THE ROYAL SOCIETY OF CHEMISTRY

Unilever

2. Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy gives information on the environment in which the nuclei of atoms are found in molecules and compounds. It is possible to derive an enormous amount of information from a single spectrum, and in many cases this will facilitate the determination of the structure of a molecule. Indeed, the NMR spectrum of a compound is frequently the first spectral information to be consulted.

The theory behind the technique is rather more complex than for mass spectrometry and infrared spectroscopy, but the interpretation of the spectra is probably no more difficult, if not easier, once a little familiarity has been gained.

The theory

A nucleus possessing a spin in the presence of an external magnetic field can align itself either with the external field (+) or against it (–) (*Fig. 1*).

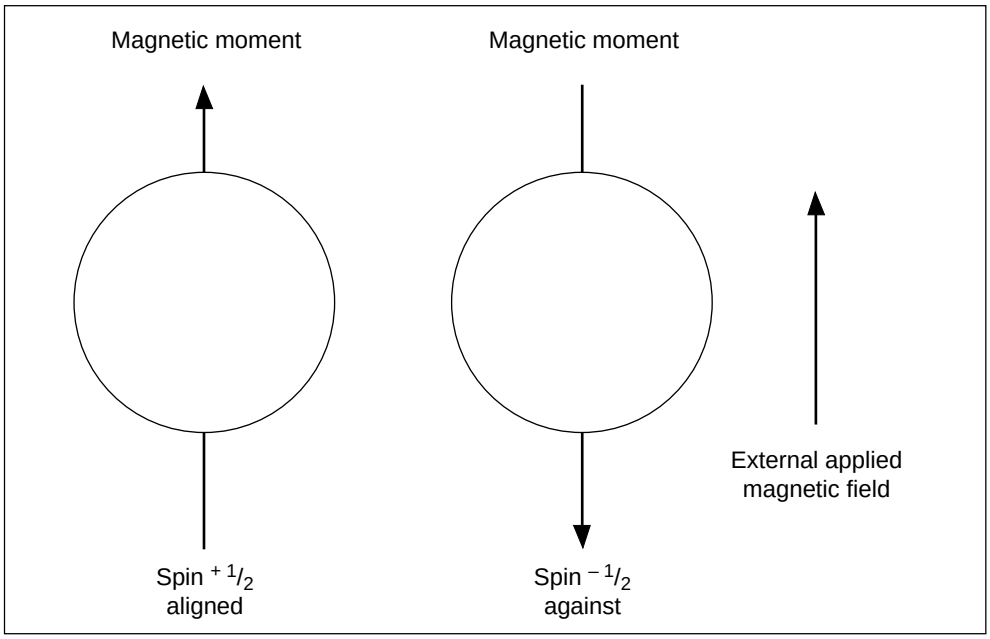


Figure 1 Nuclear magnets aligned with and against an external magnetic field

It is found that many nuclei spin about an axis. Because the nuclei are positively charged, this spin is associated with a circulation of electric charge. Circulating charges give rise to magnetic fields, so nuclei with spin also have a magnetic moment, rather like the magnet of a compass needle. When put in an external magnetic field the nuclei tend to turn (like compass needles in the earth's field) to a preferred orientation. Other, less favoured, orientations have higher energy. The nuclei obey quantum laws and for some nuclei said to have a spin quantum number of a $\frac{1}{2}$ only two orientations can be adopted. They are the most favoured and least favoured orientation (*Fig. 2*).

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

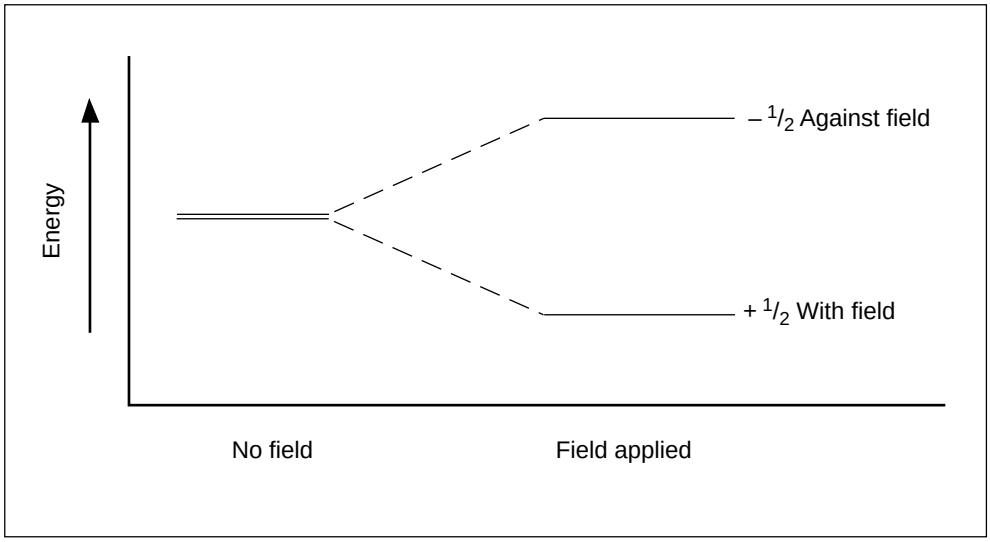


Figure 2 Splitting of energy levels in an external magnetic field

Exchange of energy between the nuclear spin and the thermal motion of the molecules containing them distributes the spins between the two energy levels in such a way that there are more nuclei in the lower than upper level.

Transitions between the two energy levels can occur if radiation of the correct frequency is absorbed.

The spin up (lower energy) state will have a higher population given by the ratio

$$\frac{N_{upper}}{N_{lower}} = e^{-\frac{\Delta E}{kT}}$$

where ΔE = difference between energy levels in joules
k = Boltzmann constant
T = temperature in kelvin
N = the number of nuclei at each energy level

Because ΔE is extremely small, the difference in populations will also be extremely small. For hydrogen nuclei it is approximately one in 10^5 for a ΔE of 6×10^{-24} J in a 2.35 tesla (T) external field. Transition from the lower to the upper state is possible by absorption of radiation of the correct frequency (*Fig. 3*).

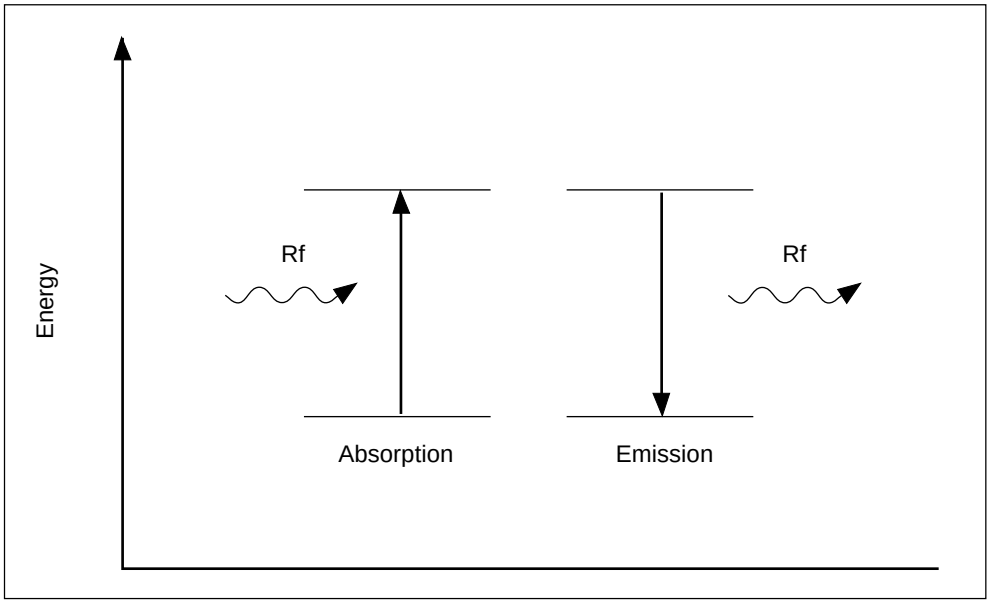


Figure 3 Absorption and emission of radiation

The radiation absorbed is in the radiofrequency region of the electromagnetic spectrum. For a 2.35 T magnetic field the radiation is typically in the range 5–100 MHz, and its precise frequency can be calculated using the formula

$$\nu = \frac{\gamma B_o}{2\pi}$$

where ν = frequency of the radiation absorbed in hertz
γ = a constant of proportionality called the magnetogyric ratio (the ratio of the magnetic moment to the angular or gyric moment) which differs according to the type of nucleus considered, and which is effectively a measure of the magnetic strength of the nucleus (units: radian T^{-1} s^{-1})
B_o = the strength of the applied magnetic field in tesla (1 T = 10^4 gauss)

The energy difference between the spin states is very small. Nuclei with a spin can be made to resonate between the spin states if:

1 a large enough external magnetic field is applied to ensure a significant difference between the energy states; and

2 radiation of the correct frequency is applied.

So far we have assumed that only two spin states are possible. This is true for all nuclei with spins of ½ – *ie* nuclei whose spin states can be +½ or -½; these include ^{1}H, ^{13}C, ^{19}F, and ^{31}P. However, other spin states do exist – *eg* ^{6}Li (which can have spin states +1, 0 or -1) and ^{23}Na (which can have spin states +3/2, +½, -½, or -3/2) have further energy levels available between which transitions can occur. However, ^{1}H-NMR is the most widely used.

The symbol for the maximum nuclear spin is *I*, and for *I*=1 three energy levels are available – *eg* ^{14}N – (*Fig. 4*), and for nuclei with *I*=3/2 four levels are available – *eg*

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

^{33}S – (*Fig. 5*). Generally for spin *I*, (2*I*+1) energy levels are available. Nuclei with no spin (*I*=0) – *eg* ^{4}He, ^{12}C, and ^{16}O – are inactive because only one energy level is available.

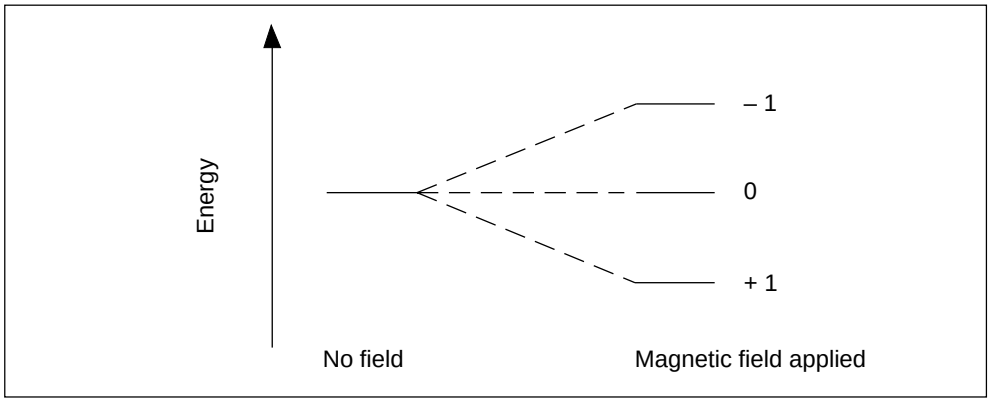


Figure 4 Energy levels available for nuclei with spin = 1

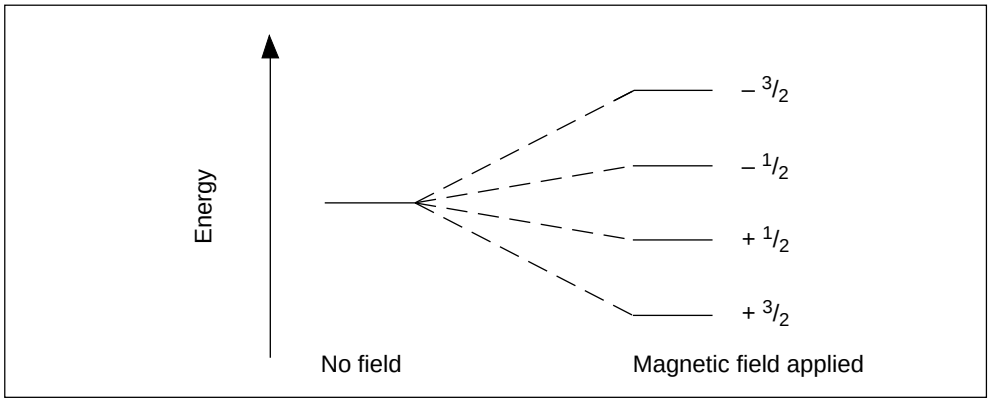


Figure 5 Energy levels available for nuclei with spin = ³⁄₂

There are no authoritative rules that enable the precise spin of all nuclei to be predicted, but some generalisations can be made. These relate the atomic and mass numbers with the observed nuclear spins.

Atomic number	**Mass number**	**Nuclear spin (*I*)**
Even or odd	Odd	½, ³⁄₂, ⁵⁄₂ ...
Even	Even	0
Odd	Even	1, 2, 3 ...

When the frequency of the radiation supplied corresponds to the energy difference between levels the population of the higher energy state increases as radiation is absorbed. The equilibrium population distribution is re-established by spin-lattice relaxation processes whereby the energy previously absorbed is shared with either the surroundings (spin-lattice relaxation) or with other nuclei (spin-spin relaxation). Spin-lattice relaxation processes are often quicker in liquid samples than solid samples because of the greater molecular mobility in the liquid phase. Most

solutions have relaxation times in the range 10^{2}–10^{-4} s, the majority of ^{1}H and ^{13}C nuclei taking a fraction of a second, whereas solid samples can take several minutes. Relaxation processes can be speeded up by the presence of a paramagnetic material – *eg* molecular oxygen or chromium(III) 2,4-pentandionate (acetylacetonate).

In the analysis of an organic sample it is the ^{1}H- or proton-NMR spectrum that is usually most useful because hydrogen atoms are present in such large numbers, bonded in a variety of environments. However, from the theory presented so far one would expect that all hydrogen atoms would resonate at the same frequency – *ie* at 100 MHz in a 2.35 T field.

When a molecule is placed in a magnetic field, the electrons surrounding the nuclei behave like perfectly conducting shells, and weak electric currents are induced in them. The currents flow in such a way as to produce a magnetic field which opposes the applied field; the nuclei at the centre, therefore, experience a fractionally smaller field than the applied external field (*Fig. 6*).

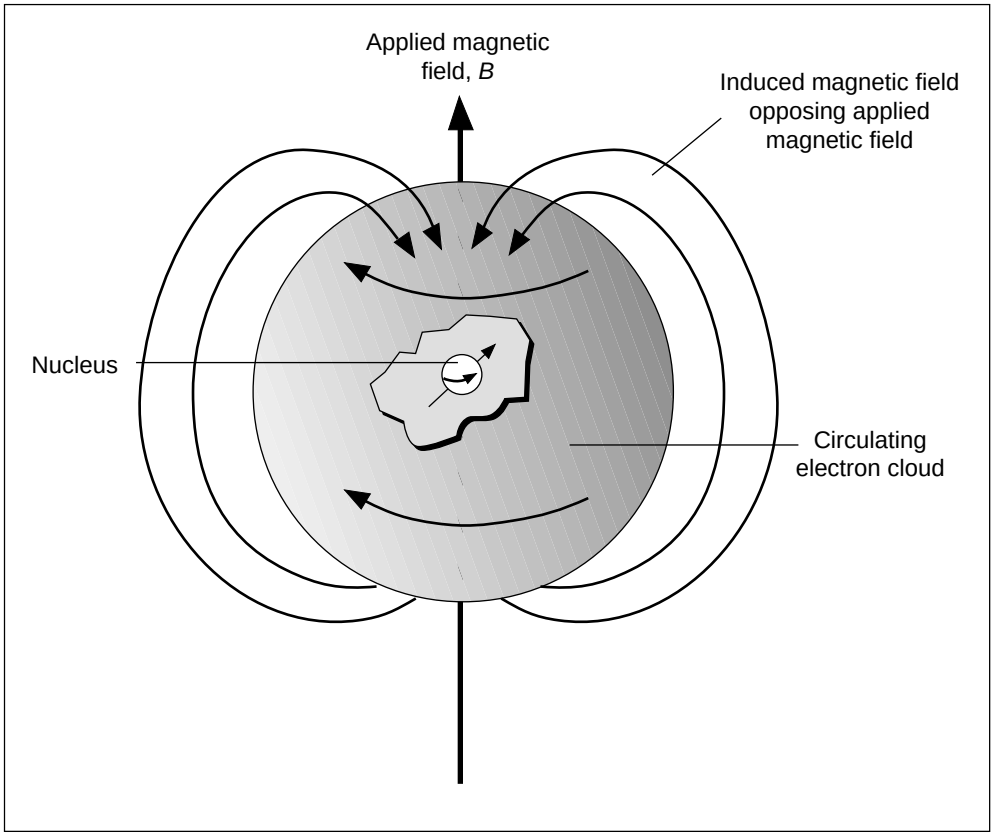


Figure 6 Shielding of an isolated nucleus by circulation of the surrounding electron cloud

Because the electron distribution around chemically different hydrogen atoms in a molecule are not the same, the induced fields vary slightly. Conseqently, the nuclei experience different magnetic fields in the same external field. The effect is, however, very small; for hydrogen atoms it is only a few parts per million. Fortunately the line widths are very small and it is still possible to measure these so-called 'chemical shifts'. The different environments of the protons in ethanol are shown by its low resolution spectrum (*Fig. 7*). Notice that the signals occur at fields differing by a few parts per million. It is for this reason that NMR spectrometers require magnetic fields that are stable and homogeneous to a few parts in 10^{8}.

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

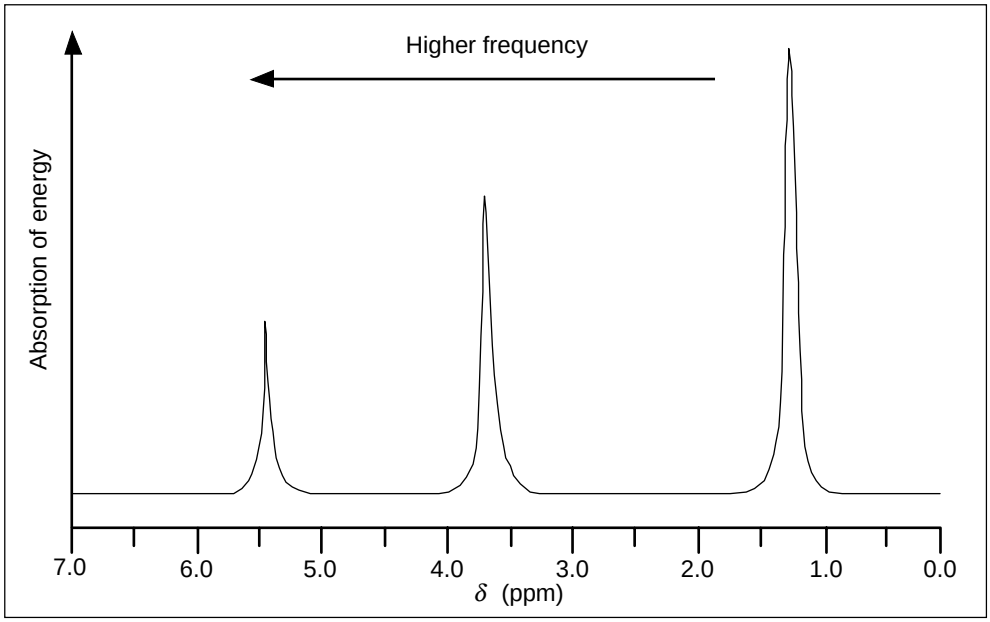


Figure 7 Low resolution spectrum of ethanol (an explanation of the scale used is given on page 36).

There are two variables that can be altered when recording an NMR spectrum:

1 the magnetic field can be kept constant and the range of radiofrequencies scanned, or

2 the radiofrequency can be kept constant and the magnetic field scanned.

A few simple instruments scan the magnetic field. A radiofrequency detector is set at right angles to the radiofrequency transmitter inducing resonance, and a recorder charts the absorption of energy as a function of the applied field or frequency (*Fig.8*). However, more advanced Fourier Transform NMR machines are the most common type of spectrometer in use today (see page 47).

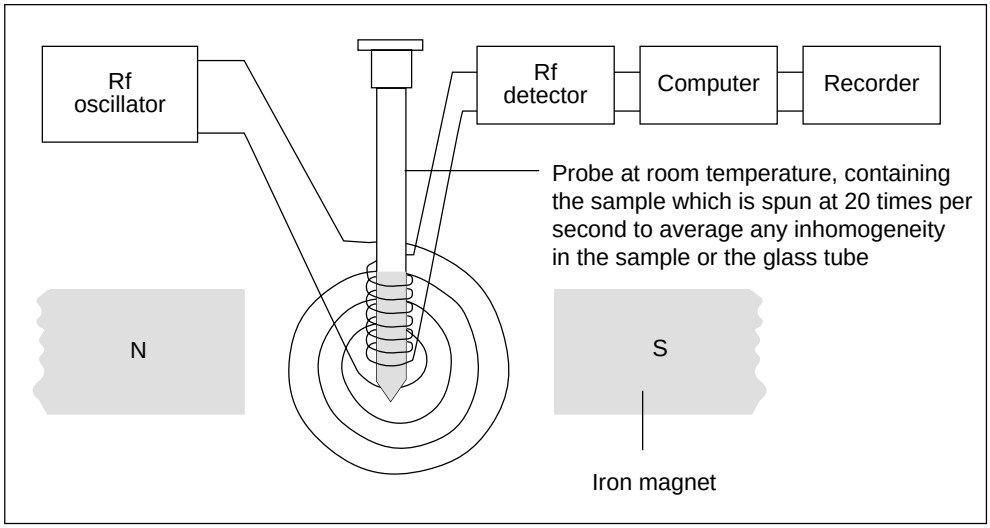


Figure 8 Diagram of a simple NMR spectrometer

THE ROYAL SOCIETY OF CHEMISTRY

Unilever

The NMR spectrum of ethanol (*Fig. 7)* shows that the absorption of energy is displayed against neither magnetic field nor frequency. Instead, it is on a scale (with no units) that increases from right to left. Peaks on this scale, δ, have the same value no matter what the magnetic field or frequency range of the instrument used because the chemical shift is induced by the applied field and is proportional to it. Values on the scale can be derived from measurements in either hertz (frequency) or tesla (magnetic field), and are always measured relative to a standard that gives a reference peak at one end of the scale. The reference material that is usually used is tetramethylsilane, TMS ($Si(CH_3)_4$).

δ can be calculated using:

$$\delta = \frac{B_{TMS} - B_{sample}}{B_{TMS}} \times 10^6 \text{ ppm}$$

or

$$\delta = \frac{\nu_{TMS} - \nu_{sample}}{\nu_{TMS}} \times 10^6 \text{ ppm}$$

where B = magnetic field strength at resonance
ν = radiofrequency at resonance

Resonances are always expressed in terms of chemical shift, measured in parts per million (ppm), so that results are reproducible no matter what machine the spectra are run on, and no matter what applied magnetic field is used (machines are available that run at different magnetic fields).

By definition the δ value of TMS is zero. Most organic proton resonances are then on a scale of 0-10 on the lower field/higher frequency side of zero. The relationship between field, frequency and shielding is shown in *Fig. 9*.

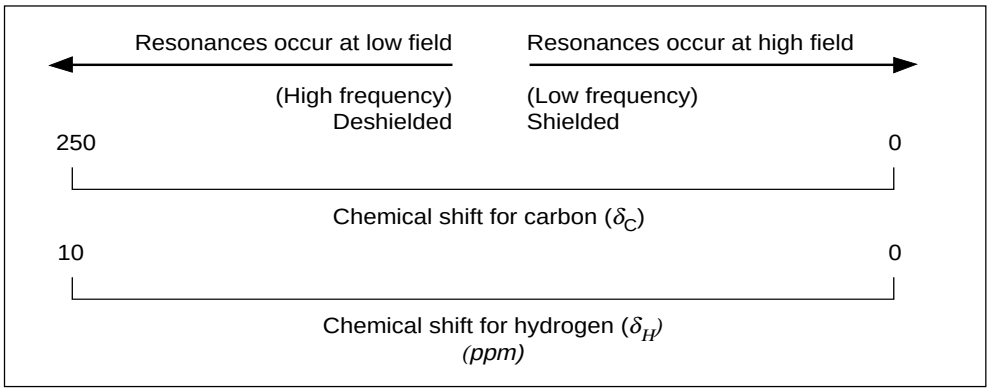


Figure 9 The relationship between field, frequency and nuclear shielding for proton and ^{13}C-NMR

Tetramethylsilane has a number of useful features:

1 it is non-toxic and inert;

2 it gives a signal that resonates well away from almost all other organic hydrogen resonances because the protons are so well shielded, and do not interfere with the spectrum;

3 because there are 12 protons in the same environment they all resonate at the same frequency so the single peak is intense and easily recognised; and

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

4 the boiling point of TMS is fairly low so it can be boiled off if the sample is required for anything else.

The high resolving power of modern spectrometers enables spectra to be produced which display far more information than that shown in *Fig. 7*. The high resolution spectrum of ethanol is shown in *Fig. 10*. Each set of peaks is centred on a δ value known as the chemical shift. The δ values vary according to the chemical environment and the δ value gives an indication of the degree of shielding experienced by the proton or protons. Tables of chemical shifts are available for protons in different environments (Tables 1 and 2).

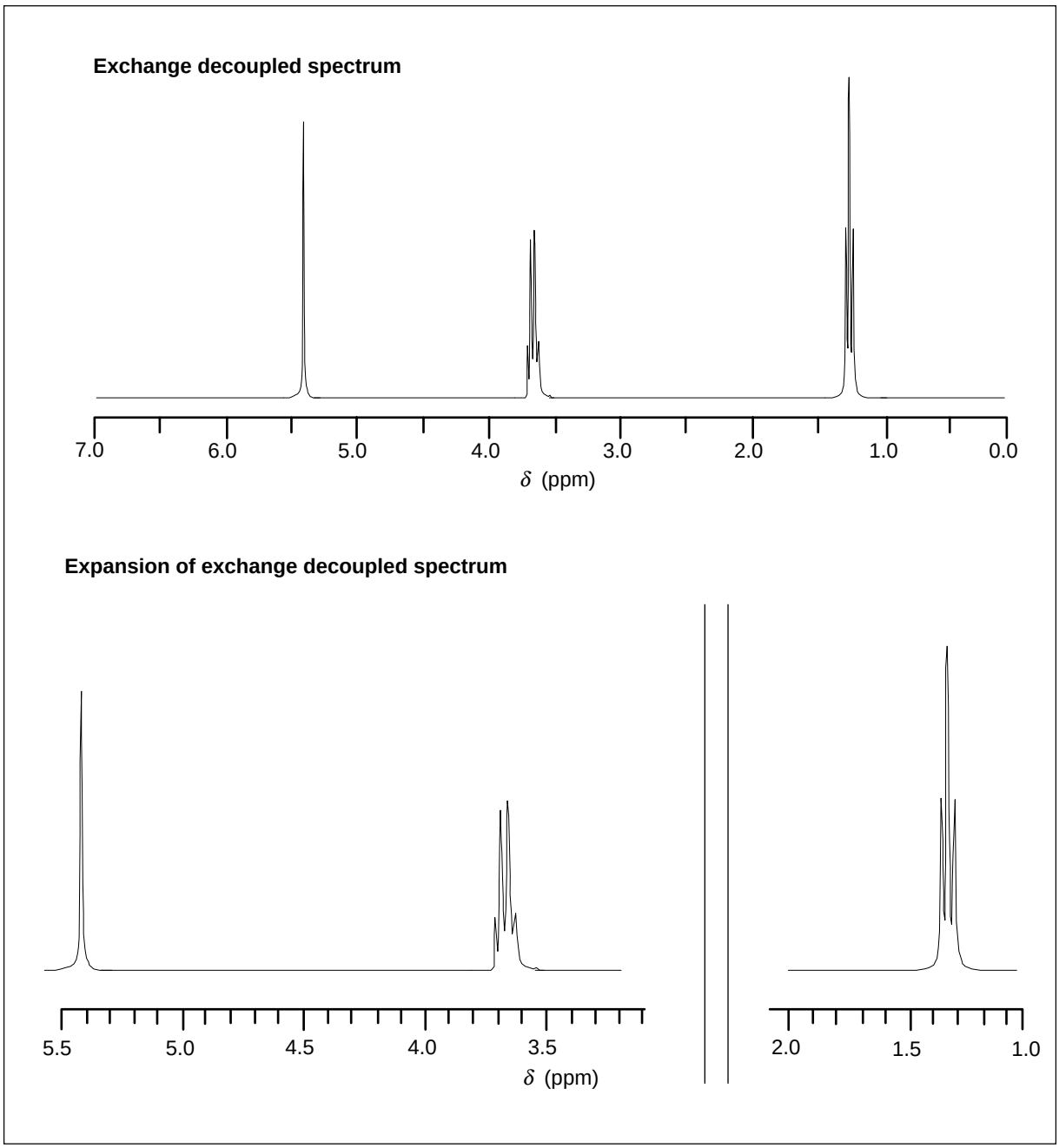


Figure 10 High resolution proton NMR spectrum of ethanol

THE ROYAL SOCIETY OF CHEMISTRY

Unilever

Chemical shift is dependent on the electronegativity of the atoms in molecules. Multiple bonding can also cause extra shielding or deshielding compared with a single bond and this causes a change in chemical shift. For example, an ethynic bond C≡C will shield adjacent protons because the circulation of the triple bond electrons reduces the apparent magnetic field. The circulation of the delocalised (π) electrons in a benzene ring deshields the protons bonded to the ring because the induced magnetic field (due to the circulation of the electrons) reinforces the applied magnetic field in the region occupied by the protons (*Fig. 11*).

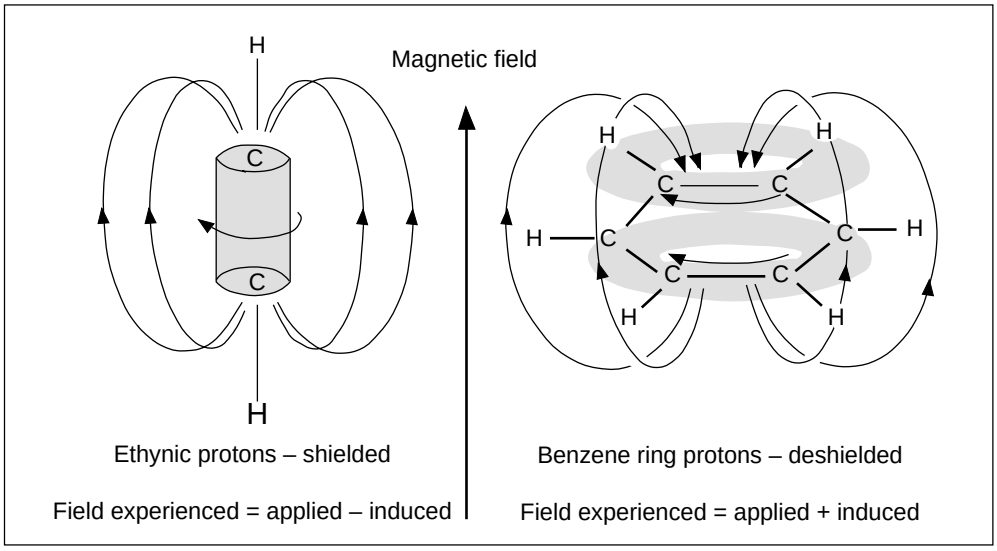


Figure 11 Shielding and deshielding of protons in ethyne and benzene

Table 1 Proton chemical shifts in aliphatic environments

These are typical values, and can vary slightly in different solvents and if induced magnetic fields within a molecule are stronger in one direction than the other (anisotropy).

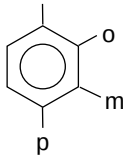
Methyl protons	δ	**Methylene protons**	δ	**Methine protons**	δ
CH_3–R	0.7–1.6	RCH_2–R	1.4	CH–R	1.5
CH_3–Ar	2.3	RCH_2–Ar	2.3–2.7	CH–Ar	3.0
CH_3–C≡N	2.0	RCH_2–C≡N	2.3	CH–C≡N	2.7
CH_3–C(=O)–R	2.2				
CH_3–C(=O)–O–R	2.0	RCH_2–C(=O)–R	2.4	CH–C(=O)–R	2.7
CH_3–C(=O)–Ar	2.6			CH–C(=O)–Ar	3.3
CH_3–C(=O)–O–Ar	2.4	RCH_2–C(=O)–Ar	2.9	CH–N–C(=O)–R	4.0
		$ArCH_2C(=O)R$	3.7		
CH_3–N–R	2.3	RCH_2–N	2.5	CH–OH	3.9
CH_3–N–Ar	3.0	RCH_2–N–C(=O)–R	3.2	CH–O–R	3.7
CH_3–N–C(=O)–R	2.9	RCH_2–Cl	3.6	CH–O–Ar	4.5
CH_3–O–R	3.3	RCH_2–Br	3.5	CH–O–C(=O)–R	4.8
CH_3–O–Ar	3.8	RCH_2–I	3.2	CH–Cl	4.2
CH_3–O–C(=O)–R	3.7	RCH_2–OH	3.6	CH–Br	4.3
CH_3–O–C(=O)–Ar	4.0–4.2	RCH_2–O–R	3.4	CH–I	4.3
		RCH_2–O–Ar	4.3		
		RCH_2–O–C(=O)–R	4.1		
		$ArCH_2$–O–C(=O)–R	4.9		

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

Table 2 Calculated proton chemical shifts in aromatic environments

Aromatic proton shifts can be calculated using the equation



$$\delta_H = 7.27 + \sum_i z_i \qquad i = o, m, p$$

eg the protons in bromobenzene would have their peaks at:

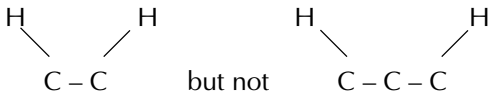
$$\delta_{ortho} = 7.27 + 0.18 = 7.35$$
$$\delta_{meta} = 7.27 - 0.08 = 7.19$$
$$\delta_{para} = 7.27 - 0.04 = 7.23$$

For more complicated systems there will be as many adjustments to the chemical shift of 7.27 for each proton as there are substituents on the ring – *eg* a proton might be *ortho* to a methyl group (- 0.20) and *meta* to a nitro group (+ 0.26).

	Zo	*Zm*	*Zp*
CH_3–	–0.20	–0.12	–0.22
CH_3CH_2–	–0.14	–0.06	–0.17
$(CH_3)_2CH$–	–0.13	–0.08	–0.18
$CH_3C(=O)$–	0.62	0.14	0.21
$H_2NC(=O)$	0.61	0.10	0.17
$HOC(=O)$	0.85	0.18	0.27
$CH_3OC(=O)$	0.71	0.1	0.21
H_2N–	–0.75	–0.25	–0.65
$CH_3C(=O)NH$–	0.12	–0.07	–0.28
O_2N–	0.95	0.26	0.38
N≡C–	0.36	0.18	0.28
HO–	–0.56	–0.12	–0.45
CH_3O–	–0.48	–0.09	–0.44
$CH_3C(=O)O$–	–0.25	0.03	–0.13
Br–	0.18	–0.08	–0.04
Cl–	0.03	–0.02	–0.09
I–	0.39	–0.21	0.00
–S–	–0.08	–0.10	–0.22
–S(=O)–	0.3	0.1	0.2
$–SO_2$–	0.76	0.35	0.45

Spin-spin coupling

In a molecule the nucleus of an atom, A, can induce in the electrons of the chemical bonds attached to it a very weak magnetic moment. This moment affects the magnetic field at a neighbouring atom, B's, nucleus. It would increase the field when the atom A's nucleus is pointing one way and decrease the field when it points the other way. This interaction is known as coupling and this causes peaks to be split into a number of lines. Protons can usually interact with other protons that are up to three bonds away, *ie*



THE ROYAL SOCIETY OF CHEMISTRY

Unilever

Protons with the same chemical shift do not show coupling with each other – *ie* coupling is not seen between protons in the same chemical environment (*nb* coupling over greater distances does exist but is much smaller.)

Thus in the spectrum of iodoethane, CH_3CH_2I, the CH_3 protons will interact with each of the CH_2 protons as follows. There are three energy states available to the two protons, depending on whether their spins are up or down, the combinations being:

1 both aligned with the field;

2 one aligned with the field and one against it (2 combinations); and

3 both aligned against the field.

ie

↑↑ ↑↓ ↓↓
↑↓

The methyl protons can therefore interact with each of these three energy states, at slightly different frequencies. Because there are two combinations having one nucleus spin up and one spin down this peak has twice the intensity (area under the peak) of the other two. This triplet of peaks is centred on a chemical shift of 1.8. (This value is downfield [larger chemical shift] of the range for methyl groups bonded to other alkyl groups because of the deshielding effect of iodine.)

Similarly the two CH_2 protons can couple (interact) with the three methyl protons in four ways:

↑↑↑ ↑↑↓ ↑↓↓ ↓↓↓
↑↓↑ ↓↑↓
↓↑↑ ↓↓↑

This gives a quartet of peaks with intensities in the ratio 1:3:3:1 according to the combination of spins. It is centred on the chemical shift expected of the CH_2 protons bonded to an alkyl group and an iodine atom ($\delta = 3.2$). The spectrum of iodoethane is shown in *Fig. 12*.

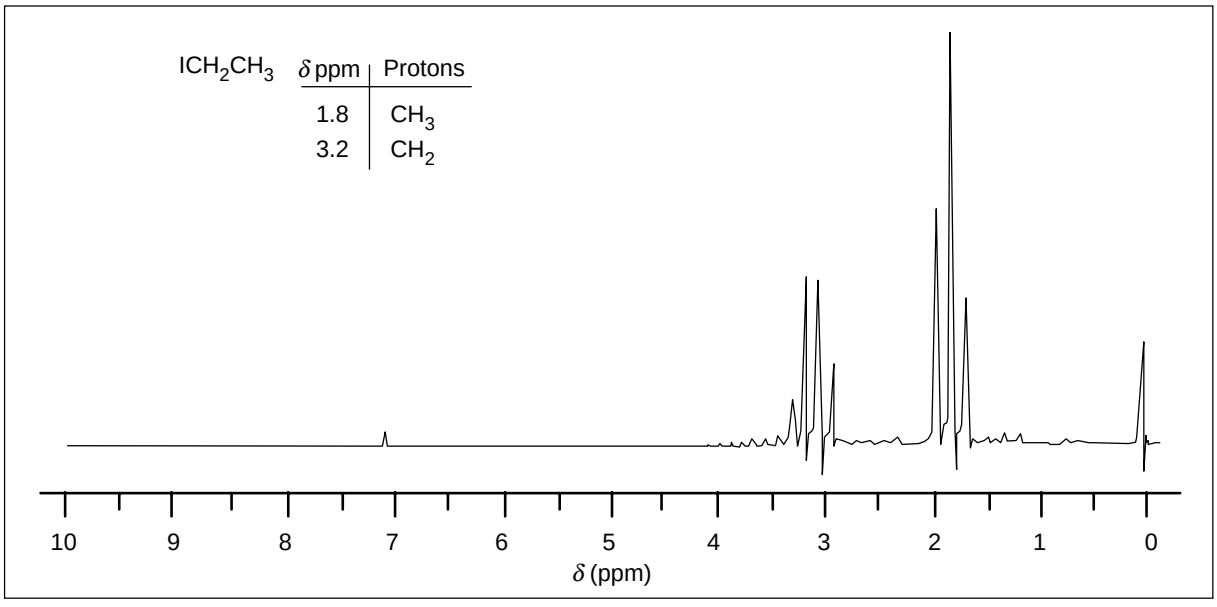


Figure 12 High resolution proton NMR spectrum of iodoethane

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

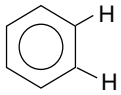
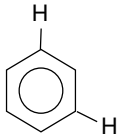
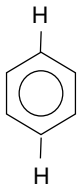
In general, if there are *n* protons three bonds away from the resonating group, the absorption will be split into a multiplet of *n*+1 lines. Their expected intensities can be predicted using Pascal's triangle:

Coupling with 0 protons:	1
Coupling with 1 proton:	1 1
Coupling with 2 protons:	1 2 1
Coupling with 3 protons:	1 3 3 1
Coupling with 4 protons:	1 4 6 4 1

Within a multiplet, the spectral lines are separated by differences which are constant for particular types of interaction between resonating nuclei (spin-spin coupling), and tables of these constants are available – *eg* Table 3. These coupling constants have the symbol *J*, and are always described in terms of frequency, whether the spectrum is obtained by scanning the frequency or the magnetic field.

Such *J* values are constant if expressed as a frequency – *ie* they are independent of magnetic field.

Table 3 Typical spin-spin coupling constants, *J* (Hz)

	*J(ortho)* = 6–9 (1,2)
	*J(meta)* = 1–3 (1,3)
	*J(para)* = 0–1 (1,4)
CH_2–CH_2	*J* = 5–8
CH_3–CH_2	*J* = 6–8
CH_3–CH	*J* = 5–7
CH–CH	*J* = 0–8

THE ROYAL SOCIETY OF CHEMISTRY

Unilever

If two groups of spectral lines have similar chemical shifts then the relative line heights may not be in the predicted ratio. The intensities of the line on the sides of the peaks that are closest together can be greater than predicted. This is known as 'roofing' – *eg Fig. 13* – and this can cause confusion of an otherwise straightforward spectrum.

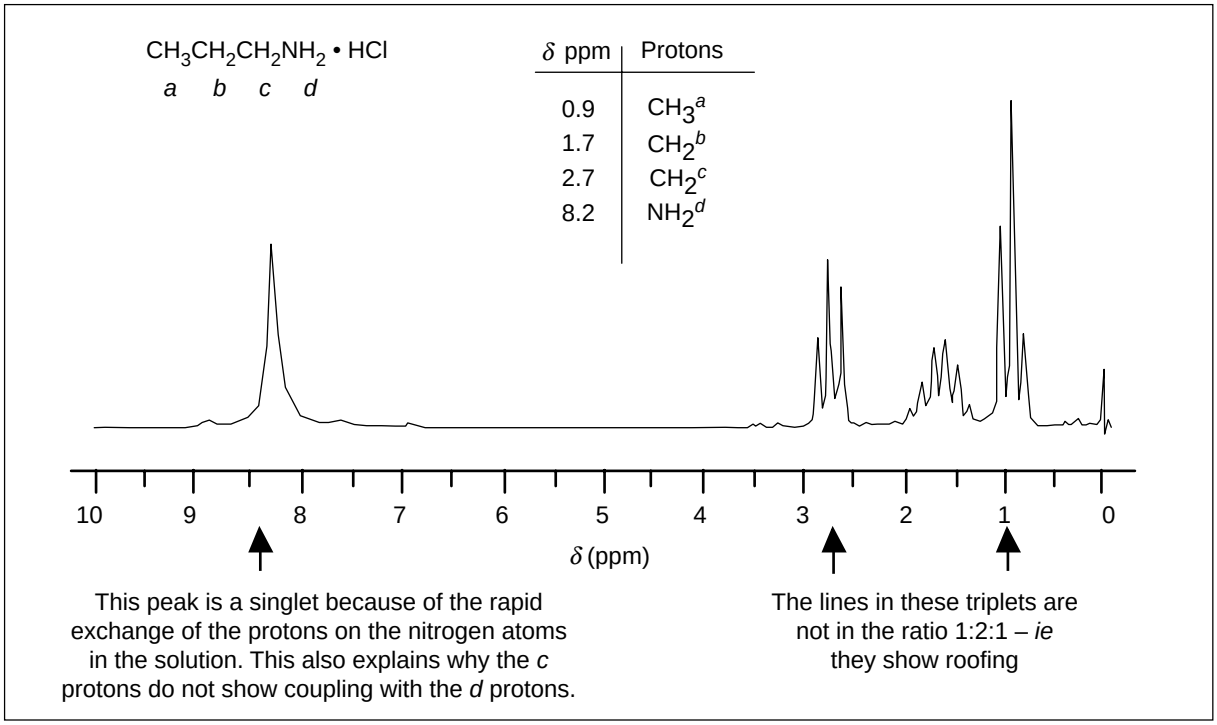


Figure 13 Proton NMR spectrum of propanamine hydrochloride showing skewing, or 'roofing', of multiplet lines

In the hypothetical case of two doublets separated by a large chemical shift compared with their coupling constant the 'roofing' effect is small, but as the chemical shifts get closer together the spectrum becomes more and more distorted until the chemical shifts are identical and only a singlet is seen (*Fig. 14*).

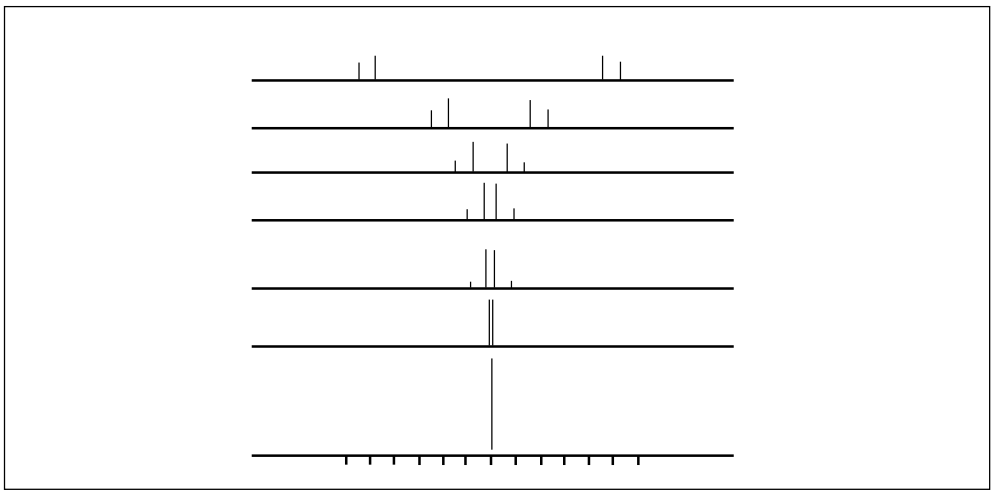


Figure 14 The effect on a spectrum of bringing sets of peaks closer together

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

So if the resonating protons couple with more than one set of protons more complex splitting patterns will be observed – *eg* the CH_2 protons in propanal (CH_3CH_2CHO), $\delta = 2.5$, couple with the methyl protons to give a quartet, and with the aldehydic proton to give a doublet at the same time *ie* this part of the spectrum therefore appears as a quartet of doublets (*Fig. 15*).

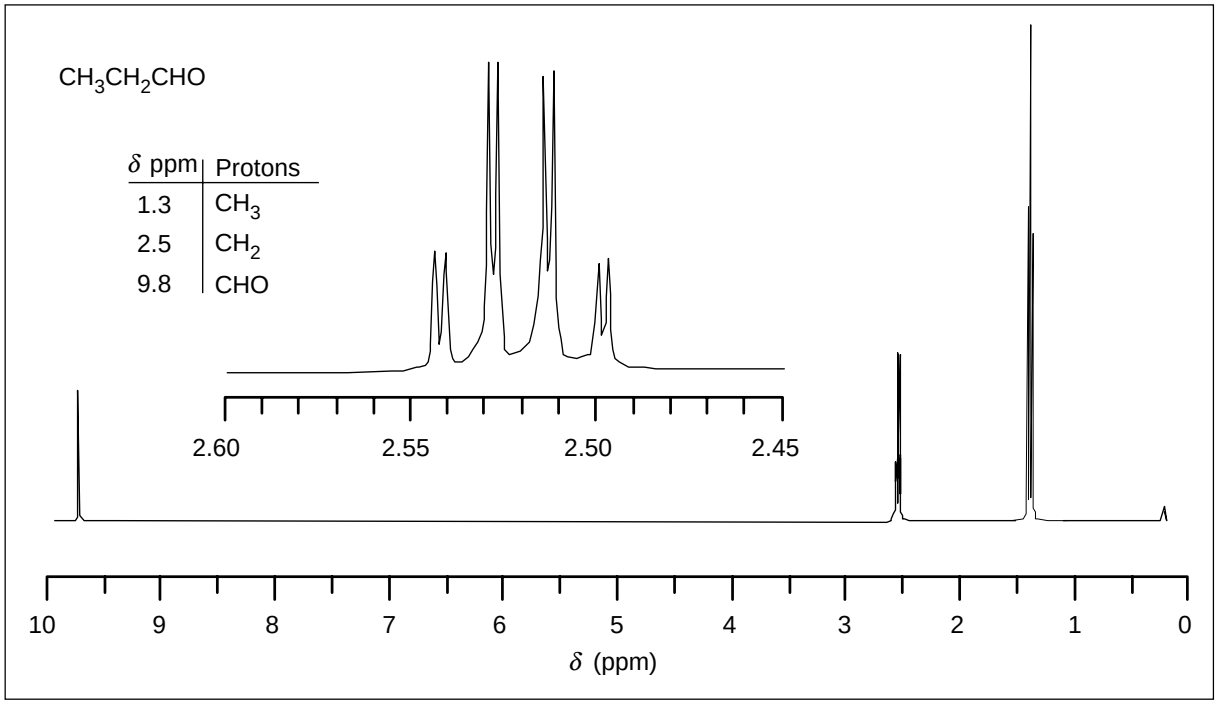
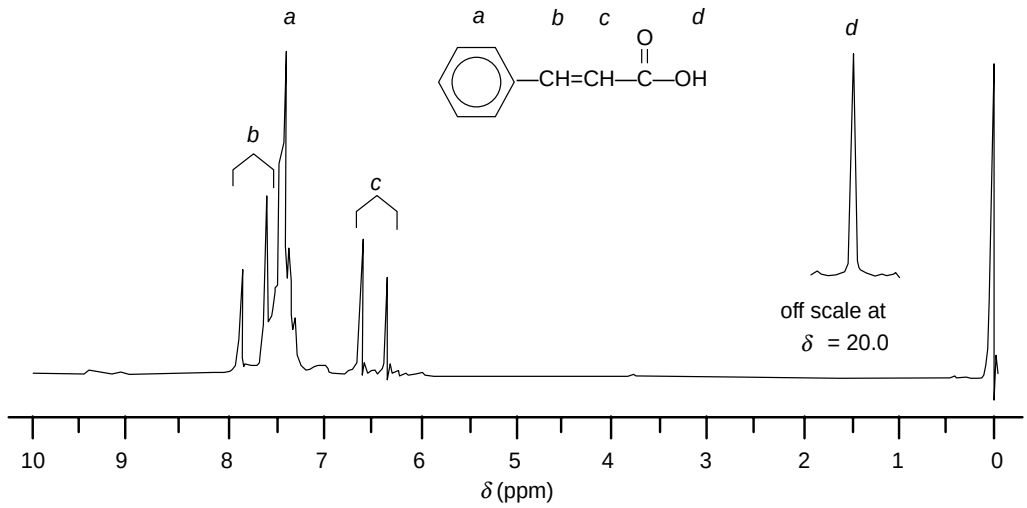


Figure 15 Proton NMR spectrum of propanal showing a quartet of doublets for the CH_2 protons

The three protons in a methyl group show coupling only with other protons, and not with each other – this makes spectral interpretation much simpler. However, if because of stereochemistry, chemical environments are not identical, slightly different patterns can be observed – *eg cis* and *trans*-3-phenyl-2-propenoic (cinnamic) acid ($C_6H_5CH{=}CHCOOH$) have different ***J*** values for the coupling between the protons attached to the double bond carbons. The coupling constant of *cis* protons is generally smaller (7-12 Hz) than that of *trans* protons (13-30 Hz). *Figure 16* shows the NMR spectrum of *trans*-3-phenyl-2-propenoic (cinnamic) acid, with the coupling constant calculation.

THE ROYAL SOCIETY OF CHEMISTRY

Unilever



The doublet at 6.4 is separated by 0.23 ppm. The spectrum was generated on a 60 MHz instrument so 1 ppm ≡ 60 Hz. Therefore 0.24 ppm ≡ 14 Hz and the coupling constant between protons *b* and *c*, *J* is 14 Hz

Figure 16 NMR spectrum of *trans*-3-phenyl-2-propenoic (cinnamic) acid

The coupling patterns for aromatic protons depend on the positions of ring substituents. *Figure 17* shows the spectral patterns expected from substituted ring protons, and *Fig. 18* shows the spectrum of 1-bromo-4-iodobenzene, which illustrates the 1,4- disubstituted pattern.

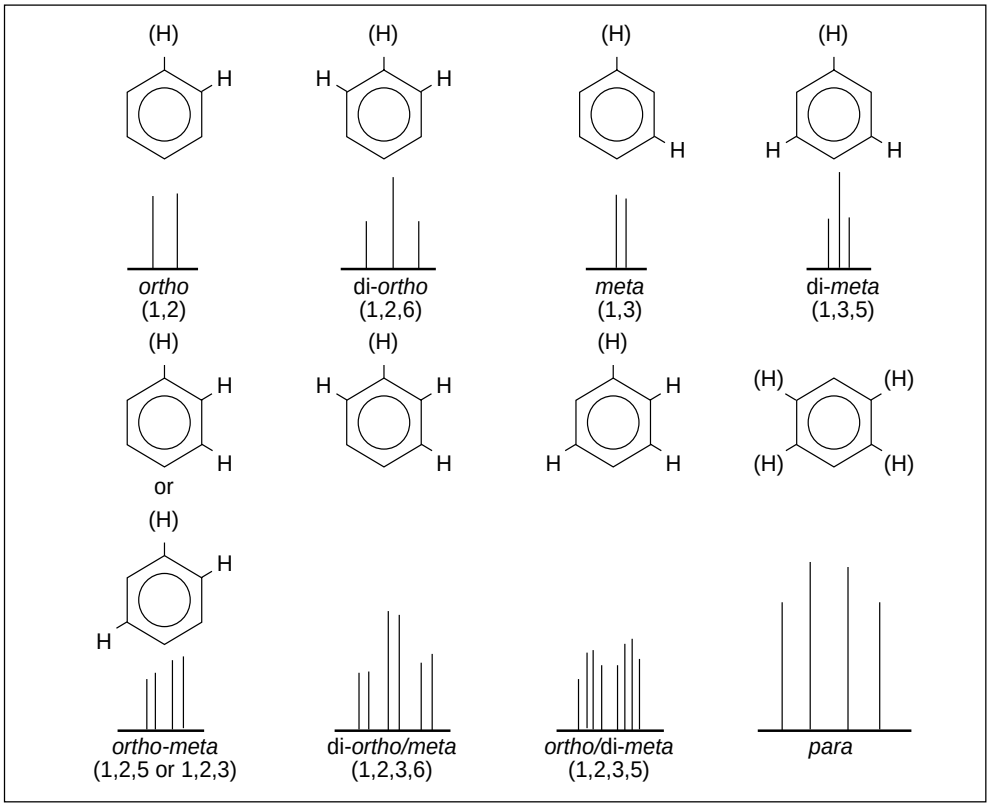


Figure 17 NMR aromatic coupling patterns as shown by the proton in brackets (terms refer to position of protons)

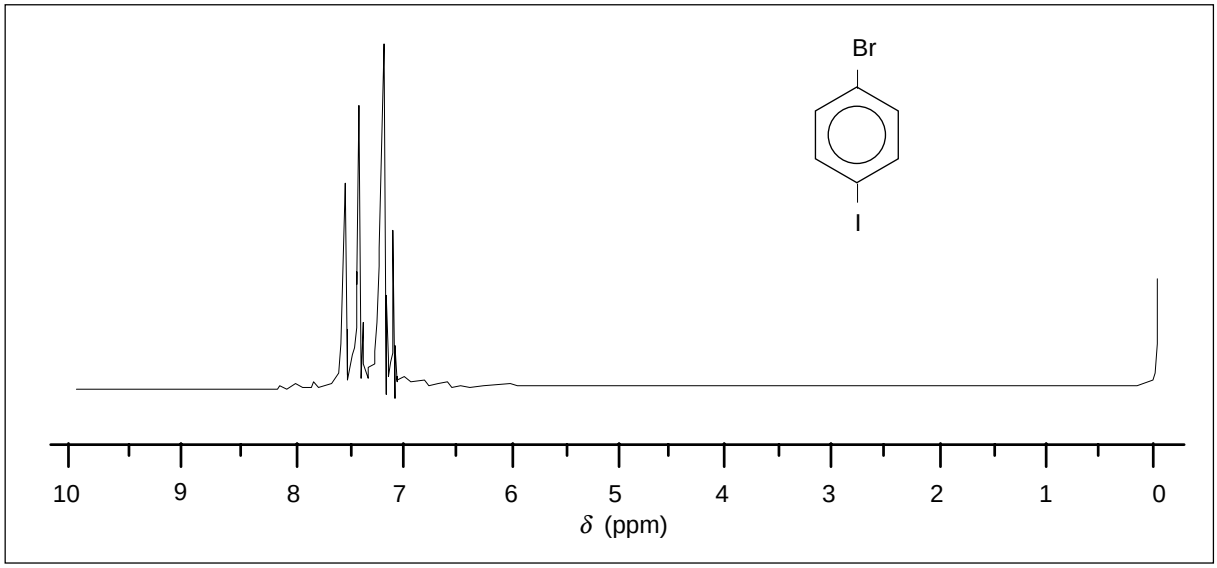


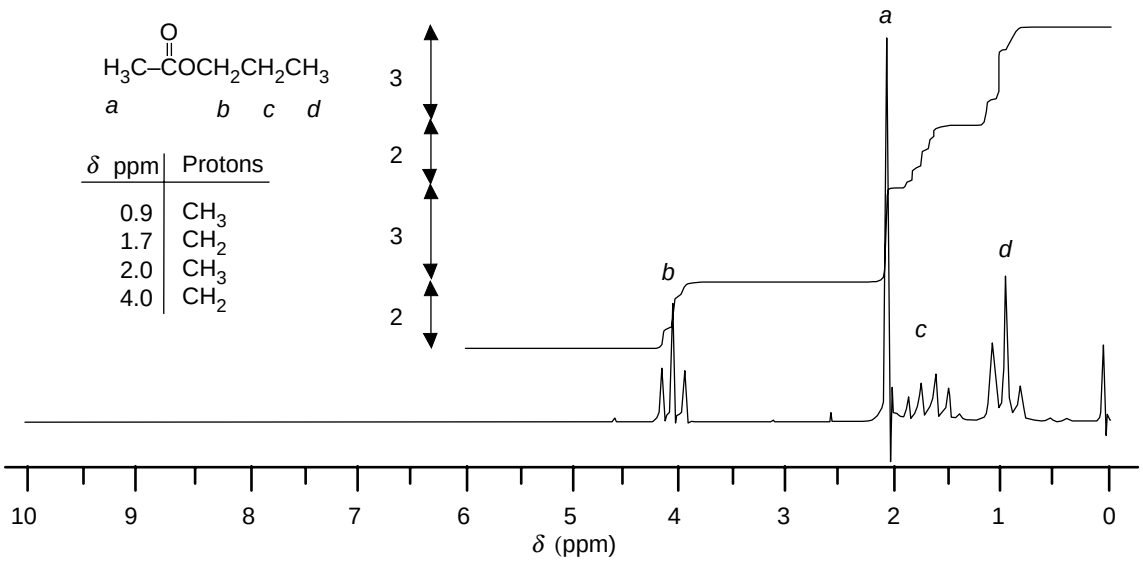
Figure 18 NMR spectrum of 1-bromo-4-iodobenzene showing 1,4-disubstituted aromatic coupling

Integration of peaks

The amount of energy absorbed at each frequency/magnetic field strength is proportional to the number of protons absorbing. Consequently, the area under each set of spectral lines is proportional to the relative number of protons absorbing. Many instruments will give this information directly by also plotting an integration curve on the spectrum. By measuring the height of the integration curve at each set of peaks the ratio of protons absorbing can be determined. The NMR spectrum of propyl ethanoate shown in *Fig. 19,* illustrates how the integration curve is used.

THE ROYAL SOCIETY OF CHEMISTRY

Unilever



The ratio of the heights of the steps on the integration curve tells us the ratio of the numbers of protons resonating at each frequency. In this case the ratio of protons (not necessarily the absolute number of protons) *b:a:c:d* is 2:3:2:3

Figure 19 NMR spectrum of propyl ethanoate showing the integration curve and how it is used to obtain information on the number of protons resonating at each frequency

The latest machines produce a numerical computer printout which gives the area of each peak without the need to measure the height of the integration trace.

An unexpectedly simple peak appears in the spectrum of compounds containing an OH group. In the spectrum of ethanol *(Fig. 10)*, a single peak appears for the resonance of the hydroxy proton, at a chemical shift of 5.4. A triplet would normally be expected because the proton couples with the two CH_2 protons. However, the hydroxy proton is rapidly lost and replaced by other protons from hydroxy groups in its vicinity (*ie* exchanges), so no coupling is observed and only a single peak is seen in the spectrum. No coupling is observed because the hydroxy proton spends too short a time on each molecule. For instance if $J = 7$ Hz, to observe coupling the hydroxy proton must remain on a molecule in the order of 1/7 of a second. The CH_2 protons would be expected to give a quartet of doublets but the lack of coupling with the hydroxy proton means that only a quartet is observed. In *Fig. 20* the spectrum shows all of the coupling because no exchange of the OH proton occurs. These peaks are seen if the spectrum is run by putting the ethanol in a solvent that prevents proton exchange – *eg* DMSO (dimethyl sulphoxide).

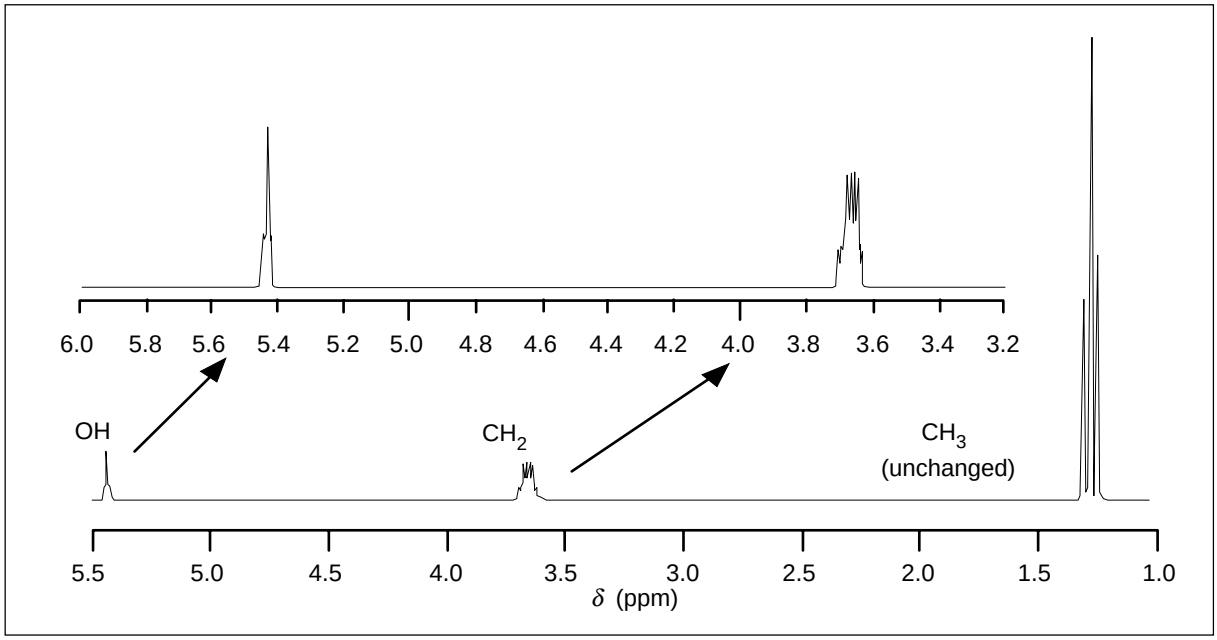


Figure 20 Non-exchange decoupled NMR peaks of ethanol

Practical considerations

About 20 mg of the sample is dissolved in 0.4 cm^3 of a solvent which has no hydrogen or is deuterated – *eg* CCl_4, $CDCl_3$, C_6D_6, d_6-DMSO (hexadeuterodimethyl sulphoxide, $(CD_3)_2SO$), or D_2O. The choice of solvent depends on the relative solubility of the sample in the various solvents. The reference material, usually tetramethylsilane (TMS) is added, and the solution is placed in a precision ground glass tube of 5 mm diameter to a depth of 2 or 3 cm. (2.5 mm and 10 mm diameter tubes can be used when only a small volume of solution is available or a large amount of sample has to be used.) The sample tube is then lowered into a probe, at room temperature (*Fig. 8*). The probe in older machines has both a transmitter and receiver coil connected to it, however, in modern Fourier Transform (FT) machines one coil performs both functions. In both machines the probe is immersed in the magnetic field.

The field in FT machines is often provided by a superconducting magnet that consists of alloy coils maintained at liquid helium temperature (4 K) but the magnet is insulated so that the sample tube remains at room temperature. The magnet retains its field strength once it has been energised with an electric current, provided the coils do not warm up or the energising current is lost.

The effect of slight variations in the magnetic field is minimised by spinning the sample at 20-30 revolutions per second and mounting 'shim coils' which can be used to reduce any inhomogeneity, in the magnet bore. This can be done manually or by computer control because the field has to be consistent to roughly one part in 10^9.

Older spectrometers relied on iron magnets and scanned the range of fields, and the receiver coils gave the spectrum directly. However, modern FT NMR spectrometers have a constant magnetic field and the range of frequencies is transmitted simultaneously for a few microseconds (typically 5 μs for ^1H and 10 μs for ^{13}C spectra). All the protons in the sample are excited, and each sends out radiofrequencies of the type shown in *Fig. 21*, as they relax. This is analogous to what

happens when a bell is hit. A sound characteristic of the bell is given out and dies away with time. The time taken for complete decay depends on the relaxation time of the system.

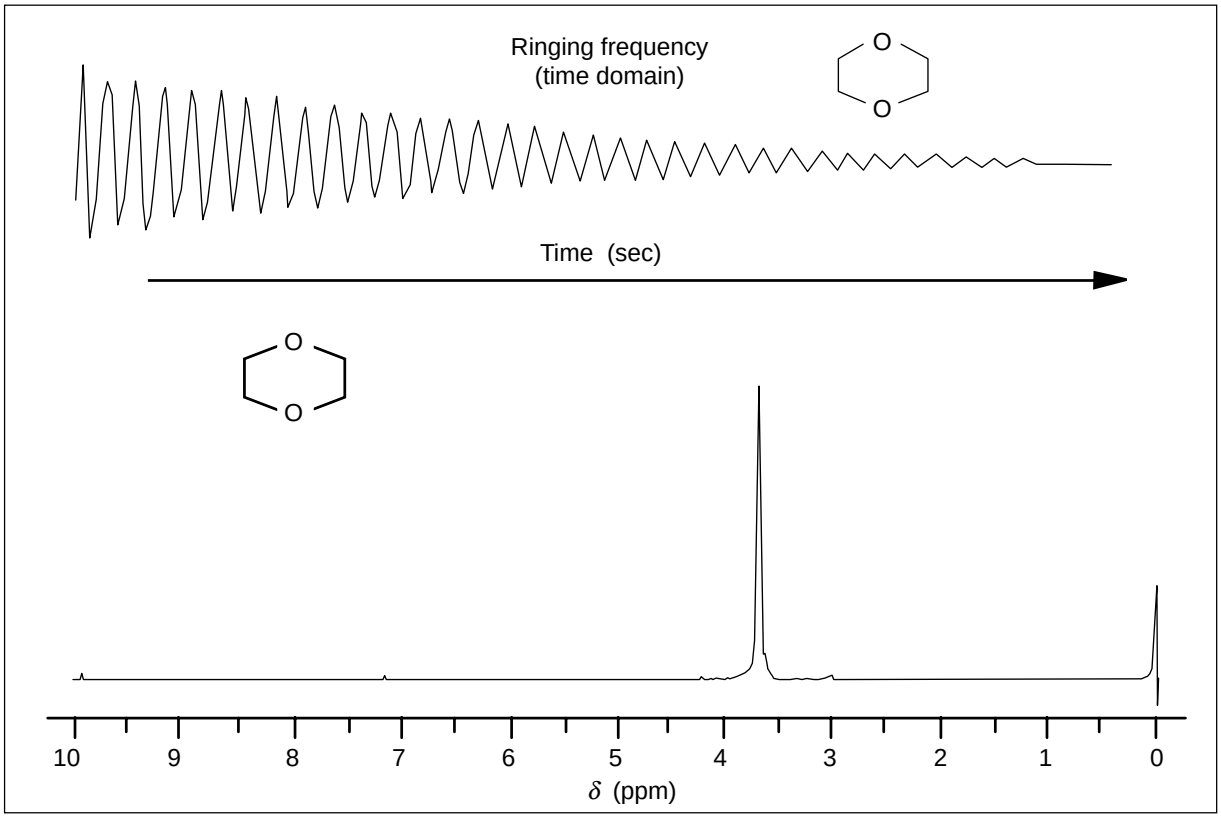


Figure 21 Time domain and final spectrum of dioxane

The amplitude of the decay curve is dependent on the degree of absorption, and all of the ringing frequencies transmitted will be superimposed on each other. A computer calculates the frequency (chemical shift) and intensity of each absorption and the spectrum is produced by a mathematical process known as Fourier transformation. The whole technique is known as pulsed NMR and typically the pulse is transmitted once a second until sufficient information has been collected. For ^{1}H-NMR spectra a 20 mg sample might require only one scan, while 1 mg might need 100–200 scans to collect enough data, depending on the relative mass of the compound and the strength of the magnetic field used. The individual scans can be added to produce the final spectrum. Pulsed NMR-FT spectra have the advantage that the background noise becomes less significant as more scans are added, because it is easier to register scans and put one scan on top of another to average them.

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

More advanced techniques

Spectra are often complicated by protons coupling with groups on both sides of them, and might be difficult to interpret. For instance, the C_b protons in propan-1-ol,

$$\underset{a}{CH_3}\underset{b}{CH_2}\underset{c}{CH_2}OH$$

couple with those of C_a and C_c. It is possible to simplify the spectrum by saturating the sample with radiation of the frequency at which one of the groups resonates. If the resonant frequency of the methyl protons (C_a) is transmitted while the data for the spectrum are being collected the protons on C_b show no coupling with the C_a protons. A triplet is then observed, and not a triplet of quartets (*Fig. 22*). In general, by removing the coupling of a known group it is possible to simplify the spectrum and decide precisely which other groups certain protons couple to. The technique is known as spin decoupling.

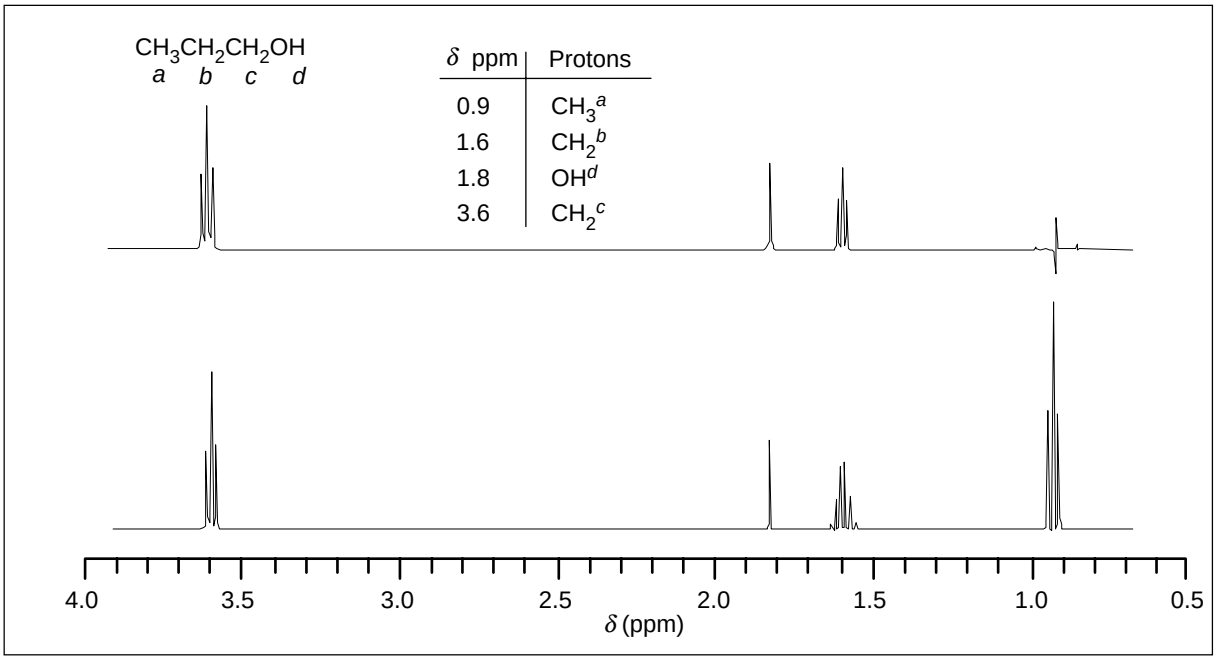


Figure 22 NMR and spin decoupled NMR spectra of $CH_3CH_2CH_2OH$

COSY

Spin decoupling has now largely been replaced by COSY (COrrelated SpectroscopY). In this method the spectrum is drawn horizontally and vertically and a contour or a three dimensional plot is constructed. This has the advantage over spin decoupling in that it shows all the coupling relationships. The full spectrum can be seen along the diagonal, and the coupling relationships are seen off-diagonal – *eg Fig. 23* where the NMR and COSY spectra of ethyl 4-methylbenzoate are shown.

Other NMR spectra

Although a number of nuclei other than ^1H will give NMR spectra, the most useful one is ^{13}C. The low abundance of this isotope (1.1 per cent) means that the probability of two ^{13}C atoms being bonded to each other is very small, so coupling is not observed and even if it were, the coupled signal would be too small to detect. ^{13}C-^1H coupling is possible, but this complicates the spectrum, so spin decoupling is frequently used to simplify it. Consequently the ^{13}C spectrum usually appears as a series of single peaks, each peak signifying the presence of a carbon atom in a

THE ROYAL SOCIETY OF CHEMISTRY

Unilever

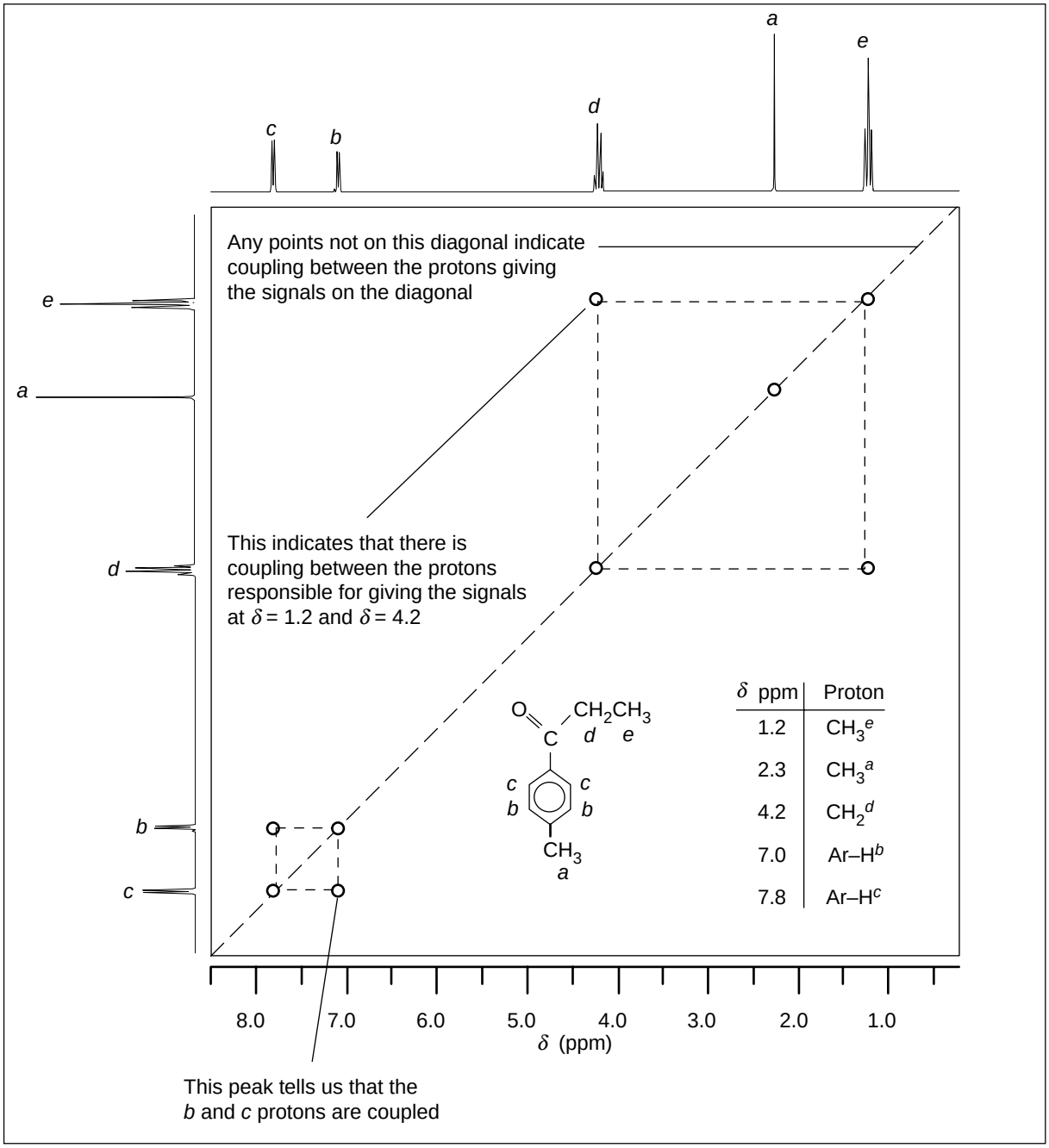


Figure 23 NMR and COSY spectra of ethyl 4-methylbenzoate

different chemical environment. In this type of NMR spectroscopy a useful integration peak may not be obtained because the signal strength is dependent on the environment of the carbon atoms. Therefore, these factors have to be taken into account when determining the number of equivalent carbon atoms in a particular position.

While proton NMR chemical shifts are in the 0-10 ppm range, ^{13}C chemical shifts are mostly in the 0-250 ppm range. The frequency at which ^{13}C resonates is also different – in a 2.35 T magnetic field protons resonate at 100 MHz, but ^{13}C resonates at 25.14 MHz. The ^{13}C spectrum of ethyl ethanoate is shown in *Fig. 24*. One

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

similarity between the two techniques is that tetramethylsilane (TMS) is a useful reference material for ^{13}C NMR spectroscopy too, and by definition gives a chemical shift of zero.

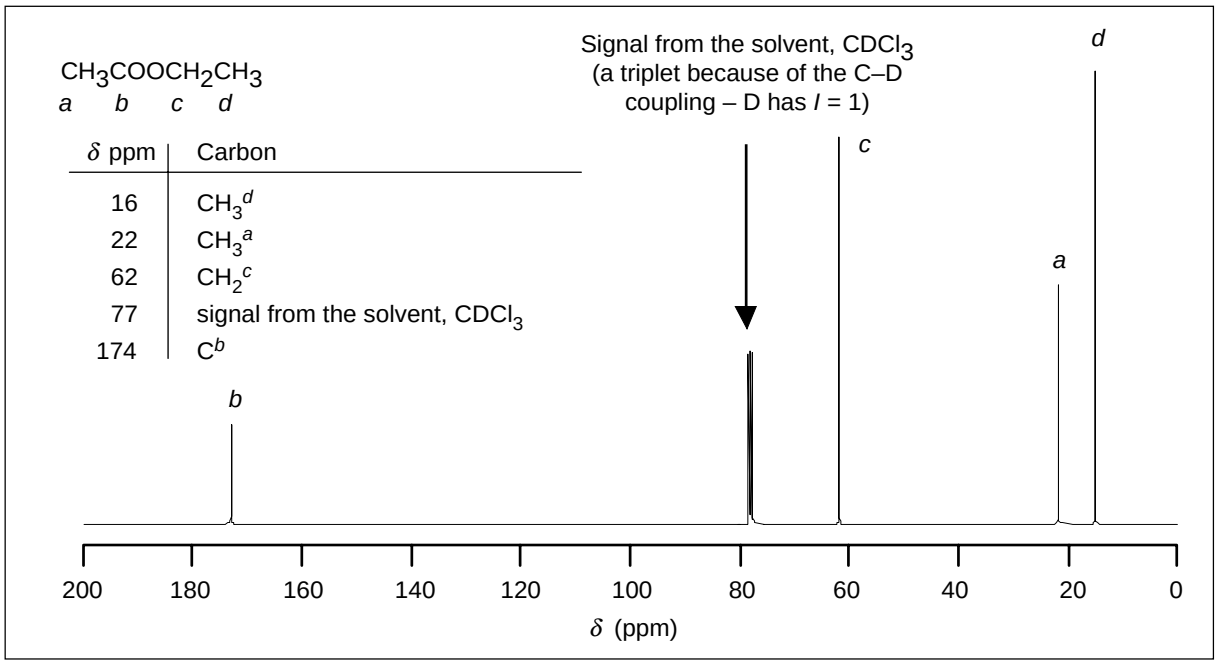


Figure 24 ^{13}C-NMR spectrum of ethyl ethanoate

Applications of NMR

NMR is commonly used for structure determination, but some other important uses do exist. Relaxation NMR spectroscopy can be used to evaluate the proportions of solid and liquid phase components in fatty foodstuffs such as margarines and low fat spreads. A plot of the signal intensity from the protons (recorded by the radiofrequency detector) with time is generated, and appears as one curve superimposed on another *Fig. 25*.

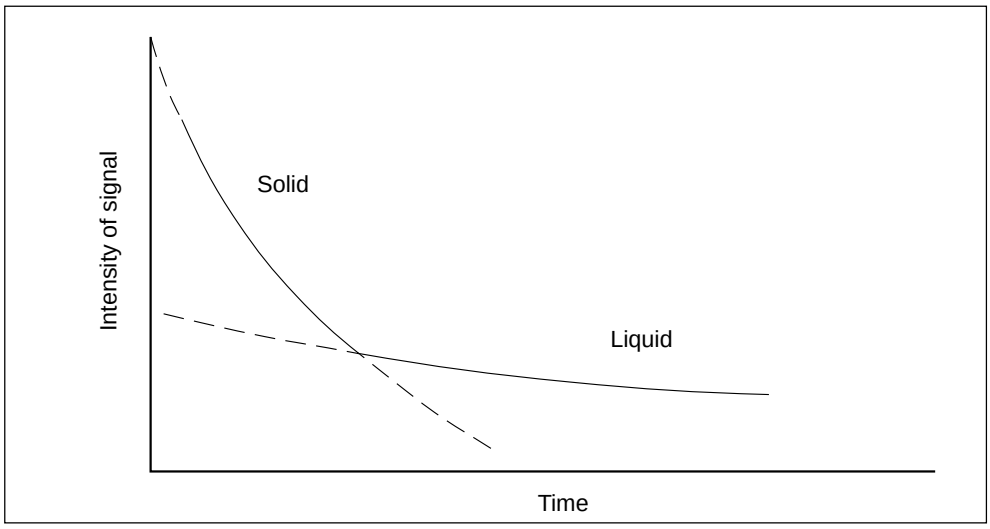


Figure 25 Intensity of NMR signal with time from a fatty foodstuff

This technique relies on the spin-spin (T_2) relaxation times of protons in the liquid phase being longer than those in the solid phase. Consequently, when the two curves are extrapolated back they give intensity values which are proportional to the relative amounts of protons in the solid and liquid phases. If the temperature of the sample is changed, the relative proportions may change, and if the experiment is repeated over a range of temperatures, a melting profile can be obtained.

The application of NMR in medicine is becoming increasingly common, from simple dynamic studies to complex diagnosis of tissue abnormalities. Pioneering work has been done at a number of institutions, including Hammersmith Hospital and Aberdeen and Oxford Universities. The studies described here were carried out in the NMR Imaging Facility at Queen Mary and Westfield College, London.

^{31}P-NMR of blood and cell fluids enables the kinetics of pH control in diabetics to be monitored. An absence of insulin can lead to harmfully high cell acidity levels, and an infusion of sodium hydrogencarbonate can help to re-establish the body's normal cell pH. This can be monitored by measuring the chemical shift separation between the organic and inorganic phosphorus signals. This is because the inorganic phosphorus NMR signal is highly pH dependent and can move as much as 1 ppm per pH unit. The area under each peak can also be used to gain data on the relative concentrations of each biochemical (*eg* ATP), and hence the metabolic status of the tissue.

Using ^{1}H-NMR in body scanning has become quite common. The intensity of ^{1}H-NMR signals depends both on the protons' density and their relaxation times. Consequently protons in water, proteins, lipids, carbohydrates and most body sites would be expected to give different signals. However, the main species, in the liquid state, with proton densities high enough to give an appreciable signal are water and lipids. The environments of the resonating nuclei give them different relaxation times, and hence different signals. Consequently different organs in the body can be differentiated.

Magnetic resonance images, as they are called (the 'nuclear' is dropped to avoid any association with nuclear radiation) look similar to X-ray images (*Figs. 26-28*). Magnetic resonance images can be acquired from a limb, the head or the whole body. Images from soft tissue can be acquired in any plane, and these complement the information from hard tissue data (such as X-rays). The scanning takes approximately 20 min, so the subject has to remain still with the part of their anatomy being scanned inside a large bore magnet. With animals this is achieved by light anaesthesia.

There are no known side effects associated with this technique, which means that subjects can be scanned regularly, including the young and the frail, to monitor any changes in condition.

This technique is now available in many clinics and has been used to diagnose successfully and monitor a variety of conditions such as cancer, hydrocephalus and multiple sclerosis.

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

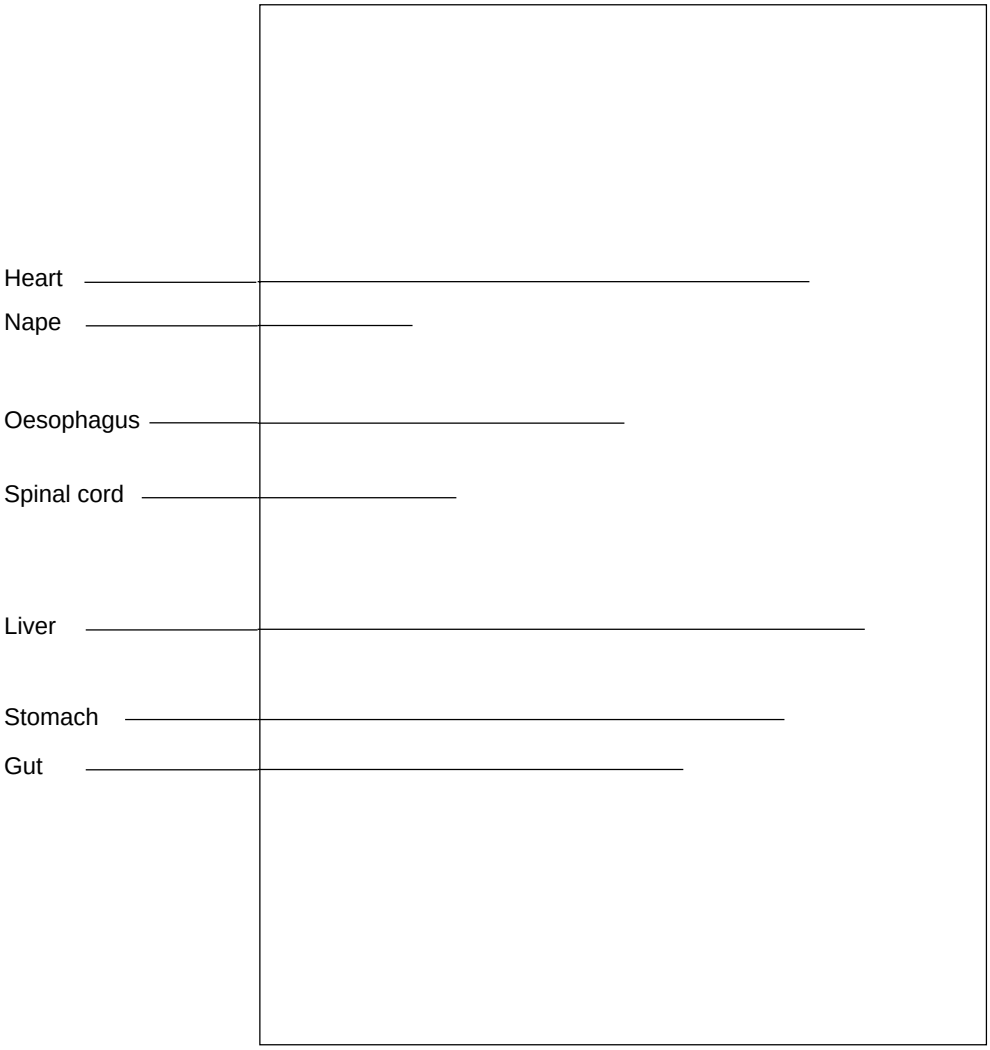


Figure 26 Saggital slice ^{1}H MR image of a live, anaesthetised guinea pig from a slice of 1.5 mm thickness with an in-plane resolution of 100 mm. Note the clear delineation of soft tissue structures such as the spinal cord, oesophagus, stomach and liver as well as heart, gut and subcutaneous fat at the nape of the neck

THE ROYAL SOCIETY OF CHEMISTRY

Unilever

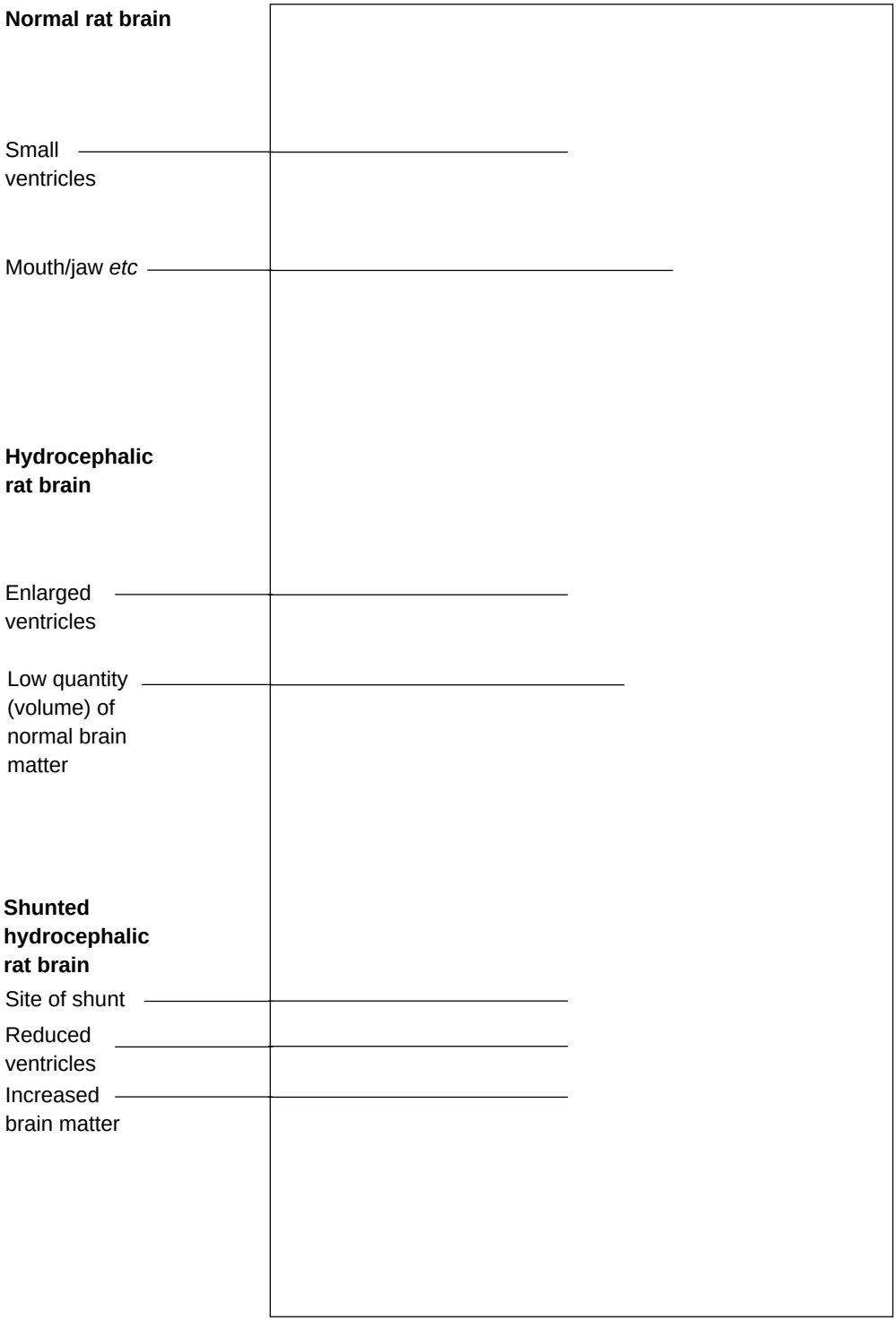


Figure 27 Coronal slice ^{1}H MR images of rats born with congenital hydrocephalus (water on the brain) from a 2 mm slice thickness. Top – normal animal with small ventricles. Middle – litter mate of affected animal with enlarged ventricles depicting build up of fluid. Bottom – another animal from the same litter that also suffered with the same disease but has been shunted in a similar manner to the techniques currently used in affected children. Note the reduced ventricle size and the increase of brain tissue relative to the diseased but untreated animal

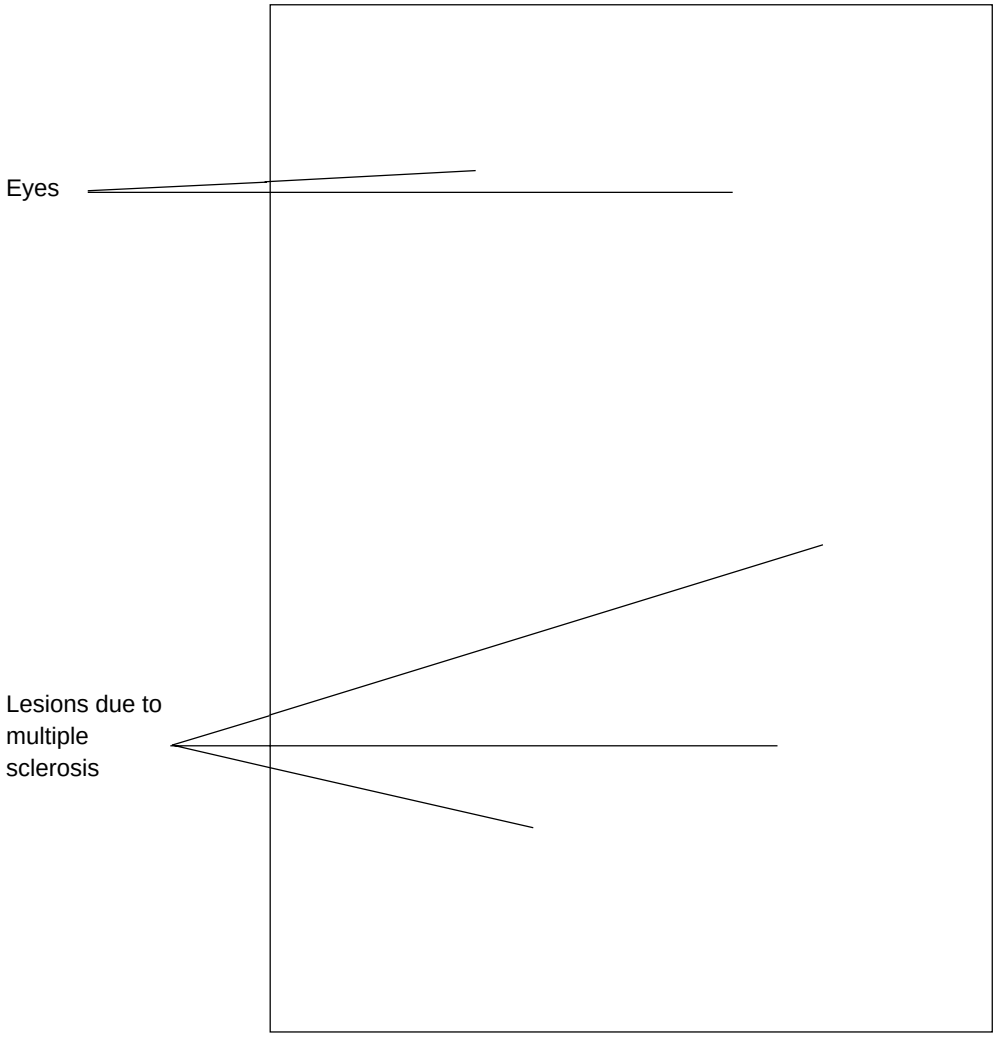


Figure 28 Transverse slice ^{1}H MR image of 5 mm thickness from human brain at the level of the eyes. The bright, white areas in both hemispheres relate to oedema formation in demyelinating lesions due to multiple sclerosis

THE ROYAL SOCIETY OF CHEMISTRY

Unilever

Exercises

By using Table 1 (page 38) and any other information given, it should be possible to determine the structures of each of the unknowns in the examples given below.

Exercise 1

A hydrocarbon, liquid at room temperature. The empirical formula of the compound is C_8H_{10}.

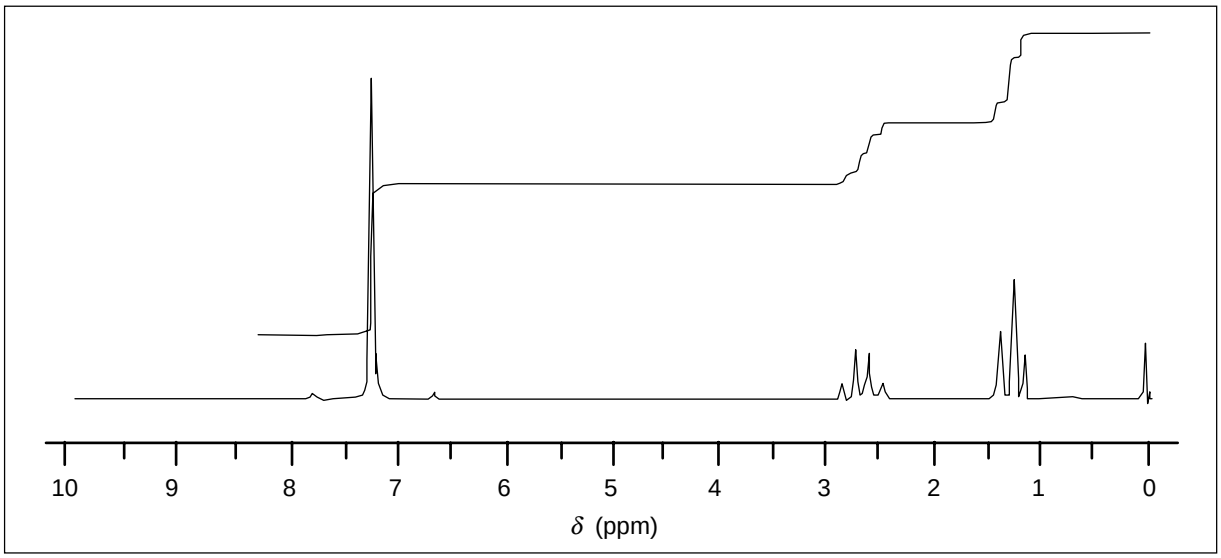


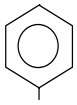
Figure 29

Chemical shift (δ)	Multiplicity (no of lines)	Integration
1.2	Triplet	3
2.6	Quartet	2
7.2	Singlet	5

The singlet at chemical shift (δ) = 7.2 indicates a benzene ring, and the fact that the integrated peak gives a ratio of 5 suggests that it is monosubstituted. The quartet at δ = 2.6 tells us that whatever is resonating at this value is coupling with three other protons. The integration curve reveals that two protons are resonating, so it would be reasonable to assume that a CH_2 group is involved. From Table 1 the only reasonable possibility is R-CH_2-Ar.

The triplet at δ =1.2 is due to protons coupling with two other protons – the CH_2 protons – and the integration curve reveals that there are three protons resonating at this shift – *ie* a CH_3 group is present.

Putting all this information together, the structure is ethylbenzene.



CH_2–CH_3

Ethyl groups that have no other coupling always give a triplet (the CH_3 protons) and a quartet (the CH_2 protons). However, the chemical shifts of these two peaks change with the electronic environment.

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

Exercise 2

A colourless mobile liquid at room temperature, with a sweet smell, having the empirical formulae $C_4H_8O_2$.

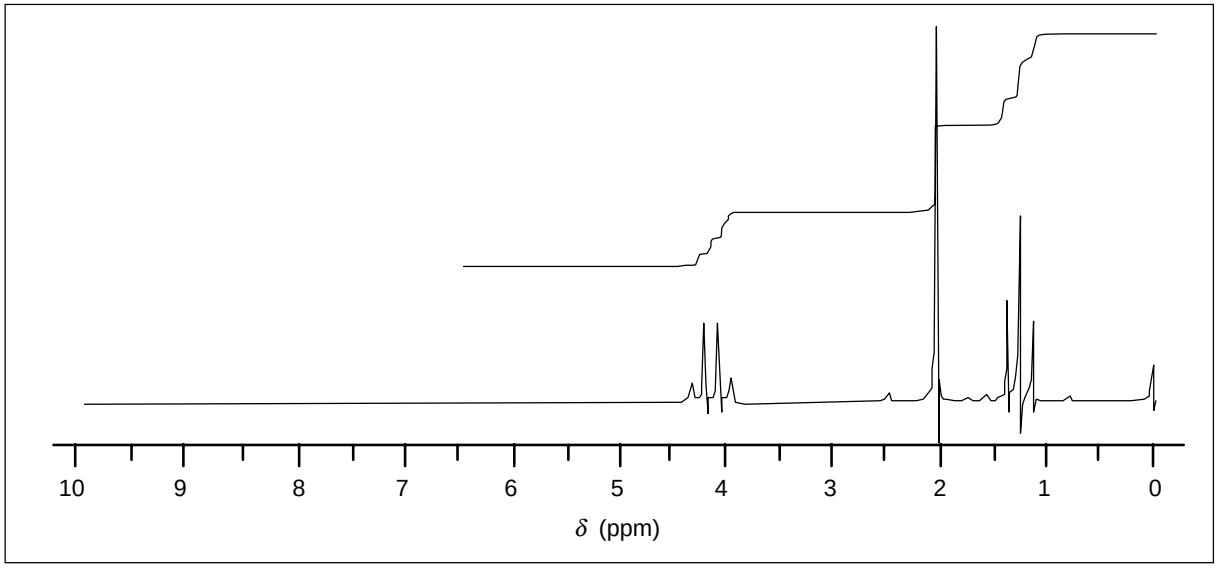


Figure 30

Chemical shift (δ)	Multiplicity (no of lines)	Integration
1.2	Triplet	3
2.0	Singlet	3
4.1	Quartet	2

From Table 1 the only realistic resonances at $\delta = 4.1$ are due to C$\underline{H}_3$–O–CO–Ar and R–C$\underline{H}_2$–O–CO–R′. Because the peak is split into a quartet, the resonating protons must be coupling with three other protons, and as the integration value is 2 the most sensible assignment would be R–C$\underline{H}_2$–O–CO–R′, where R must be CH_3. This would also account for the triplet at $\delta = 1.2$, where the methyl protons couple with the two CH_2 protons. The integration curve supports this, revealing that three protons are resonating at this value. Further evidence for dismissing the first structure suggested is that there are no aromatic protons present in the molecule – there is no peak in the range expected from benzene ring protons ($\delta = ca$ 6.5 – 8.2).

So far we have the structure CH_3–CH_2–O–CO–R′, an ester. The integration of the singlet at $\delta = 2.0$ reveals that three protons are present, suggesting a methyl group. This is supported by the value in Table 1.

Thus, *Fig. 30* is the ^{1}H-NMR spectrum of CH_3–CH_2–O–CO–CH_3, *ie* ethyl ethanoate.

THE ROYAL SOCIETY OF CHEMISTRY

Unilever

Exercise 3

This compound has a relative formula mass of 122, and is a solid at room temperature. It contains carbon, hydrogen and oxygen as the only chemical elements.

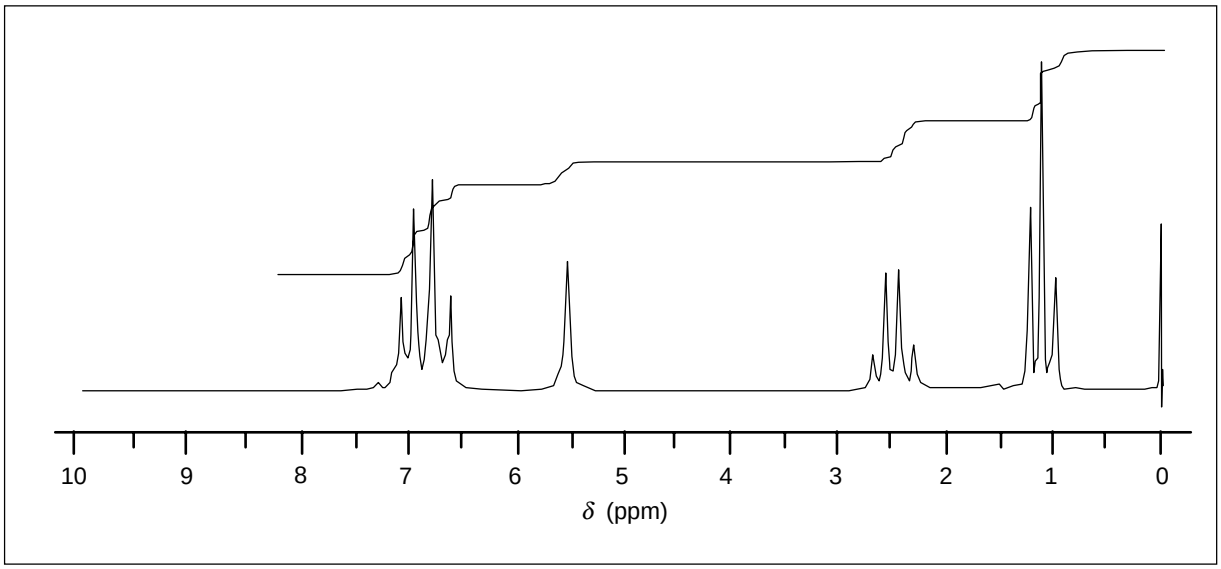


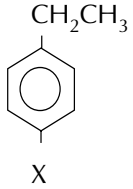
Figure 31

Chemical shift (δ)	Multiplicity (no of lines)	Integration
1.2	Triplet	3
2.5	Quartet	2
5.5	Singlet	1
6.8	Quartet	4

From Table 1, the three proton triplet centred on $\delta = 1.2$ is due to a methyl group attached to and coupled with a CH_2 group. The latter group appears at $\delta = 2.5$.

The CH_2 protons must also couple with the methyl protons to give a quartet, as is seen at $\delta = 2.5$. The shift value also suggests that the CH_2 unit is bonded to a benzene ring.

The multiplet at $\delta = 6.8$, with integration curve data showing four protons, indicates a benzene ring. The splitting pattern is that expected for a 1,4- disubstituted ring. So far we have



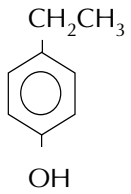
X

The relative mass of the components identified so far is $C_6H_4 + C_2H_5 = 105$. This leaves 17 mass units unaccounted for, and the only reasonable unit that corresponds to this mass is OH.

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

Thus if X is OH the molecule must be 4-ethylphenol,



Assigning the singlet at chemical shift = 5.5 would have been difficult in the absence of any other information because hydroxy protons can resonate over a range of shifts, depending on factors such as solvent, pH and temperature as well as the chemical environment of the proton.

Exercise 4

A solid at room temperature, containing carbon, hydrogen and oxygen only. The structure of this compound can be determined from its spectrum (*Fig. 32*).

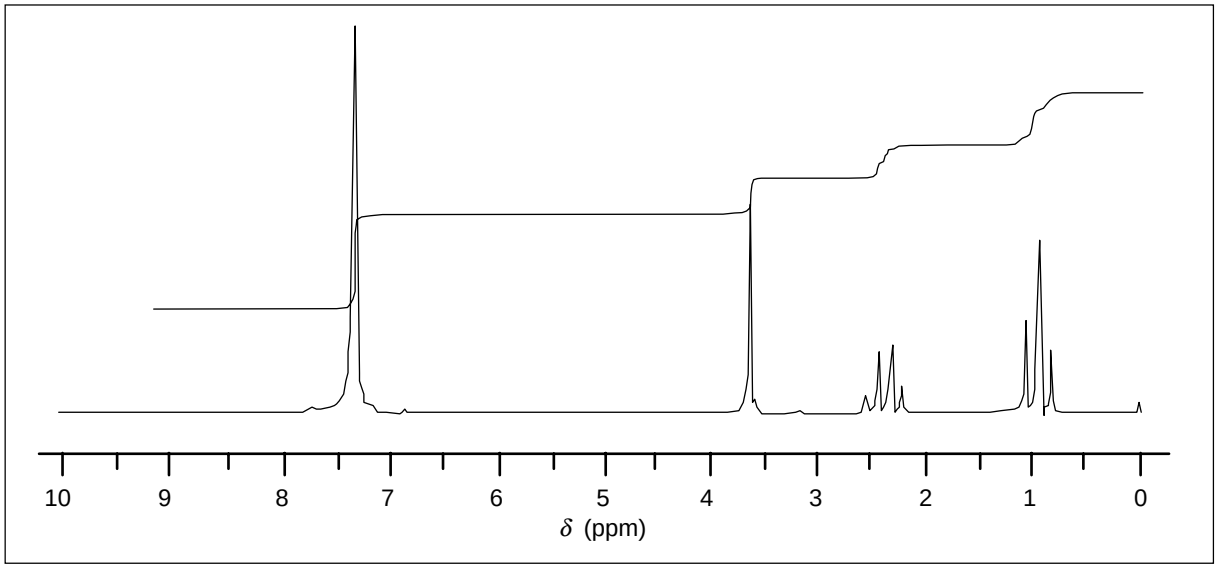


Figure 32

Chemical shift (δ)	Multiplicity (no of lines)	Integration
0.9	Triplet	3
2.35	Quartet	2
3.6	Singlet	2
7.2	Singlet	5

The singlet with integration ratio 5, at $\delta = 7.2$ indicates a monosubstituted benzene ring. A convenient way of determining the nature of the side chain is to start with the quartet at $\delta = 2.35$. Whatever is resonating at this value is coupling with three protons (from the multiplicity), and there are two protons (from the integration curve) resonating. A group that would conform to this is a CH_2 group bonded to a CH_3 group. The chemical shift should give some information about what else is bonded to the CH_2 unit. From Table 1 the possibilities are:

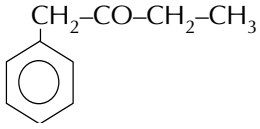
THE ROYAL SOCIETY OF CHEMISTRY

Unilever

$CH_3–CH_2–Ar$ or $CH_3–CH_2–CO\text{-}R$

The first possibility can be discounted because if the molecule is ethylbenzene there would be no peak $\delta = 3.6$. This peak could be from another side chain, but in that case the ring protons would give a more complicated splitting pattern and the integration curve would suggest only four (and not five) ring protons.

Therefore, the ethyl group must be bonded to a carbonyl carbon atom. However, the structure is still not solved, because the R function on $CH_3–CH_2–CO–R$ has not been determined. The only peak not assigned so far is the singlet at $\delta = 3.6$ – representing two protons. These protons must be adjacent to groups that they cannot couple with, otherwise the peak would not be a singlet. One group is obviously the carbonyl and the other will be the benzene ring. The final structure is therefore 1-phenyl-2-butanone.



It is possible that there is a carbonyl or an ester function between the CH_2 group and the benzene ring, but this would shift the resonance of the ring protons further downfield (see Table 2 for values).

Exercise 5

This compound has percentage composition C 73.2 per cent, H 7.3 per cent and O 19.5 per cent by mass. It has two oxygen atoms per molecule and its ^{1}H-NMR spectrum is shown in *Fig. 33*.

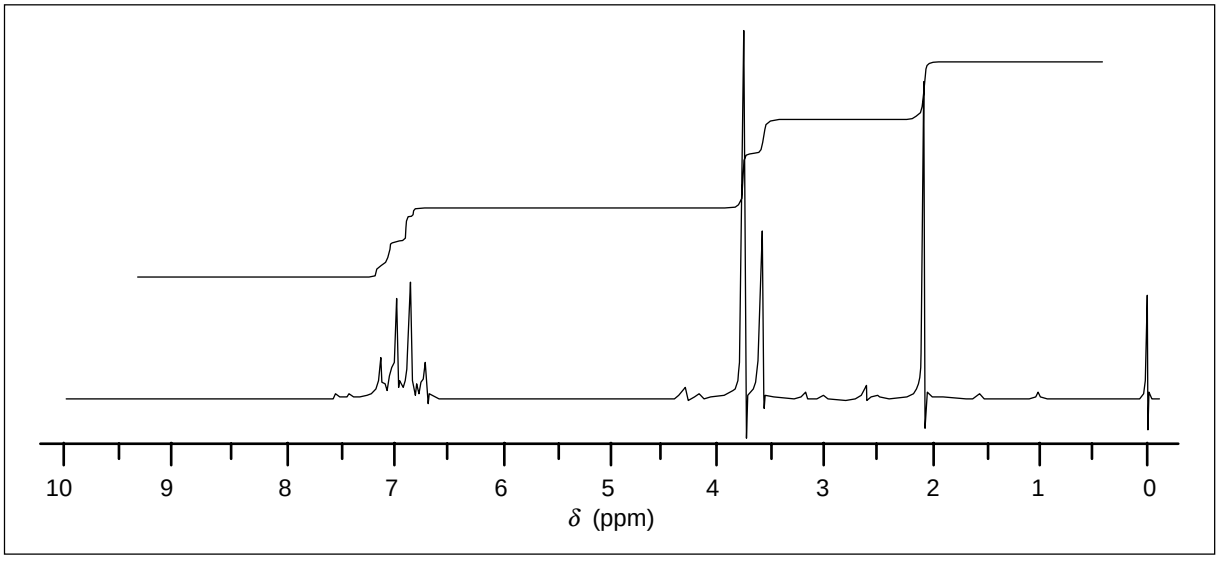


Figure 33

Percentage composition data gives the empirical formula C_5H_6O. Each molecule contains two oxygen atoms, so the molecular formula must be $C_{10}H_{12}O_2$. The information can be obtained from the integration of the NMR peaks because the ratios add up to 12.

Unilever

THE ROYAL SOCIETY OF CHEMISTRY

Chemical shift (δ)	Multiplicity (no of lines)	Integration
2.1	Singlet	3
3.55	Singlet	2
3.7	Singlet	3
6.85	Quartet	4

Apart from the quartet at $\delta = 6.85$ all the peaks in this spectrum are singlets. Consequently, apart from the four ring protons (the quartet) no other protons couple with each other. The splitting pattern of the ring protons suggests that it is 1,4-disubstituted.

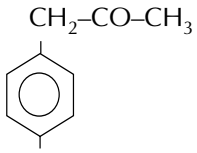
The signal from the protons at $\delta = 2.1$ is likely to be from one of the following:

CH_3–CN, or CH_3–CO–R, or CH_3–CO–O–R

The nitrile can be dismissed because nitrogen is not present in the compound, but the other two are possibilities. If the unknown is an ester, it must have at least one CH_2 unit between the ester function and the benzene ring (the chemical shift is for an alkyl ester and not an aromatic ester). Thus a signal would be expected at $\delta = 4.9$ from the CH_2 protons in Ar–CH_2–O–CO–R (Table 1). This is not observed, so the compound must contain CH_3–CO–R.

From Table 1, the singlet from the two protons at $\delta = 3.55$ could be due to either Ar–CH_2–CO–R or R–CH_2–OH. The latter is not possible because R–CH_2– involves coupling between the protons, and this is not observed.

Combining the information so far, we have:



X

and have accounted for C_9H_9O. This leaves CH_3O unaccounted for. Two possibilities are: OCH_3 and CH_2OH. The latter would give two NMR signals, and we only have one peak at $\delta = 3.7$ representing three protons which is unassigned. This corresponds to OCH_3.

Thus the structure of the compound is 4-methoxyphenylpropanone.