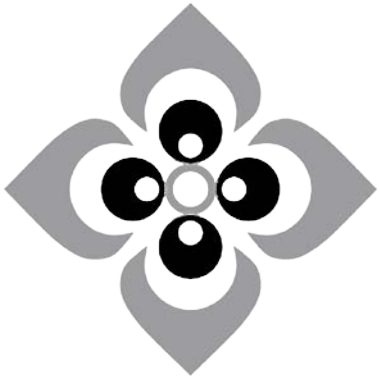

Consortium for Educational Communication

Module on

Nuclear magnetic resonance spectroscopy (NMR)

By

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TEXT

NMR introduction:

Nuclear magnetic resonance spectroscopy, also known as NMR spectroscopy, is an analytical technique that is used to determine the physical and chemical properties of atoms and molecules by analyzing the behaviour and properties of their nuclei in externally applied magnetic field. It is a powerful non-selective and non-destructive analytical tool that enables you to ascertain molecular structure including relative configuration, relative and absolute concentrations, and even intermolecular interactions of an analyte. The entire phenomenon employs nuclear magnetic resonance and can be used not only to provide detailed information about the structure but also the dynamics, reaction state and chemical environment of molecules. The technique was first of all observed in 1946 by research groups at Stanford and M.I.T., in the USA, although the history of nuclear spin and magnetic moments was known since 1925. The entire technique is based on the fact that within a molecule, the nuclei of atoms have magnetic properties and, the magnetic field around each atom is known to change the resonance frequency that can be utilized to yield chemical and electronic structure of a molecule. However, the technique is applicable to any kind of sample that contains nuclei possessing a spin.

NMR spectroscopy is a widely used technique to investigate the properties of organic molecules ranging from small compounds analyzed with 1-dimensional proton or carbon-13 NMR spectroscopy, to large proteins and nucleic acids using 3- and 4-dimensional techniques. Several advantages of NMR spectroscopy include a wide range of information that can be obtained and its good resolution. The technique is suitable for both solids and solutions. In addition to this, NMR spectra for small molecules are unique, well-resolved, analytically traceable and often highly predictable. The technique has been employed for different applications like confirming

the identity of a chemical substance, making distinguish between the different functional groups and also the identical functional groups which are surrounded by different neighbouring constituents and give different signals. In this way, NMR has largely replaced traditional wet chemistry tests such as colour reagents identification. The technique has also some disadvantages like a relatively large amount of purified sample about 2–50 mg is required to start the analysis, although it may be recovered later on. The sample that is dissolved in a solvent is preferred. It has a relatively long timescale and, thus, it is not suitable for observing fast phenomena. In addition of being expensive, NMR is inherently not very sensitive for detecting impurities.

Principle of NMR:

The basic principles are usually covered in the physical chemistry and the concepts of electricity, magnetism, and classical mechanics which are further extended with quantum mechanical treatments. The simplest nucleus is that of hydrogen atom, which consists of one particle only i.e., the proton. The protonic mass (1.67×10^{-27} kg) and charge ($+1.60\ 10^{-19}$ C) are taken as the elementary units of mass and electric charge, respectively. Another particle which is a constituent of all nuclei (except hydrogen nucleus) is the neutron. It has unit mass which is equal to that of a proton and no charge, however, a spin of 1/2. Thus, if a particular nucleus is composed of 'p' number of protons and 'n' number of neutrons, its total mass is p + n, its total charge is +p and its total spin will be a vector combination of p + n spins, each of magnitude 1/2. The atomic mass is usually specified for each nucleus by writing it as a prefix to the nuclear symbol, e.g. ^{12}C indicates the nucleus of carbon having an atomic mass of 12. This nucleus contains six protons and six neutrons to make up a mass of 12. The nucleus of ^{13}C (an isotope of carbon), on the other hand has six protons and seven neutrons. Each nuclear isotope, being composed of a different number of protons and neutrons, will have its own total spin value which cannot be predicted (McLauchan, 1972). For example, deuterium (^{2}H), an isotope of hydrogen containing one proton and one neutron might have a spin of 1 or zero depending on whether the proton and neutron spins are parallel or

opposed, respectively. Quantum mechanically subatomic particles (protons, neutrons and electrons) have a spin. In some atoms (eg. ^{12}C, ^{16}O, ^{32}S), these spins are paired and cancel each other out so that the nucleus of the atom has no overall spin. However, in many atoms (^{1}H, ^{13}C, ^{31}P, ^{15}N, ^{19}F etc), the nucleus does possess an overall spin. To determine the spin of a given nucleus one can use the following rules:

- If the number of neutrons and the number of protons are both even, the nucleus has no spin i.e., nuclei with both p and n 'even' have zero spin e.g. ^{4}He, ^{12}C, ^{16}O
- If the number of neutrons plus the number of protons is odd, then the nucleus has a half-integer spin like 1/2, 3/2, 5/2, and so on, or in other words nuclei with odd mass number have half-integral spins e.g. ^{1}H, ^{15}N etc.
- If the number of neutrons and the number of protons are both odd, then the nucleus has an integer spin like 1, 2, 3, and so on. In other words, nuclei with both p and n 'odd' and mass even, have integral spin e.g. ^{2}H, ^{14}N etc.

Therefore, it can be understood that all the nuclei are not magnetically active and thus not detectable by NMR. In quantum mechanical terms, the nuclear magnetic moment of a nucleus will align with an externally applied magnetic field of strength, say 'B_o' either with or against the applied field B_o. For a single nucleus with nuclear spin = 1/2, only one transition is possible between the two energy levels. The energetically preferred orientation has the magnetic moment aligned parallel with the applied field (spin= +1/2) and is often given the notation α, whereas the other having anti-parallel orientation (spin = -1/2) with respect to the external magnetic field is higher energy referred to as β. The rotational axis of the spinning nucleus cannot be, however, orientated exactly parallel (or anti-parallel) with the direction of the applied field magnetic field (defined in our coordinate system as about the *z* axis) but must precess (motion similar to a gyroscope) about this field at an angle, with a certain angular velocity as shown in the fig. 1 given:

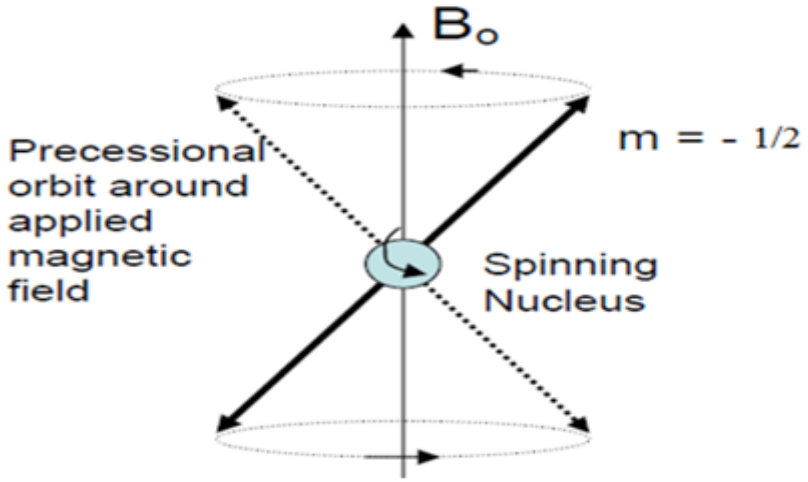


Fig. 1: Nucleus Precessing

The orientations that the magnetic moment of a nucleus can take against an external magnetic field are not of equal energy. Spin states which are oriented parallel to the external magnetic field are lower in energy than that, these would exhibit in its absence. In contrast, spin states whose orientations oppose the external magnetic field are higher in energy than that these would exhibit in its absence, as shown in fig. 2:

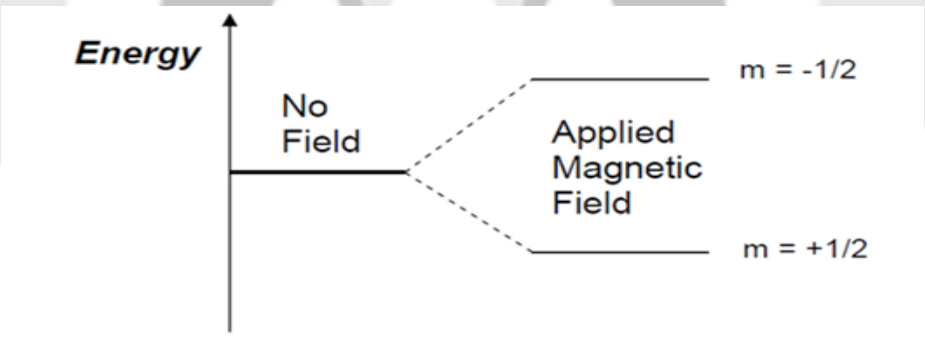


Fig. 2. Energy levels of nucleus with spin quantum number greater than zero.

If 'I' is taken as a symbol for magnetic spin, B_o for externally applied magnetic field, for I =1/2, the diagrammatic representation of energy levels can be given as:

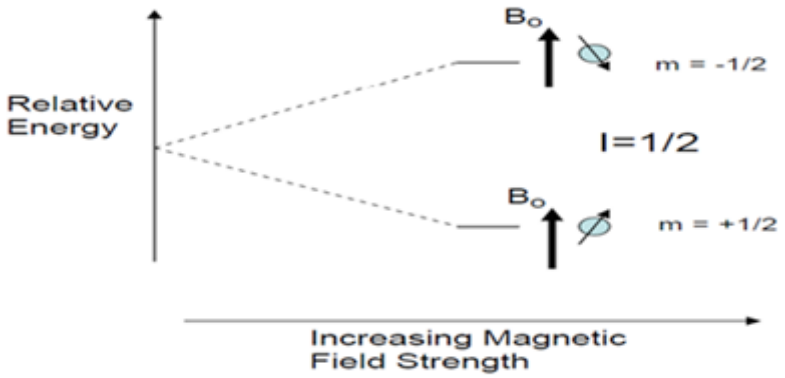


Fig. 3: Energy levels of nuclei

An energy separation that exists between the two states creates a possibility to induce a transition between the various spin states. By irradiating the nucleus with electromagnetic radiation of the correct energy which is determined by its frequency, a nucleus with a low energy orientation can be induced to undergo a "transition" to an orientation with a higher energy. The phenomenon is also called as 'spin flip' of nucleus from lower energy orientation to higher energy orientation. The absorption of energy during this transition forms the basis of the NMR method. The difference in energy (ΔE) between the two spin states increases as the strength of the external magnetic field increases, but this difference is usually very small, leading to the requirement for strong NMR magnets (usually 1-20 T for modern NMR instruments).

Population Distribution:

In a given sample of a specific NMR-active nucleus, the nuclei will be distributed throughout the various spin states available. As the energy separation between these states is comparatively small, energy from thermal collisions is sufficient to place many nuclei into higher energy spin states. The number of nuclei in each spin state is described by the Boltzmann distribution equation, given below:

$$N_{upper} / N_{lower} = e^{-\gamma Bo/kT}$$

Where the 'N' values are the numbers of nuclei in the respective spin states, 'γ' is the magnetogyric ratio that relates the magnetic moment and the spin number for a specific nucleus (i.e., γ = precession rate/strength

of the externally applied magnetic field), **h** is Planck's constant, $\mathbf{B_o}$ is the external magnetic field strength, **k** is the Boltzmann constant, and **T** is the temperature. As demonstrated from the mathematical calculations, the population ratio of the two energy states of nuclei at room temperature is approximately 0.999, which implies that the upper and lower energy spin states are almost equally populated with only a very small excess in the lower energy state that represents spins aligned with the applied field. The important consequence of this nearly equal population distribution is relatively weak signals that NMR yields.

Chemical shift:

Even if all protons have the same magnetic moments, they do not give resonant signals at the same frequency values. This is due to the differing electronic environment of the nucleus of interest. Electrons are negatively charged particles that surround and move around the nuclei within a molecule generating a magnetic field. Upon application of an external magnetic field, these electrons move in response to this magnetic field and generate their own local magnetic fields and lines of force (magnetic moment) that run in the opposite direction as the lines of force generated by the external magnetic field to oppose it. In other words, the electron's magnetic field runs anti-parallel to the external magnetic field. This has the effect of reducing the net magnetic moment affecting the proton. These local fields are thus known to "shield" the proton from the applied magnetic field, which must therefore be increased in order to achieve resonance (absorption of energy). This causes the NMR signal generation to occur at a higher external magnetic field setting. The NMR signal is shifted upfield, and the protons are said to be electronically shielded. The word shielded is used because the electronic magnetic moment actively shields the proton from the external magnetic field such that the effect of the external field is not as great as it could have been, if the proton was removed from an electronic environment.

Chemical Shift and the TMS Standard:

We have now determined that chemically different protons have different

electronic environments. Differences in the electronic environments cause the protons to experience slightly different applied magnetic fields owing to the shielding/deshielding effect of the induced electronic magnetic fields. In order to standardize the NMR scale, it is necessary to set a zero reference point to which all protons can then be compared. The standard reference chosen for this purpose is tetramethylsilane (TMS). This compound has four CH_3 methyl groups single bonded to a silicon atom. All of the protons on the methyl groups are in the same electronic environment. Therefore, only one NMR signal will be generated. Furthermore, the electronegativity of the carbon atoms is actually higher than the silicon atom to which they are bonded. It results in the sigma electrons being shifted toward the carbon atoms in the methyl groups and consequently, the protons will be heavily shielded causing the one signal to be generated at a very high magnetic field strength setting. It is that signal that all other NMR signals of a sample are referenced to. This association with the reference signal is called the chemical shift. This shift is measured in parts per million (ppm). NMR signals occurring near the TMS resonance are said to be in an upfield position while those shifted away by deshielding are said to be downfield (as shown in figure below). Virtually all NMR signals will be further downfield from the TMS signal because of the heavily shielded nature of the methyl protons in the TMS molecule, as shown in below:

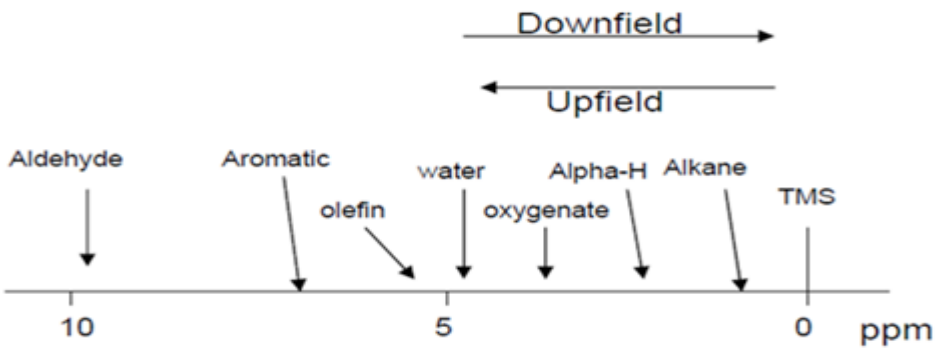


Fig. 4: Chemical shift.

Spin-spin coupling:

Beyond the interaction with the magnetic field generated by the moving electrons, the spinning nuclei also interact with other nuclear spins in their neighbourhood leading to the so called 'fine structure' or J-J splitting.

It results in mutual splitting of the NMR signal from each nucleus into multiplets depending upon number of other nuclei interacting with the nucleus and their spin quantum numbers. One of the important properties of the multiplet formed is that the difference between any two of its adjacent components is the same and yields a constant value known as spin-spin coupling constant or 'J' constant (measured in hertz). It is independent of magnetic field strength. Therefore, an increase in the magnetic field strength will increase the chemical shift difference between two peaks in hertz, but the coupling constant 'J' will not change. The influence of the spin of a neighbouring proton on the spin of absorbing proton is called spin-spin coupling. This can be shown with the help of an example of NMR spectra of ethanol:

^{1}H NMR spectrum (1-dimensional) of ethanol is plotted below as signal intensity vs. chemical shift. There are three different types of H- atoms in ethanol regarding NMR. The hydrogen (-H) on the –OH is not coupling with the other H atoms and appears as a singlet, but the $–CH_3$ and $–CH_2$-hydrogens are coupling with each other, resulting in a triplet and quartet respectively, as shown in the figure:

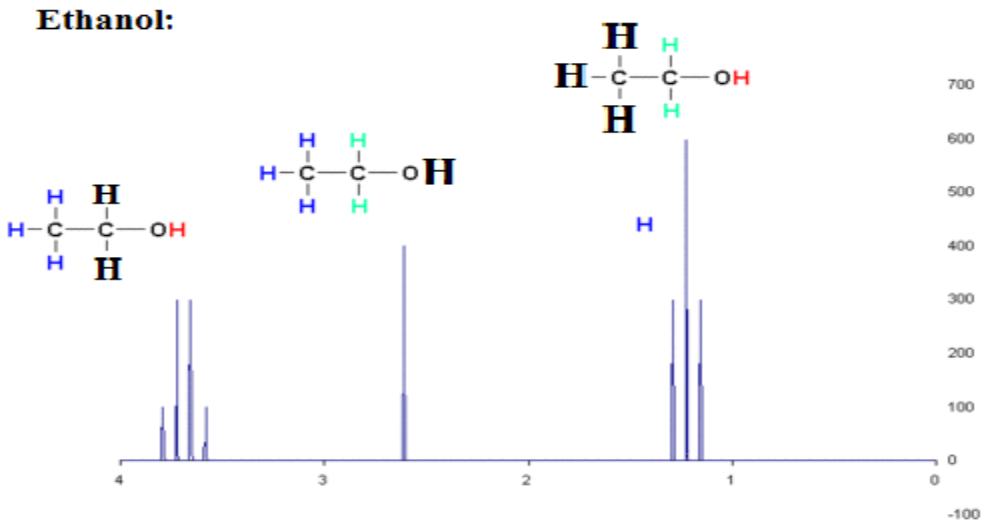


Fig. 5: NMR spectra of ethanol.

Relaxation:

The NMR process is an absorption process. Nuclei in the excited state must also be able to "relax" and return to the ground state. The timescale for this relaxation is crucial to the NMR experiment. For example, relaxation of electrons to the ground state in uv-visible spectroscopy is a very fast process and approximately in the order of picoseconds, however, in NMR, the excited state of the nucleus can persist for minutes. This is because the transition energy between spin levels is very small and equilibrium is attained for a much longer timescale. The timescale employed for this relaxation process dictates the execution and success of the NMR experiment. There are two processes through which this relaxation is achieved in NMR, i.e., longitudinal (spin-lattice) relaxation and transverse (spin-spin) relaxation. In longitudinal relaxation, energy is transferred to the molecular framework i.e., the lattice and is lost as vibrational or translational energy. The half-life for this process is called the spin-lattice relaxation time (T_1). Dissipating the energy of NMR transitions into the sample, which are tiny compared to the thermal energy of the sample, is not difficult, however, T_1 values are often long. In transverse relaxation, energy is transferred to the neighbouring nuclei. The half-life for this process is called the spin-spin relaxation time (T_2). This process exchanges the spin of one nucleus with the spin of another nucleus in its neighbourhood, leading to no net change in spin of the sample. The peak widths in an NMR spectrum are inversely

proportional to the lifetime.

Instrumentation:

In general, NMR instruments can be of two types i.e., continuous wave and Fourier transform.

- **Continuous wave NMR spectrometers:**

In such instrumental systems, the sample is held in a strong magnetic field, and the frequency of the source is slowly scanned in a way similar in principle to optical-scan spectrometers. However, in some instruments, the source frequency is held constant while the magnetic field is scanned. These systems are currently obsolete except for a few wideline experiments that are performed in specialty solid-state NMR applications.

- **Fourier Transform (FT) NMR instruments**

Fourier Transform (FT) NMR is an advanced instrument which is based on the principle of the integration of spectra in order to overcome the disadvantage of low sensitivity of NMR. As the magnitude of the energy transition between the lower and higher energy state involved in NMR spectroscopy is very small, it is a probable cause of the limited sensitivity in case very low concentrations of the sample is taken into consideration. One way to increase sensitivity would be to record many spectra, and then add them together. In FT-NMR, all frequencies in a spectral width are irradiated simultaneously with a radio frequency pulse. A single oscillator (transmitter) is used to generate a pulse of electromagnetic radiation of frequency ω_o but the pulse is truncated after only a limited number of cycles (corresponding to a pulse duration 'τ' or tau). This pulse has simultaneous rectangular and sinusoidal characteristics. In response to the pulse, the magnetic moments of nuclei find themselves in a non-equilibrium condition having precessed away from their alignment with the applied magnetic field. They begin the process of relaxation, by which they return to their thermal equilibrium. A time domain emission signal, called free induction decay (FID) is recorded by the instrument as the nuclei magnetic moments relax back to equilibrium with the applied magnetic field. A frequency

domain spectrum that we are familiar with is then obtained by Fourier transformation of the FID.

Early experiments were conducted with continuous wave (CW) instruments, and in 1970 the first Fourier transform (FT) instruments became available. This type now dominates the market, and currently we know of no commercial CW instruments being manufactured at the present time. A general construction of the equipment is shown as below:

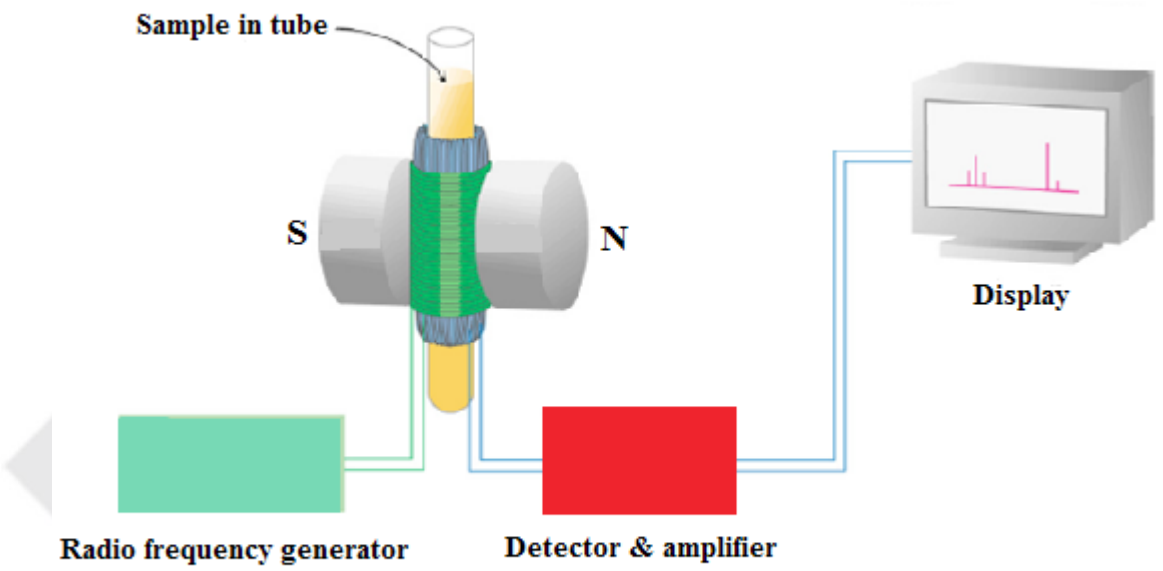


Fig. 6: Construction of NMR equipment.

A Simple NMR machine consists of:

- A powerful, super-cooled magnet (stable, with sensitive control, producing a precise magnetic field).
- A radio-frequency transmitter (emitting a very precise frequency).
- A detector to measure the absorption of radiofrequency by the sample.
- A recorder (to plot the output).

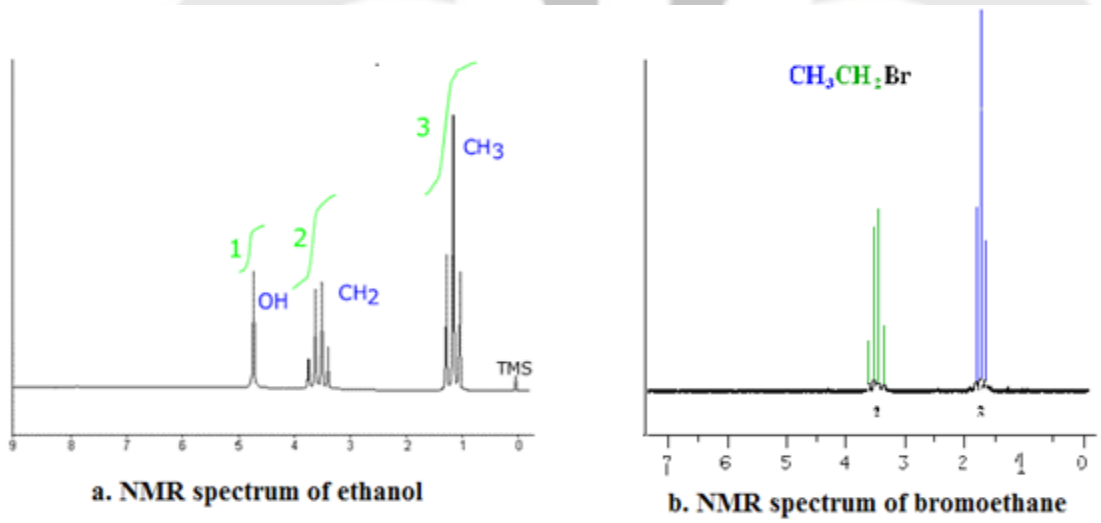
Interpretation of NMR signal:

Four rules need to be understood for interpretation of NMR spectra:

1. Number of signals:

Protons within a compound experience different magnetic environments, which give a separate signal in the NMR spectrum. Protons that reside in the same magnetic environment are termed as chemically equivalent protons. As a general rule of thumb, H's in CH_3 and CH_2 groups are usually equivalent. Symmetrical compounds, such as benzene, are also equivalent; however, since many compounds are not symmetrical, it is important to know how to identify non-equivalent protons. Protons that are different in any way even in their stereochemistry are not equivalent and will absorb at different frequencies producing a separate signal on the NMR spectra.

The compounds given below are known to produce different signals in their NMR spectrum:



a. NMR spectrum of ethanol

b. NMR spectrum of bromoethane

Fig. 7: NMR spectra of different compounds

2. Position of signals (chemical shift):

The position on the horizontal frequency scale at which the equivalent proton signals appears is called a chemical shift. Protons generally sense 3 different magnetic fields: magnetic field of the Earth, the magnetic field that is externally applied and the field produced by the different protons in the molecule. Since the magnetic fields of the Earth and external magnetic field are felt similarly by all the protons in the molecule, the chemical shift

depends only on the varying local magnetic fields from the neighbouring protons. In order to standardize the NMR spectra, the chemical shifts are positioned in relation to a reference proton set at 0.00 ppm, produced by Tetramethylsilane i.e., $(CH_3)_4Si$ which is the standard for H_1NMR. TMS is taken as a reference compound because of its inert quality that prevents it from reacting with the sample and its highly volatile nature that makes it easy to evaporate out of samples. Few compounds have a lower frequency reading than TMS and it has 12 equivalent protons that read strongly on the NMR spectra.

Under an applied magnetic field, circulating electrons in the electron cloud produce a small opposing magnetic field, ultimately decreasing the effective of magnetic field felt by the proton, shifting the signal to the right (or *upfield*). This effect, in which the electron cloud "shields" the proton from the applied magnetic field, is called local diamagnetic shielding. However, electronegative or electron seeking atoms when present in the compound tend to remove electrons from the electron cloud, which decreases their density and results in less shielding; hence, electronegative atoms are said to deshield the proton and cause it to have a higher chemical shift, moving it to the left (or *downfield*).

3. Relative Intensity of Signals (Integration):

The area under the signals corresponds to the number of protons responsible for that signal. Therefore, the relative intensities of the signal are proportional to the relative number of proton equivalents. It is important to remember that integration only provides ratios of protons, not the absolute number. For convenience in calculating the relative signal strengths, the smallest integration is set to 1 and the other values are converted accordingly.

4. Splitting of signals (spin-spin coupling):

NMR signals are not usually single triangles, but a complex pattern of split triangles labelled as doublets for two peaks, triplets for three peaks, quartets for four peaks, etc. The distance between the splitted peaks are

called coupling constants, denoted by J^{13}. When the spin of the nucleus of one proton is close enough to affect the spin of another, called spin-spin coupling, the splitting of signals is seen. Splitting is always reciprocated between the protons for example if H_a splits H_b, then H_b must also split H_a and in this way an information is seeked about the neighbours of a proton within the molecule.

Applications in food technology:

- NMR microscopy can provide valuable information about the fruit ripening, the best conditions for food handling and even the best cooking temperature conditions.
- NMR spectroscopy can be used in the verification of the wine aging and authenticity as well as in the identification of the fatty constituents of oils (Belton, 1995).
- It can also contribute in the investigation of the mechanisms that are responsible for food decomposition without the need of sample destruction which is common with the classical chemical analysis techniques.
- High resolution NMR techniques have found interesting applications in the analysis of complex mixtures of various extracts of natural products (Gerothanassis, *et al.,* 1998; Exarchou *et al.*, 2001) for example rosmarinic acid (figure given below) that has been reported as a potent active substance against human immunodeficiency virus type one (HIV-1) (Mazumder *et al.,* 1997).
- It has been used for determination of water activity of pork.

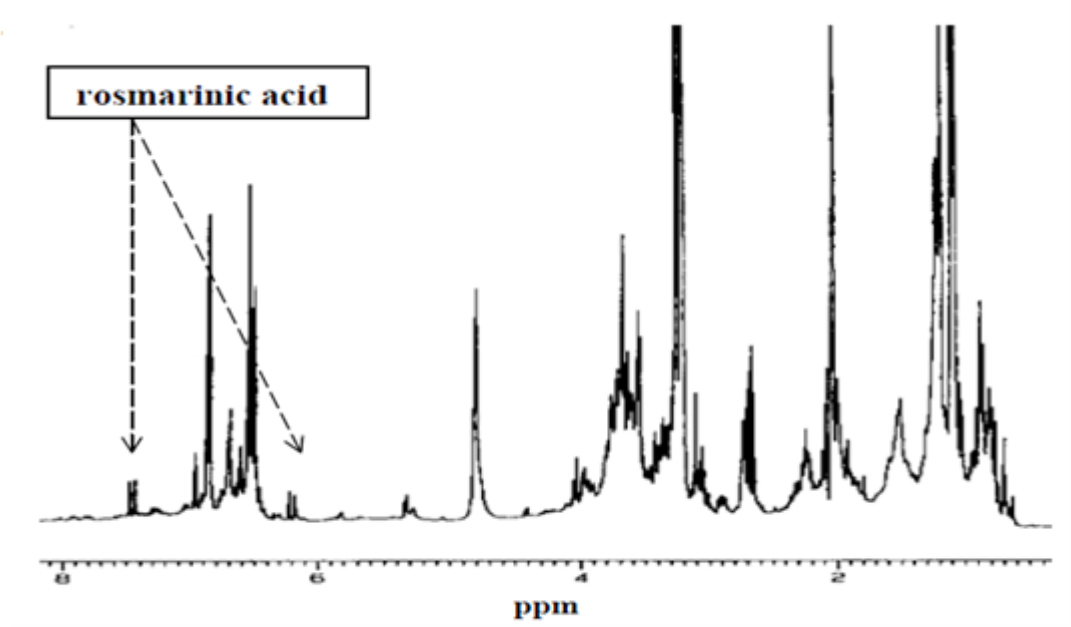


Fig. 8: NMR spectra of rozmarinic acid

Other applications:

- Materials science

NMR spectroscopy, in solid state, can be used to investigate new materials of great technological importance such as glasses, ceramics, polymers, synthetic membranes and superconductors. Furthermore, it can be used to investigate reactions taking place on catalytical surfaces.

- Medical Imaging:

The potential use of NMR tomography is seen in medicine. NMR tomography offers a non-invasive and non-destructive imaging method for biological tissue which easily penetrates a human body. As compared to X-ray tomography, NMR offers unique capabilities beyond the simple imaging of tissue density called Nuclear Magnetic Resonance Tomography. It makes possible the identification of different kind of tissues as well as the visualization of movement of the organs which is a very important field of medicare applications, knowing as *magnetic tomography* or *Magnetic Resonance Imaging*, *MRI*.

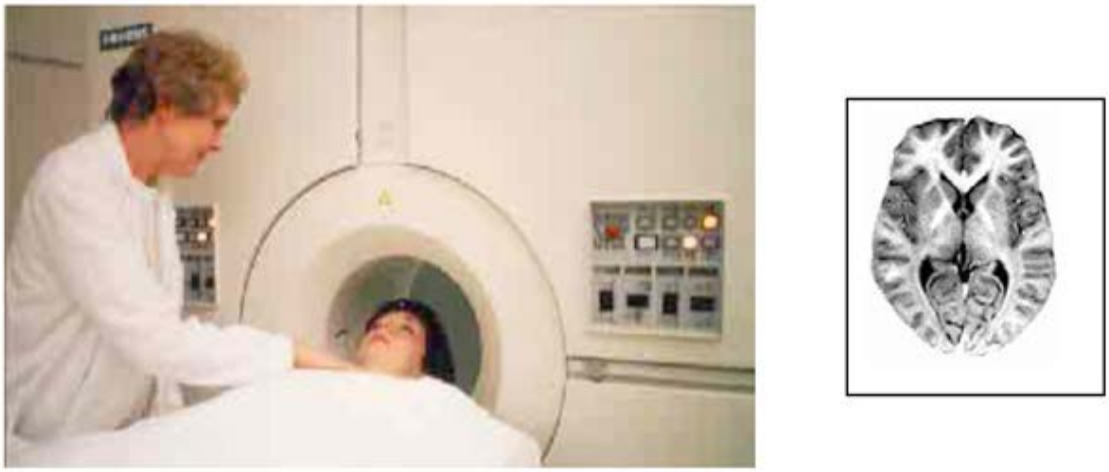


Fig. 9: MRI imaging of human brain

- Clinical applications

NMR has found numerous applications in the localization and characterization of metabolites in biological fluids in vivo and in vitro and, thus, it can be utilized in the diagnosis of many kinds of diseases for instance rapid screening of urine by NMR spectroscopy can provide information about both the identity of the poison and the resulting abnormal pattern of endogenous metabolites.

- NMR as a microscope

All the examples mentioned above show that NMR spectroscopy in the hands of chemists, physicists and biologists is perhaps the most powerful tool for studying the matter at a molecular level. A new category of applications is the so-called NMR microscopy or NMR imaging, which is based on the same basic principles as the classical NMR. NMR microscopy exhibits a variety of technological applications such as the detection of microscopic defects in plastic tubes, the diamond localization in order to avoid breakings during the procedures that follow their excavation etc.