Nuclear Magnetic Resonance Spectroscopy (NMR)

What is it ?

An instrumental method that gives very detailed structural information about molecules. It can tell us

- how many of certain types of atom a molecule contains
- where these atoms are located in the molecule

It works because the nuclei of certain atoms have a property we call "spin". Their spins can be "up (↑)" or "down (↓)". Normally these two spins have the same energy, but when the nuclei are in a magnetic field, the spins align with the field. The spin state which is aligned with the field is of lower energy than the spin state which opposes the field, so there is an energy gap – nuclei can be excited, using a pulse of radio frequency energy, from the lower energy spin state to the higher energy spin state, and can relax back to the lower energy spin state by releasing energy. The energies involved are tiny, so NMR instrumentation has to be extremely sensitive.

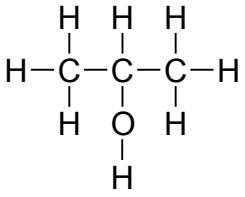
The commonly-used nuclei for NMR are ^{1}H, and ^{13}C although it is also possible to do NMR with ^{31}P and ^{19}F amongst other atoms.

Carbon-13 NMR – signals arise from the carbon atoms in the molecule

1.1% of all naturally occurring carbon atoms are ^{13}C atoms. They occur randomly in any position in the molecule where a carbon atom exists, so the signal from the ^{13}C atoms gives information about all the C atoms present in the sample.

If each carbon nuclei was entirely on its own, separated from the influence of any other nuclei, it would produce an NMR signal at exactly the same energy. In reality the carbon atoms have other atoms bonded around them. The electrons in these atoms subtly affect the magnetic field the nucleus experiences, and therefore the signal occurs at a different energy. The energy of an NMR signal is measured in terms of how far away it is (the Chemical Shift, δ) from a known reference signal, in units of parts per million (ppm).

Consider a molecule of propan-2-ol:



Each carbon atom produces an NMR signal, but how many different carbon-atom environments are there ? The first and third carbons are in identical environments, so produce signals at the same chemical shift. The second carbon will produce a signal at a different chemical shift. We will therefore see two peaks in the NMR spectrum.

Using your datasheet you should now be able to predict the number of peaks in the 13C spectrum of propan-1-ol and sketch its 13C NMR spectrum.

In carbon-13 NMR the size of the peaks DOES NOT tell us anything useful. (This is different from in ^{1}H NMR (proton NMR) which we will look at next.

The data sheet shows which chemical shift values to expect for carbon atoms in different environments – this is used to decide which NMR signals correspond to which carbon nuclei in the molecule. Notice how the more electronegative atoms bonded to the C atom increase its chemical shift. The solvent, and concentration of the molecule being sampled cause the chemical shifts to vary slightly also.

Environment	**Approx. chemical shift (δ) in ppm**
C – C (aliphatic)	5 – 55
C – Cl, C – Br	30 – 70
C – N	35 – 60
C – O	50 – 70
C=C	115 – 140
C ⁻C (aromatic)	110 – 165
C=O also bonded to O or N	160 – 185
C=O	190 – 220
	(but check against A2 datasheet)

Practice:

1: Using the data sheet, predict what the carbon-13 NMR spectrum of propan-1-ol will look like.

[Ans: 3 peaks, C-O at 65ppm C-C at 25 and 10 ppm]

2: What can you tell about the ketone which produced this ^{13}C NMR spectrum ? Can you identify the ketone from this data ?

Peak at 200 ppm

Peak at 50 ppm

Peak at 15 ppm

[Ans: There are 3 distinct proton environments
The C=O carbon shows up at 200ppm
The other two peaks are two different aliphatic C - C environments
- so it can't be propanone (only 2 environments)
- it can't be butanone (4 different environments)
- it can't be hexanone or anything bigger – too many environments
- pentan-2-one has 4 different C environments
- but pentan-3-one has exactly 3 different environments]

3: An alcohol with formula C_3H_7OH was oxidized. The ^{13}C NMR spectrum of the product showed three peaks: at 170ppm, 45ppm and 25ppm. Using the data table of chemical shifts, what can we say about the product and about the alcohol ?

Ans: The peak at 170ppm corresponds to the carbon in COOH rather than in C=O so the product is likely to be propanoic acid.

The two other peaks (aliphatic C-C) confirm that the product is not propanone (only one aliphatic C-C environment).

We can therefore be sure that the oxidized alcohol was primary – propan-1-ol.

More about Chemical Shift

NMR spectra are calibrated using the δ scale, in parts per million. This is based on the frequencies used by the NMR spectrometer. For example, a medium-sized NMR instrument may be referred to as a 200MHz instrument, this refers to the frequency of the rf energy needed to excite the nuclei when they are in the magnetic field of the that instrument. A chemical shift of 1 part per million means a shift of 200Hz (one millionth) in the frequency at which a specific nucleus gives its signal.

The signals are all measured from a reference zero point – the NMR signal from a reference substance. This substance has to be chosen so that its signal is strong and so that there is only a single peak in the spectrum due to this substance – i.e. the nuclei giving the signal are all in identical environments. The substance also has to be chemically unreactive, and volatile so it can be removed from the sample after running the spectrum. Tetramethylsilane (TMS) $(CH_3)_4Si$ is used because it has four identical carbon environments.

When NMR spectra of a substance are recorded, a small amount of TMS is added so that the spectrometer can be calibrated. The signal from the TMS is taken as the δ=0ppm chemical shift point from which the chemical shifts of other peaks are measured.

Proton (^{1}H) NMR – signals arise from the hydrogen atoms in the molecule

The terms "proton" and "hydrogen" are used interchangeably in NMR. A hydrogen-1 nucleus consists of one proton. When referring to proton NMR we are NOT talking about the protons in any other atoms' nuclei !

Proton NMR is similar to ^{13}C NMR in a number of important ways

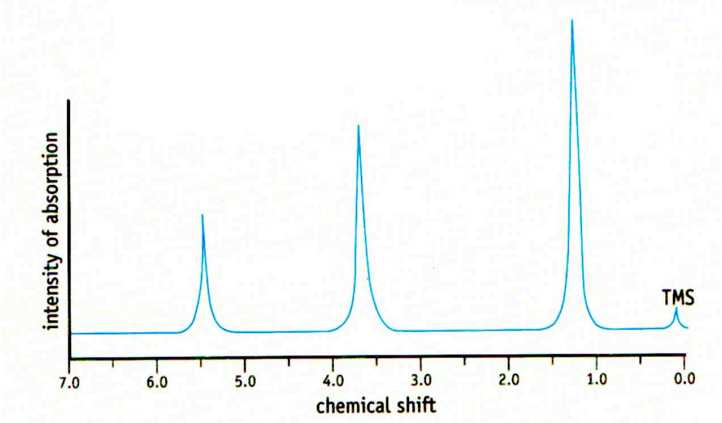
- each equivalent hydrogen atoms environment produces a peak in the spectrum
- the peaks positions are measured in chemical shift (δ) in ppm
- the reference is still TMS, which has 12 equivalent hydrogen atoms

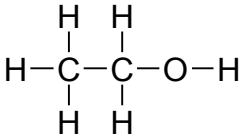
There are some important differences too

- the area under a peak tells us how many hydrogen atoms there are in tha environment
- each peak also gives information about how many protons are in adjacent environments (spin-spin coupling, see later)

The ^{1}H isotope is much more abundant (99%) than the ^{13}C isotope, so the NMR signals are much stronger (meaning that much smaller sample sizes can be used). The chemical shifts from protons in different environments are much smaller than found in ^{13}C NMR, and the chemical shift ranges for each signal tend to overlap more.

Starting to interpret a proton NMR spectrum:

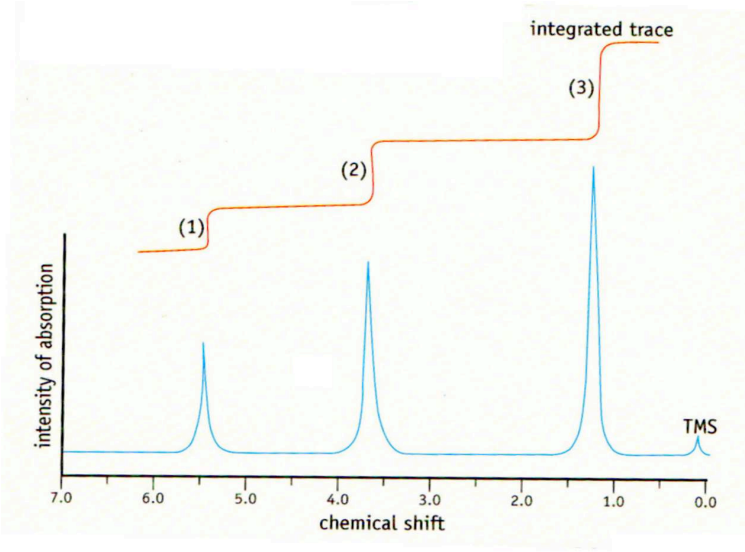




Here's the proton NMR spectrum of ethanol. We can use the chemical shift table to work out which peak belongs to which hydrogen atoms in the molecule:

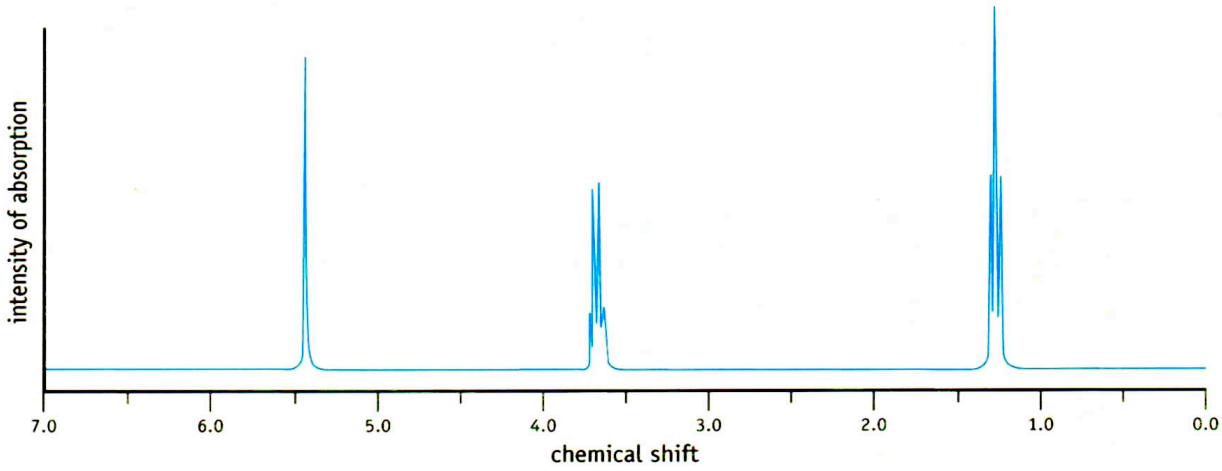
- The peak at 5.4ppm is outside the range of C-H and is going to be the O-H proton
- The peak at 3.7ppm corresponds to protons on a carbon with an O also connected (O-CH_2-R)
- The peak at 1.2ppm corresponds to an R-CH_3 proton environment.

Notice that the peak heights follow the same pattern as the number of protons contributing to each peak. In fact it's the area under the peak that tells us about the numbers of protons. NMR spectra are often supplied with the areas under the peaks calculated and labeled:



Spin-Spin Coupling

Few NMR spectrometers are so low in resolution that they produce the kind of proton NMR spectrum you have seen so far – higher resolution spectrometers show an extra kind of information – spin-spin coupling. Compare the previous ethanol spectrum with that produced by a more normal higher resolution instrument:



Notice that the peak at 1.2ppm has been split into thee finer peaks – a triplet – and that the peak at 3.7ppm has been split into four finer peaks – a quadruplet. The pattern of peak heights is characteristic too: the triplet has heights 1:2:1 and the quadruplet has heights 1:3:3:1.

Spin-spin coupling arises from the interaction of the protons giving rise to the peak, with non-equivalent protons on adjacent carbon atoms. The splitting pattern tells you how many protons there are bonded to the adjacent carbon atom. Note that –OH, -NH (and –SH) groups do not cause splitting and are not themselves split.

We use an "n+1" rule: If there are n non-equivalent protons on the adjacent carbon then the splitting will be into n+1 finer lines.

- a quadruplet/quartet tells us there are 3 hydrogen atoms on the adjacent carbon
- a triplet tells us there are 2 hydrogen atoms on the adjacent carbon
- a doublet tells us there is 1 hydrogen atom on the adjacent carbon
- a singlet tells us there are no hydrogen atoms on the adjacent carbon
  (or no adjacent carbon !)

More complicated splitting patterns (multiplet) are sometimes seen when there are very similar but not equivalent proton environments e.g. in a substituted benzene ring, and here we rely on the peak area and chemical shift information rather than trying to interpret the multiplet.

See how this works for the ethanol spectrum:

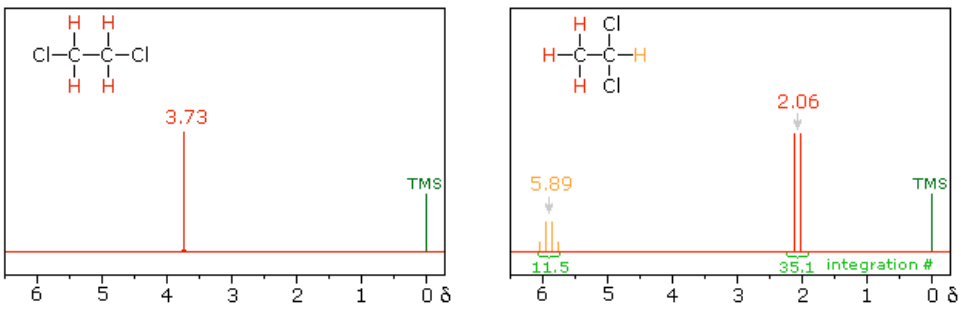
- For the peak at 1.2ppm: It has an area of 3 so it arises from three equivalent protons in an R-C**H** environment, so we are look at a CH_3 group. It is split into a triplet because there are two non-equivalent hydrogens on the adjacent carbon atom, so we can identify this signal as coming from: $–CH_2-C\mathbf{H}_3$

- For the peak at 3.7ppm: It has an area of 2 so it arises from two equivalent protons in an O-C**H** environment, so we are looking at an $-O-C\mathbf{H}_2-$ fragment of the molecule. It is split into a quadruplet because there are three non-equivalent hydrogens on the adjacent carbon, which therefore must be a CH_3 group, so we can identify this signal as coming from $–O-C\mathbf{H}_2-CH_3$

- Note that the quadruplet/triplet pattern we see here is commonly seen, and characteristic of an ethyl group in the molecule ($-CH_2-CH_3$).

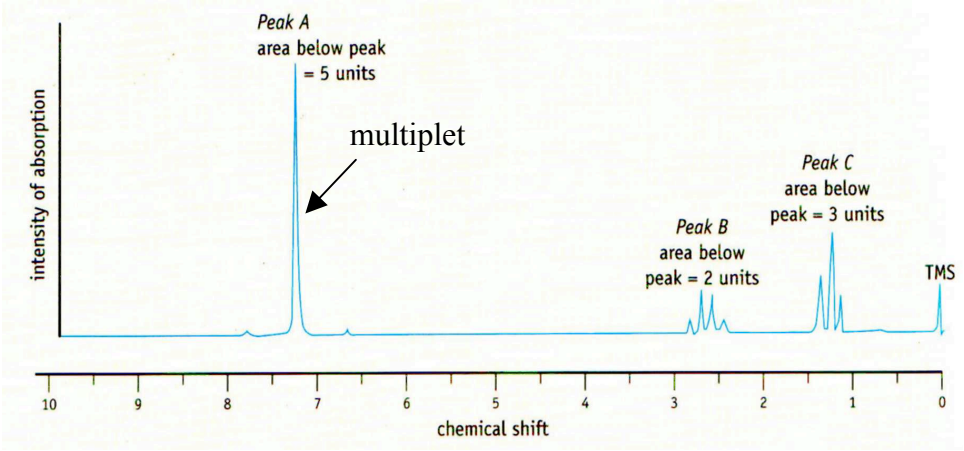
- For the peak at 5.4ppm: It has an area of 1 so it arises from a single proton in an –OH environment. It is a singlet (no splitting) as –OH groups are not split and do not cause splitting.

Spin-spin coupling only occurs between non-equivalent protons on adjacent carbon atoms. A good illustration of this is given by the ^1H NMR spectra of dichloroethane isomers – the spectrum of 1,1-dichloroethane consists of a quadruplet (peak area = 1) and a doublet (peak area 3) corresponding to the $–CHCl_2$ and $-CH_3$ carbons respectively; but the spectrum of 1,2-dichloroethane contains a single peak, unsplit, not two overlaid triplets.



Worked example:

What can you work out about this spectrum?



NUMBER OF PEAKS = NUMBER OF ENVIRONMENTS

There are thee peaks, so there are three different proton environments

FOR EACH PEAK:

Chemical shift = what environment

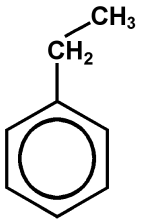
Peak area = how many hydrogens in that environment … specify fragment

Splitting = information about adjacent environment … extend the fragment

Peak at δ=7.2ppm: Chemical shift indicates protons on a benzene ring.
Area indicates 5 of them, so $C_6\mathbf{H}_5$- fragment.
Splitting is a multiplet, the hydrogens on adjacent carbons around the ring cause this splitting, but it is too complex to analyse using N+1 rule. (This is typical for substituted benzene rings).

Peak at δ=2.6ppm: Chemical shift indicates hydrogens on a carbon next to a benzene ring.
Area indicates 2 of them, so $-C\mathbf{H}_2-C_6H_5$.
Quartet splitting indicates 3 non-equivalent hydrogens on an adjacent carbon. The carbon of the benzene ring where this $-CH_2-$ group connects has no hydrogens on it, so these are three non-equivalent protons in the form of a CH_3- group bonded to the $-CH_2-$, as $CH_3-C\mathbf{H}_2-$.

Peak at δ=1.3ppm: Chemical shift indicates hydrogens in an R-C**H** environment.
Area of 3 indicates that this is a $-C\mathbf{H}_3$ group.
Triplet splitting indicates two non-equivalent hydrogens on an adjacent carbon, so $-CH_2-C\mathbf{H}_3$.



COMBINE FRAGMENTS FOR FINAL MOLECULE:

More about solvents for NMR Spectroscopy

NMR is usually carried out in solution, so a solvent is needed. Organic solvents contain C and H atoms, and these would produce NMR signals that would complicate the spectra.

Instead, it is common to use **deuterated** solvents. The ^{1}H isotope is only one of the isotopes of hydrogen. The ^{2}H isotope (with one proton and one neutron in its nucleus) is called deuterium and has the chemical symbol D. Because they are isotopes of the same atom, hydrogen and deuterium have the same chemical properties.

Deuterium does not produce an NMR signal in an NMR spectrometer tuned to proton frequencies, so solvents with deuterium atoms where the hydrogen atoms would have been will do the same job of dissolving the sample, but produce no peak in the spectrum.

Solvents such as $CDCl_3$ are commonly used for proton and 13C NMR (in the latter case it is easy to remove the one peak from the C in the solvent). Because $CDCl_3$ is volatile its easy to evaporate off the solvent and recover the sample after the NMR experiment. Remember that a small amount of TMS is also added; not as a solvent but as a reference.

NMR spectra from compounds with –OH and –NH groups
(alcohols, amines, carboxylic acids, phenols)

The peaks arising from –OH and –NH protons are often broad, and occur over a wide range of chemical shift values. This makes them hard to identify unambiguously.

There is a clever technique we can use to check if a peak which we think arises from an –OH or –NH group actually does. This makes use of D_2O – water molecules with both hydrogen atoms replaced with deuterium atoms (sometimes referred to as "heavy water" since its M_r is 20, not 18).

1. Run the normal NMR spectrum and make peak assignments as best you can
2. Add a small amount of D_2O to the sample and shake (because the sample sizes are really tiny, a small amount of D_2O still constitutes an excess)
3. Run the NMR spectrum again – any peaks which correspond to –OH or –NH protons will disappear from the spectrum

This works because the hydrogen atoms on –OH and –NH are **labile** – they rapidly exchange with the deuterium atoms in the D_2O – and of course the D atoms don't give a ^{1}H NMR signal so the peaks disappear.

e.g. $CH_3CH_2OH + D_2O \rightleftharpoons CH_3CH_2OD + HOD$

Splitting (or the lack of it) from –OH and –NH protons

As far as splitting is concerned, ignore –OH and –NH protons.

- They will show up as a singlet which is not split by adjacent protons
- They will not cause splitting of the signal from adjacent protons

The peak from –OH and –NH protons may be quite broad. Traces of water in the solvent, and hydrogen bonding between sample molecules, can form hydrogen bonds with –NH and –OH protons which affects the environment (electron cloud) in the vicinity of these protons.

Combining analytical methods

It is much more common to have the results of several different types of analysis, rather than relying on a single method. Commonly we might have the results of elemental analysis, infrared spectra, mass spectra and NMR spectra to work with.

Given such a variety of data, a systematic approach to interpreting it is needed, and the following sequence works:

CONTEXT: use the any information given in the question, and the results of elemental analysis, to suggest possibilities and eliminate others. You may be able to determine empirical formula, or identify/reject certain functional groups being present.

INFRARED SPECTROSCOPY: use the infrared spectrum to suggest functional groups present in the molecule and to eliminate the possibility of others being present.

MASS SPECTROMETRY – MOLECULAR ION PEAK: identify the molecular ion peak (at highest m/e value, but bear in mind there may be a very small m+1 peak due to 1% of carbon atoms being ^{13}C) and use this m/e value to suggest M_r for the molecule. If the empirical formula is known, the molecular formula can be found. If the empirical formula is not known, at you get a feel for the size of the molecule. The number of carbon atoms can be estimated, taking into account the mass of any functional groups you have identified as being present. *N.B occasionally a molecule can be unstable enough that every molecule fragments when hit by the electron beam in the mass spectrometer – there will not be a molecular ion peak, but you won't figure this out until the rest of the data supports a candidate molecule which does not fit with the highest m/e peak in the mass spectrum.*

NMR SPECTROSCOPY: work through the NMR data as explained already in this topic, identifying the number of different environments, and then peak by peak building up a picture of the fragments and how they are connected. Use this data to propose one (or more) candidate molecules that fit all the data, including checking that they fit the M_r value.

MASS SPECTROMETRY – FRAGMENT IONS: look at the most common fragments that would arise from breaking bonds in the candidate molecules, work out the mass of these fragments, and look for corresponding fragment ion peaks in the mass spectrum to confirm the candidate molecule or to choose between candidates.