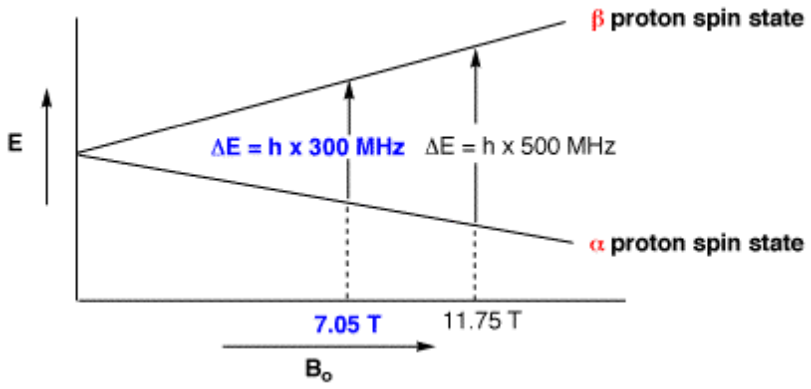
NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

In nuclear magnetic resonance (NMR) spectroscopy, the magnetic properties of certain nuclei are exploited to seek structural information of the molecule. NMR spectroscopy helps to identify the carbon-hydrogen framework of organic compounds and is one of the most powerful tools for investigation of the structure of organic molecules, thus having important medical applications. It is a qualitative technique only and it non-destructive of the sample. Like electrons, all nuclei (except even-even nuclei) have allowed spin states and this property allows them to be studied by NMR. Spinning charged nuclei generate a magnetic field. In the absence of an applied magnetic field, the nuclear spins are randomly orientated. However, when a sample is placed in an applied magnetic field, the nuclei align themselves with or against the field of the larger magnet. More energy is needed for a proton to align against the field than with it. Protons that align with the field are in the lower energy alpha spin state while those that align against the field are in the higher beta state. The energy difference between the alpha and beta state depends on the strength of the magnetic field. The greater the strength of the magnetic field to which the nucleus is exposed, the greater the energy difference between the states. By Boltzmann's distribution, the lower energy level will contain slightly more nuclei than the higher level.



**Note: Bo is the external magnetic field and ΔE is the energy difference between the states.

The energy difference between the spin states depends on the operating frequency of the spectrometer, which in turn depends upon the strength of the magnetic field and the gyromagnetic ratio (the gyromagnetic ratio is a constant that depends on the magnetic moment of the particular kind of nucleus). The magnetic field is proportional to the operating frequency, so if the spectrometer has a more powerful magnet, it must have a higher operating frequency. Because each kind of nucleus has its own gyromagnetic ratio, different energies are required to bring different kinds of nuclei into resonance. When the sample is subjected to a pulse of radiation whose energy corresponds to the difference in energy between the spin states, nuclei in the alpha spin state are promoted to the beta spin state. This transition is called splitting the spin. Because the energy between the alpha and beta spin states is so small, only a small amount of energy is needed to flip the spin. The radiation required is in the radiofrequency region of the electromagnetic spectrum. When the nuclei undergo relaxation (i.e. return to their original state), they emit electromagnetic signals whose frequency depends on the difference between the alpha and beta spin states. The NMR spectrometer detects these signals and displays them as a plot of signal frequency versus intensity – an NMR Spectrum.

**Note: resonance, here, refers to the flipping back and forth of nuclei between the alpha and beta spin states in response to the radiofrequency radiation. The radiofrequency value is varied until it matches the frequency required to flip the spins. At this point, the protons are “in resonance” with the radiofrequency radiation and they absorb and re-emit the energy which is detected by the radiofrequency receiver of the NMR spectrometer.

Now, if all the protons of a sample required the same energy difference for resonance, a 1H NMR spectrum would only have one peak and would be useless. However, the energy difference between the two states depends on the actual magnetic field experienced by each proton, which is affected by the tiny magnetic fields of electrons of atoms adjacent to that proton. Thus the energy difference of each proton depends on the electrons in the adjacent atoms – C atoms, electronegative atoms, multiple bonds and aromatic rings – in other words, on the specific molecular environment. As a result, the 1H NMR spectrum is unique to that compound.

The 1H NMR spectrum of a compound consists of a series of peaks that represent the resonance of each proton as a function of the changing magnetic field. The chemical shift of the protons in a given environment is where a peak appears; it represents the ratio of the frequency at which resonance occurs for that proton to the magnitude of the external magnetic field. The chemical shifts are shown relative to that of an added standard, tetramethylsilane, TMS, (CH3)4Si, which has 12 protons bonded to four carbon atoms that are bonded to one Si atom. TMS is chosen because:

- It is highly inert and does not interact with most organic compounds
- It is easily removed from the sample after the measurement
- It gives a strong and sharp signal at a very high field because the methyl protons are in a more electron-dense environment (well-shielded) compared to other organic compounds
- All the 12 protons are chemically and magnetically equivalent
- All carbon atoms are in the same chemical environment
- It has low boiling point (27oC) (highly volatile) and is soluble in most organic solvents and hence it can be easily removed or separated from other organic compounds or solvents after the spectrum is recorded

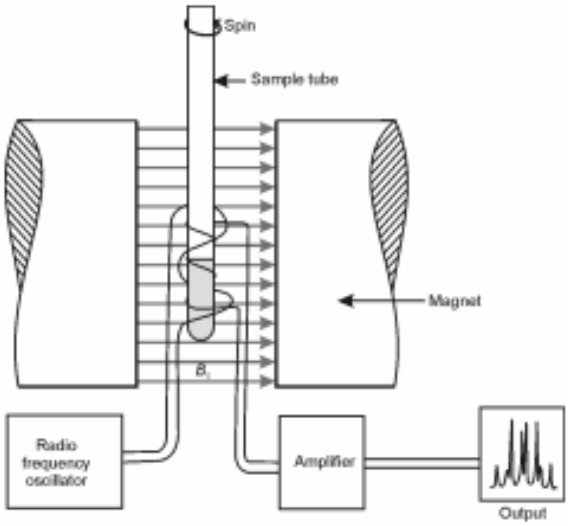
The first piece of information required for the deduction of an NMR spectrum is the number of resonance signals that can be expected. In the presence of a homogenous magnetic field, every 1H nucleus shows upon irradiation with an oscillating electromagnetic field of the correct frequency a resonance signal. The resonance signal of a 1H in a chemical compound is shifted compared to the signal of an isolated 1H, since the external magnetic field will be modulated by the secondary magnetic fields induced in the electrons in the neighbourhood of the proton. There 1H with identical electronic environments will show identical chemical shifts, i.e. signals at identical resonance frequencies. So the number of signals in an NMR spectrum is equal to the number of sterically (spatially) equivalent groups of protons.

Since a nucleus is embedded in a cloud of electrons, these electrons partly shield the nucleus from the applied magnetic field. Since the shielding varies for different protons within a molecule, all protons do not experience the same magnetic field. In a magnetic field, the moving electrons about the nuclei induce a local magnetic field that opposes the applied magnetic field. This means that the greater the electron density of the environment in which the proton is located the more the proton is shielded from the applied magnetic field. Thus protons in electron dense environments require a lower frequency to come into resonance – that is, flip their spin – because the energy difference between the alpha and beta spins states is smaller. Protons in electron poor environments require a higher frequency to come into resonance (i.e. a shielded nuclei will be more “upfield” (right-hand side) while a deshielded nuclei will be more “downfield” (left-hand side)). In summary, we see a signal in an NMR spectrum for each proton in a different environment. Protons in the same environment are called chemically equivalent protons.

To obtain a NMR spectrum, a small amount of the sample is dissolved in about 0.5mL of solvent (along with an inert reference compound) in a long, thin glass tube which is placed within a powerful magnetic field. Solvents with protons cannot be used since the signal for the solvent protons would be very intense because there is more solvent than compound in a solution. As a result, deuterated solvents like CDCl3 and D2O are commonly used. Spinning the sample tube along its long axis averages the positions of the molecules in the magnetic field and thus greatly increases the resolution of the spectrum.

**Note: a reference compound (usually TMS) is added to the sample and the resonance frequency of each proton in the sample is measured relative to the resonance frequency of the protons in the reference compound. This enables a frequency difference to be measured rather than an exact frequency. The positions of the signals in an NMR spectrum are defined according to how far they are from the reference compound. The position at which a signal occurs in an NMR spectrum is called the chemical shift. The chemical shift is a measure of how far the signal is from the reference TMS signal. The most common scale for this is the delta scale which has the units of parts per million (ppm) of the operating frequency. Factors that influence chemical shift include: electronegativity, shielding/deshielding and hydrogen bonds.

**Note: the data book contains a table of the approximate values of the chemical shifts for different kinds of protons that exist, the 'R' represents an alkyl group (like methyl, ethyl etc.) which may have other things substituted on it.



The size of the signals in an NMR spectrum is proportional to the number of protons that gives rise to the signal. The area under the curve can be determined by integration calculated electronically by a computer linked to the spectrometer. The integrals are printed out as number on the spectrum and they can also be displayed by a line of integration on the original spectrum. The height of each integration step is proportional to the area under that signal, which, in turn, is proportional to the number of protons giving rise to the signal. The integration tells us the relative number of protons that give rise to each signal, not the absolute number.

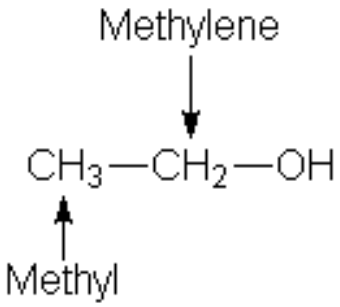
**Note: methyl group is –CH3 while methylene group is –CH2–

A low resolution spectrum looks much simpler to a high resolution spectrum because it can't distinguish between the individual peaks in the various groups of peaks. However, in a high resolution spectrum, the splitting of the peaks observed in high resolution NMR spectroscopy results in peaks that can be singlets, doublets, triplets, quartets, quintets, sextets, septets, octets and so on. Splitting is caused by protons bonded to adjacent (i.e. directly attached) carbons. The splitting of a signal is described by the n+1 rule where n is the number of equivalent protons bonded to adjacent

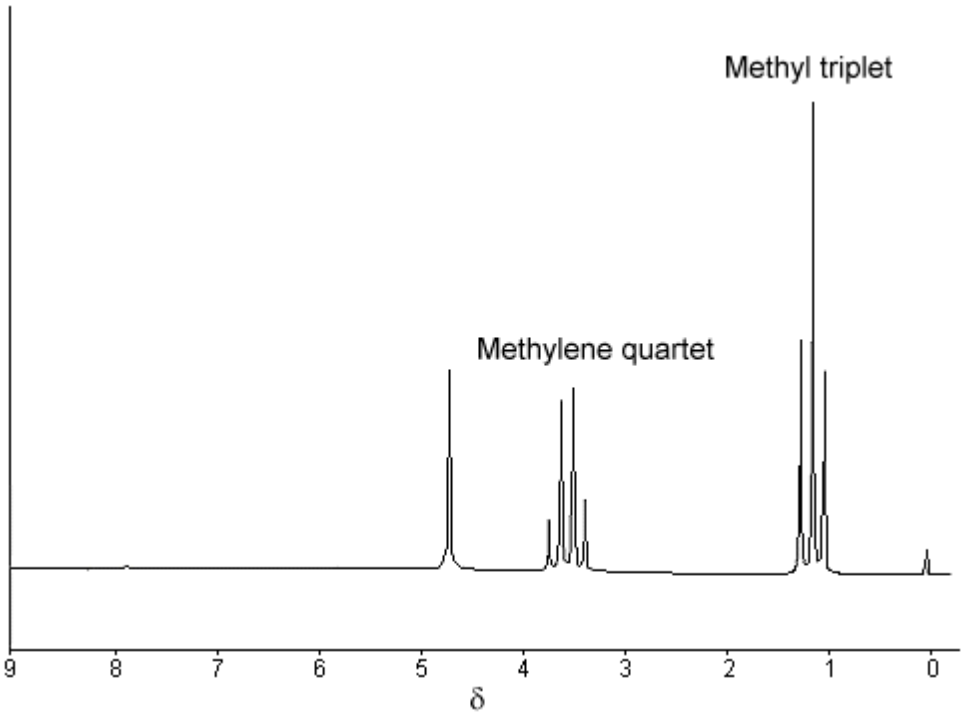
atoms. By equivalent protons, we mean that the protons bonded to an adjacent carbon are equivalent to each other, but not equivalent to the proton giving rise to the signal. The number of peaks in a signal is called the multiplicity of the signal. Splitting is always mutual: if the a protons split the b protons then the b protons must split the a protons. Coupled protons split each other's signal.

**Note: remember it is not the number of protons giving rise to a signal that determines the multiplicity of the signal, rather, it is the number of protons bonded to the immediately adjacent carbons that determines the multiplicity.

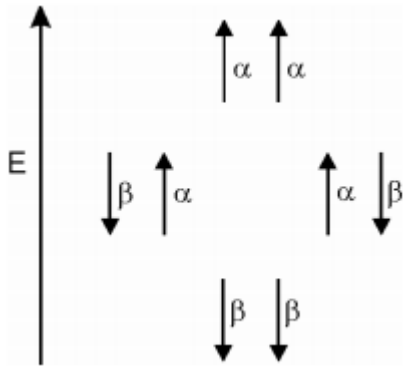
For example, consider the structure of ethanol:



The 1H NMR spectrum of ethanol below shows the methyl peak has been split into three peaks (a triplet) and the methylene peak has been split into four peaks (a quartet). This occurs because there is a small interaction (coupling) between the two groups of protons.

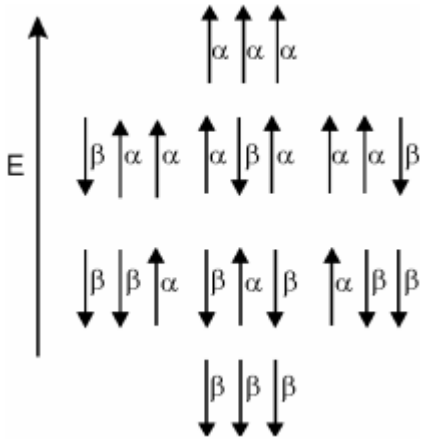


To see why the methyl peak is split into a triplet, let's look at the methylene protons. There are two of them, and each can have one of two possible orientations (aligned with or opposed against the applied field). This gives a total of four possible states:



In the first possible combination, spins are paired and opposed to the field. This has the effect of reducing the field experienced by the methyl protons; therefore a slightly higher field is needed to bring them to resonance, resulting in an upfield shift. Neither combination of spins opposed to each other has an effect on the methyl peak. The spins paired in the direction of the field produce a downfield shift. Hence, the methyl peak is split into three, with the ratio of areas 1:2:1.

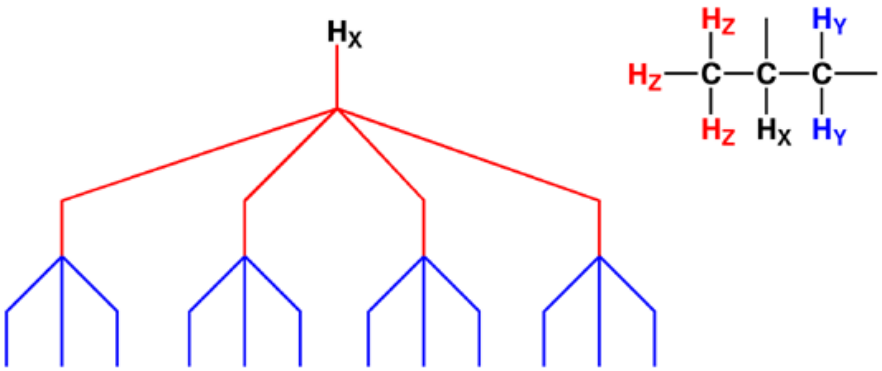
Similarly, the effect of the methyl protons on the methylene protons is such that there are eight possible spin combinations for the three methyl protons.



Out of these eight groups, there are two groups of three magnetically equivalent combinations. The methylene peak is split into a quartet. The areas of the peaks in the quartet have the ration 1:3:3:1. The separation of these peaks in frequency units is called coupling constant. It is denoted by J and is a measure of the strength of the coupling interaction. The coupling constant is independent of the strength of the applied magnetic field.

**Note: the relative intensities of the signals obey the mathematical mnemonic known as Pascal's triangle (e.g. a doublet is 1:1, a triplet is 1:2:1, a quartet is 1:3:3:1 and so on).

However, the splitting will multiply if a single hydrogen atom is adjacent to hydrogen atoms on either side that are in different chemical environments. Thus, if you have a hydrogen atom X between one –CH2- and one –CH3 group, it should be split into an amazing (2+1) x (3+1) = 12 signals. The reason why we do not see 12 peaks on a spectrum is because some of them overlap.



The signal of H_X is split into 12 peaks by the combination of H_Z and H_Y when the coupling constants are different (this almost never happens)

The principles behind 13C NMR and 1H NMR are essentially the same. The number of signals in a 13C NMR spectrum tells you how many different kinds of carbon environments a compound has just as the number of signals in 1H NMR. There are, however some differences that make 13C NMR easier to interpret.

The individual 13C signals are weak because the 13C isotope of carbon that gives rise to 13C NMR signals constitutes only 1.11% of carbon atoms (the most abundant isotope of carbon, carbon-12, has nonnuclear spin). The low abundance of carbon-13 means that the intensities of the signals in 13C NMR compared to those in proton NMR are reduced by a factor of approximately 100. In addition, the gyromagnetic ratio of 13C is about one-fourth that of 1H and the intensity of the signal is proportional to the cube of the gyromagnetic ratio. Therefore the overall intensity of a 13C signal is about 6400 (100 x 4 x 4 x 4) less than the intensity of a 1H signal. This means that the signals obtained from a single scan are too weak to be distinguished from background electronic noise. However, we employ Fourier transform techniques, as Fourier transform scans can be repeated rapidly so a large number of scans can be recorded and added. 13C signals stand out when hundreds of scans are added, because electronic noise is random, so its sum is close to zero.

One advantage of 13C NMR is that the chemical shifts range over 220 ppm compared with about 12 ppm for proton NMR. This means that signals are less likely to overlap. TMS is once again employed as the reference compound. A disadvantage of 13C NMR is that, unless special techniques are used, the area under a 13C NMR signal is not proportional to the number of atoms giving rise to the signal. Thus the number of carbon atoms giving rise to a 13C NMR signal cannot be determined by integration. Also, the signals are not normally split by neighbouring carbons because there is little likelihood of an adjacent carbon being a 13C (due to its low abundance). Thus all the signals are singlets in an ordinary 13C NMR spectrum.

**Note: carbons in electron-dense environments produce low frequency signals and carbons close to electron-withdrawing groups produce high frequency signals.