMR spectroscopy are useful in determining the structures of organic compounds.

a The three peaks of highest mass in the mass spectrum of organic compound **L** correspond to masses of 142, 143 and 144. The ratio of the heights of the M : M+1 peaks is 43.3 : 3.35, and the ratio of heights of the M : M+2 peaks is 43.3 : 14.1.

i Use the data to calculate the number of carbon atoms present in **L**.

ii Explain what element is indicated by the M+2 peak.

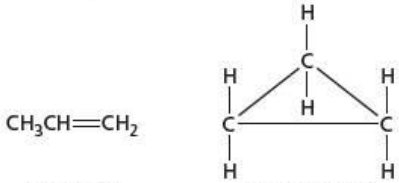
iii Compound **L** reacts with sodium metal. The NMR spectrum of compound **L** is given below.



What does the NMR spectrum tell you about the number of protons in **L** and their chemical environments?

iv Use the information given and your answers to **i**, **ii** and **iii** to deduce a structure for **L**. **Explain** how you arrive at your answer. [7]

b The molecular formula C_3H_6 represents the compounds propene and cyclopropane.



i Suggest **one** difference in the fragmentation patterns of the mass spectra of these compounds.

ii Suggest **two** differences in the NMR spectra of these compounds. [3]

[Cambridge International AS & A Level Chemistry 9701, Paper 42 Q7 June 2013]