NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

NMR is defined as the study of the magnetic properties of nuclei under the influence of a strong magnetic field and an oscillating radio frequency field. The nuclei are charged particles moving on their own axis. This motion of nuclei makes them behave like dipoles. These dipoles are randomly oriented, but when placed under the influence of a strong magnetic field, they are split into two energy states, setting up the geometry required for the phenomenon of magnetic resonance.

CONCEPT OF SPIN

The necessary condition for nuclei to exhibit the magnetic resonance phenomenon is that it should have a nett free spin. The nuclei which have the necessary features to exhibit NMR lines can be classified as follows:

a) Nuclei with odd mass number but odd or even charge number have half integral spin and will exhibit magnetic resonance phenomenon e.g. ^{1}H, ^{17}O, ^{15}N.
b) Nuclei with an even mass number and even charge number have zero spin and do not exhibit magnetic resonance phenomenon, e.g. ^{12}C, ^{16}O, ^{32}S.
c) Nuclei with an even mass number but an odd charge have integral spin and do not exhibit magnetic resonance phenomenon, e.g. ^{2}H, ^{14}N.

The nuclei are charged particles, which are spinning. These spinning charges make the nuclei to behave as dipoles, and a magnetization is generated along the axis of spin of these dipoles. The angular momentum of this spin is the spin quantum number or spin number and is represented as **I**. The spin number I could have two values, +½ and -½. The number of orientations the dipole could have, when placed in a strong magnetic field depends on the spin number. The general formula for this is 2nI+1, where n is the number of nuclei, e.g. in case of protons the spin number is ½ and therefore it can have two orientations.

In the ground state or the state in which the sample has not been influenced by the strong external magnetic field, these two values of spin exist as degenerate energy levels. As soon as magnetic field is applied the degeneracy is removed and these spins form two distinct energy levels, the +½ spin being of lower energy (α state) is aligned in the direction of the applied magnetic field (with the field) and the -½ spin being of higher energy (β state) is aligned in the opposite direction (against the field). There is a slight excess of population in the lower energy level according to the Boltzmann's distribution law. Thus the nett magnetization in the lower energy level is slightly more in the lower energy level. This difference in the magnetization between the two energy levels is known as **Macroscopic Magnetization**. This macroscopic magnetization is responsible for giving the NMR signals.

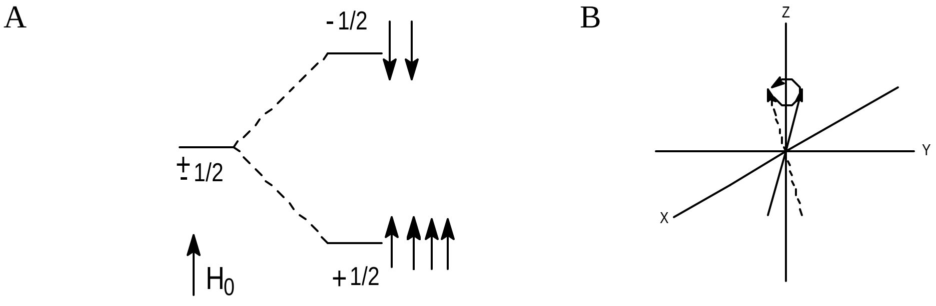


Fig.1 A Spins under the influence of magnetic field. B Precisional Motion

Apart from spinning on their own axis, the nuclei also have a precisional motion in which the axis of spin moves about the axis of the applied magnetic field. The frequency with which nuclei precess is known as the Precisional Frequency or Larmor Frequency. It is this precisional frequency of nuclei, which is in the range of radio frequency.

CONTINUOUS WAVE TECHNIQUE

The conventional method of acquiring the NMR spectra is the continuous wave technique. In this method, the total range of resonance of protons is scanned evenly starting from the low field to the high field region, i.e., from the left of the spectra to the right. According to the basic NMR equation, it is possible to keep either the radio frequency or the magnetic field constant and vary the other. Practically, it is difficult to sweep the radio frequency or the strong magnetic field at an even rate. In most spectrometers the radio frequency is kept constant while the magnetic field is swept with the help of sweep coils which act as electro-magnets and generate a small amount of magnetic field. The magnetic field of the sweep coils is in addition to the magnetic field of the strong electro or superconducting magnets. This is just like tuning the radio and moving the needle in an even manner from left to right.

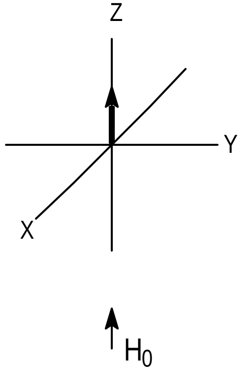
At particular positions there is resonance between the precisional frequency and the radio frequency. At the resonance point the macroscopic magnetization, which is aligned along the Z axis tilts towards the XY plane and in doing so generates an EMF in the receiver coil in accordance with the Faradays Laws, which states that a moving charge generates an EMF in a coil around it. The protons being charged particles also generate the EMF in form of NMR signals. These signals are then plotted to get the NMR spectra.

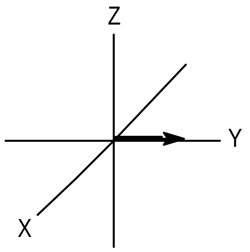
The CW technique has got certain disadvantages such as

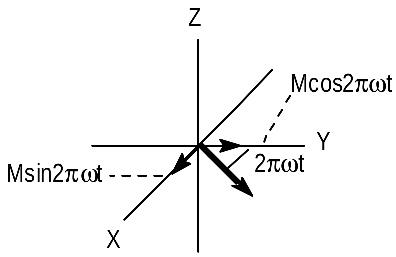
1. **It is time consuming**: The optimum rate of acquiring a spectrum by the CW technique is 1Hz/sec. At this rate, a spectrum having spectral width of 1000 Hz on a 100MHz spectrometer will require 1000seconds or nearly 15 min for a single experiment.
2. **Large quantity of sample required**: The normal CW experiment requires about 40 to 50 mg of the sample. Compounds isolated from natural products are obtained in very small quantities and will be difficult to study.
3. **Nuclei having low natural abundance cannot be studied**: Nuclei such as ^{13}C, which has a very low natural abundance (only 1.08% of the total population of ^{12}C) cannot be studied by this method. This is so because these nuclei will give very weak signals that cannot be distinguished from the noise generated by the spectrometer.
4. **High resolution NMR not possible**: As the magnetic field strength is increased, it becomes difficult to sweep the entire range of resonance thereby making high resolution impossible.

FOURIER TRANSFORMATION

To overcome the problems of the CW technique, the Fourier Transformation technique was proposed. This technique involved application of the entire range of radio frequency simultaneously in the form of a pulse. This results in the irradiation of all the spin systems at the same time. This can be better explained if we consider a three coordinate system.







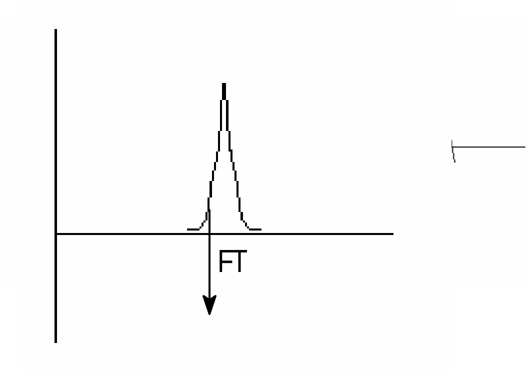
If **M** is the macroscopic magnetization aligned along the Z axis or the longitudinal plane, and the receiver coil being aligned along the XY plane or the transverse plane, the initial magnetization in the transverse plane is zero. As the pulse is applied, the macroscopic magnetization tilts towards the transverse plane. Here the spins evolve under the influence of chemical shifts and coupling constants. The magnetization M is split into two vectors, the one aligned along the Y axis is **Mcos2ðùt** and that aligned along the X axis is **Msin2ðùt**. Initially when t=0, the magnetization vector along the Y axis has a minimum value while that along the X axis has a maximum value. As the value of t increases, the X magnetization decreases while the Y magnetization increases. The motion of the magnetization vector is spiral as it moves to regain its ground state. In this process, the value on M in the transverse plane decreases with time. Thus if a graph is plotted between the intensity and time for both, the cos as well as the sin components, the spectra will be in form of a free induction decay (FID) which is the time domain spectra and on being Fourier transformed will give the frequency domain spectra. This frequency domain spectra is a plot of intensity against time. This spectra is converted into the frequency domain spectra with the help of Fourier Transformation which is done by the help of the computer attached to the spectrometer. The expression for Fourier transformation is

$$\mathbf{f(u)= \int f(t)e^{-i\grave{u}t}\,dt}$$

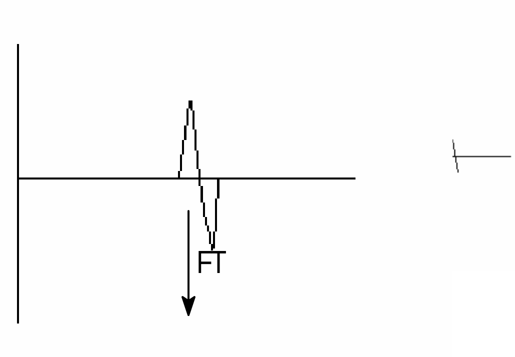
Fourier transformation of the Sin component will give an absorptive spectra (A) while that of the Cos component will dive a dispersive spectra (B).

A.

B.



A. Sin component (absorptive)



B. Cos component (dispersive)

CHEMICAL SHIFT AND RESONANCE FREQUENCY

All protons have the same gyromagnetic ratio and therefore are expected to exhibit the same type of magnetic behavior. Had this been the case, all the protons would give only one signal and the purpose of NMR would have been defeated. The thing that makes the protons behave differently is the electronic environment around it. The electrons are charged particles, which are moving in their orbits. This circular motion of electrons generates its own magnetic field, which is always opposing the applied magnetic field. The proton therefore experiences the magnetic field of the electrons in addition to the externally applied magnetic field. Thus if the electron density around a proton is high, the magnetic field generated by the electrons will be quite strong and will oppose the external magnetic field more. In fact it will shield the proton from the external magnetic field and the signal will appear upfield that is at a higher frequency. Thus the electronic environment makes the proton signals resonate within a range of 1000 Hz at a magnetic field of 2.3 tesla (100 MHz).

Chemical shift is defined as the difference in the resonance position of a signal with respect to a reference signal**. **The resonance frequency is defined as the frequency difference between the reference signal and a proton signal.

The chemical shift is determined by dividing the total range of proton resonance which is 1000 Hz on a 100 MHz spectrometer, into 10 equal parts. Then each part is numbered starting from zero at the extreme right of the spectra. Each part therefore comprises of a frequency of 100 Hz on a 100 MHz spectrometer.

The relationship between the resonance frequency and chemical shift is given by the following expression:

$$\delta = \frac{\text{Resonance Frequency (in Hz)}}{\text{Spectrometer Frequency (in Hz)}} \quad \text{X } 10^{6}$$

The electronegativity and inductive effect of groups attached to the vicinity of the proton effects the chemical shift. An electron-attracting group decreases the electron density around the proton thereby reducing the intensity of the electronic magnetic field. Such a proton experiences an enhanced amount of the external magnetic field. Such groups or atoms, which decrease the electron density around the proton, are said to be deshielding the proton from the applied magnetic field. Such groups make the proton signal appear at a lower frequency or in the downfield direction (high δ). Similarly an electron-repelling group enhances the electronic magnetic field thereby shielding the proton causing the proton to resonate at a higher frequency and the signal to move in the upfield direction (low δ). To remember this, it is best to keep in mind that Deshielding starts with **D** and it moves the signal in the downfield Direction which also starts with **D**.

SPIN-SPIN COUPLING AND COUPLING CONSTANT

The spin of a proton, which acquires two energy states when exposed to a strong magnetic field, is also influenced by the spin of neighboring magnetically active protons. Let us consider two protons H_a and H_b, which are present on adjacent carbon atoms in a molecule. The spin of H_a is affected by the spin of H_b. the interaction of the spins of H_a and H_b is through the intervening bonding electrons. The spin of H_b will have two orientations in the applied magnetic field. The two spin states of H_b effect the signal by partially shielding and deshielding the spin of H_a. The lower energy levels of the spin of H_b which is aligned in the direction of the applied magnetic field will cause deshielding of the signal of H_a and will result in a slight downfield shift while the higher energy level of the spin of H_b which is aligned against the direction of the applied magnetic field will cause shielding of the signal of H_a and will result in a slight upfield shift of the signal. This shifting of the signal of H_a in the upfield and downfield direction makes the signal to appear as a doublet. The signal of H_b will be affected in the same way by the spin states of H_a. This interaction of spins through bonds resulting in the splitting of the signals is called **Spin-Spin Coupling.** This is also called Scalar Coupling as the interaction takes place through the intervening bonding electrons and is limited to three bond distances only.

The general formula for splitting is given as 2nI+1 where n is the number of chemically and magnetically similar nuclei interacting with the proton under consideration, I is the spin number of the interacting nuclei. If there are more than one type of nuclei interacting, the formula becomes (2nI+1)(2mI+1)(2oI+1)… where n, m, o, etc are the number of chemically and magnetically similar nuclei in one set. Since we are studying the interactions between protons, which have I as ½, the formula could be written as (n+1)(m+1)….

E.g. in case of a molecule like CH_3-CH_2-CH_3 the methylene protons interact with six methyl protons. Since the methyl groups are identical, they belong to one set and therefore will split the methylene protons onto a septet. The signal for the methyl group will be split into a triplet due to interaction with the two methylene protons. If we replace one of the methyl protons with chlorine atom, then the two methyl groups no longer remain identical and this results in the methylene protons interacting with two sets of protons, one set containing three protons and the second set containing two protons. The signal of methylene will now be split into twelve lines [(3+1)(2+1)].

The spacing between the component peaks of a signal depends upon its interaction with the neighbouring nuclei. This spacing is called the **Coupling Constant**

HIGH RESOLUTION NMR

High resolution NMR takes advantage of the fact that the chemical shift (δ) and coupling constant (J) are independent of the applied magnetic field, but the resonance frequency of signals is not. i.e. change in the magnetic field strength will have no effect on the first two factors while the resonance frequency will vary. Chemical shift is determined on the basis of a scale which is obtained by dividing the range of proton resonance into ten equal parts taking the signal of the reference signal (normally TMS) to be at zero. Thus this scale starts from zero at the extreme right of the spectra and increasing as we move towards the left. The difference between the position of the reference signal and the proton signal in terms of this scale is its chemical shift. The frequency difference between the resonance position of the reference signal and that of the proton signal is known as the resonance frequency of the proton signal. The resonance frequency varies with change in the magnetic field strength. It is this variation of the resonance frequency, which forms the basis of high resolution NMR.

e.g. the total range of proton resonance, which is normally 1000Hz on a 100 MHz spectrometer, is divided into ten equal parts. Thus each part comprises of a frequency of 100 Hz. A signal having chemical shift (δ) value as 1 has a frequency difference of 100 Hz from the signal of TMS, which is taken to be, zero. The resonance frequency of this signal is 100 Hz. Now if the spectrum is run on a 400 MHz spectrometer, the total range of proton resonance will be 4000 Hz, since the spectrometer frequency is four times of what it was earlier. On dividing 4000 Hz into ten equal parts, each division will now comprise of 400 Hz. The chemical shift of this signal is still 1 but its resonance frequency has changed from 100 Hz to 400 Hz. Thus the frequency difference between all the signals increases as we increase the magnetic field strength.

The concept of high resolution can be better explained by taking the example of a bookshelf of 10 cm width, containing 10 books of 1 cm each. If we divide the bookshelf into 10 parts, each part will be of 1 cm and the books will fit in perfectly. The books can be given numbers, which can be compared to chemical shifts in NMR. The thickness of the books can be compared to the coupling constant. The first book is positioned at zero, the second at one and so on.. Now if the size of the bookshelf is increased to 40 cm and the shelf is divided into 10 equal parts, each part will now be of 4 cm. The same books are arranged in such a manner that the first book is kept at zero, the second at one and so on, and all the books are arranged so that their position and thickness remain unchanged, the distance between each book is now 3 cm more than what it was earlier. The distances between the books have increased and it appears that the books have moved apart. This increase can be compared to the increase in the resonance frequencies between the signals when a spectrometer of higher magnetic field strength is used.

The relationship between the spectrometer frequency and chemical shift is given by the following expression:

$$\delta = \frac{\text{Observed Frequency (in Hz)}}{\text{Spectrometer Frequency (in Hz)}} \times 10^6$$

cm book will be starting from a fresh mark The marks of the books and thickness are not changing