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Nuclear Magnetic Resonance Spectroscopy- A Comprehensive Review

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ABSTRACT:
Nuclear magnetic resonance (NMR) spectroscopy is an advanced characterization technique. It is used to determine the molecular structure at the atomic level of a sample. This article gives a brief introduction into the principles, interpreting NMR spectra, Instrumentation,2D NMR and Application
Key-words: NMR, Types, Instrumentation,2D NMR, Application

I. INTRODUCTION:

NMR is a relatively novel technique developed in the late 1940s. It gives the most descriptive picture of a compound for a chemist.
Radio frequency (rf) energy (60-600 MHz)

Interaction with matter

Irradiation of a sample with radio frequency (rf) energy (60-600 MHz) flips the lower magnetic orientation to the higher state in the presence of an external magnetic field. When the spins of the nuclei particles are not paired, the overall spin of the charged nucleus generates a magnetic dipole along the spin axis indicated by the nuclear magnetic moment μ. Most of the elements have isotopes that are magnetically active (^{1}H, ^{13}C, ^{15}N and ^{31}P) and these nuclei behaves as a tiny spinning bar magnets and when placed within a strong magnetic field the nuclear magnetic moment of the spinning nucleus will align with that stronger, external field just like a compass needle aligning with the Earth's magnetic field and pointing north. This is a highly favorable energy state for the spins. When the spins of the nuclei particles are not paired, the overall spin of the charged nucleus generates a magnetic dipole along the spin axis indicated by the nuclear magnetic moment μ. As per the quantum considerations, the nuclear magnetic moment of the spinning nucleus can align with respect to the external magnetic field of strength Bo in (2I + 1) ways. In the case of hydrogen nucleus, where I = ½, either parallel (aligned) to the external field (low energy orientation, I = +1/2) or opposing (aligned against) the external field (higher energy, I = -1/2). Like a spin precess about the gravitational axis of earth, a spinning nuclei also precess about the external magnetic field axis.[1]

The angular velocity of the precessing nuclei ω_o (Larmor frequency) is given by the equation,

$\omega_o = \gamma Bo$

Where, γ is the constant magnetogyric ratio related to magnetic moment μ and spin number I.

$\gamma = 2\pi\mu / hI$

Radiation of 60-600 MHz flips the lower magnetic orientation to the higher state in the presence of an external magnetic field. The spinning frequency does not change but the speed of precession does. The precessional frequency is directly proportional to the strength of the external field Bo.

$N \alpha Bo$

$\nu = \frac{\mu\beta Bo}{h I}$

Where, β= nuclear magnetic constant

Thus a proton exposed to an external magnetic field of 1.4 T will precess at around 60 million times per second (60 MHz). For an external field of 2.3 T, ν = 100 MHz. If an rf pulse is applied to the aligned spins, the low energy (the nuclei precessing in the aligned orientation) nuclei may absorb this energy and 'flip' into the higher energy state. However, the absorption of rf pulse takes place only if the precessing frequency of the nucleus is the same as the frequency of the radiofrequency. At this condition, the radiofrequency and nuclear precessional frequency are in resonance; hence the term nuclear magnetic resonance. Thus in NMR experiments, the protons are exposed to a powerful magnetic field, whereby they precess and the precessional frequency depends on the net external field exerted to the nucleus, which in turn depends on the environment of the particular nucleus. And thus protons in

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different molecular environments absorb energy at different frequencies. NMR spectrum is the record of this absorption of energy. The area under an NMR signal is directly proportional to the number of atoms in the sample responsible for that signal. ^{1}HNMR and ^{13}C NMR spectroscopy are the most useful for a phyto chemist.[2]

Interpreting the NMR spectra

Shielded protons: If the magnetic field of the environment of a proton opposes that of the external magnetic field, the effective field felt by the proton is diminished and the proton is said to be shielded. The internal standard TMS is highly shielded and absorb at higher field than almost all organic protons. The electrons in the C-H bonds are closer to the hydrogen and thus the hydrogen nuclei are the most shielded from the external magnetic field in this compound than in almost any other one. This demands an increased magnetic field to bring the hydrogen back into resonance.[3]

Deshielded protons: and the hydrogen nucleus feels more of the field and said to be deshielded. Electronegative groups attached to the C-H system decrease the electron density around the protons and the external magnetic field needed to bring the hydrogen into resonance will be smaller as here the electrons in the bond would be away from the hydrogen nucleus. This results in less shielding (deshielding) and the chemical shift increases [CH_4 (δ 0.23), CH_3Cl (δ 3.05), CH_2Cl_2 (δ 5.30), $CHCl_3$ (δ 7.27)]

Chemical shift: The position of the NMR signal of a particular proton depends on the shielding or deshielding exerted on the proton by its environment over the external magnetic field applied. Chemical shift is the difference in absorption position of a particular proton from the absorption position of a reference proton. The chemical shifts are measured using the δ scale and are given as ppm values relative to an arbitrary standard TMS (δ=0ppm). A peak at a chemical shift 2.0 means that the hydrogen atoms which caused that peak need a magnetic field two millionths less than the field needed by TMS to produce resonance. A peak at a chemical shift of 2.0 is said to be downfield of TMS. ^{1}H NMR appears predominantly in the range 0-10 ppm downfield from the reference signal of TMS.

Protons with neighbouring electronegative atoms such as O, F or Br will be at a larger chemical shift (O-CH_2 δ= 3.5- 4.5) than for the same proton without a neighbouring electronegative atom (C-CH_2 δ=1.5). The smaller the magnetic field needed, the higher the chemical shift (deshielded). CH_3 groups have a lower chemical shift than CH_2 groups. Hydrogen bonding shifts the resonance signal of a proton to lower field (higher frequency).

Hydroxyl proton peaks: The -OH peak varies depending on the solvent used, concentration and purity. Moreover the position of –OH peak depends on the extent of hydrogen bonding. The more hydrogen bonding, the more the proton is deshielded and the higher its chemical shift will be. Deuterium exchange is another characteristic feature, where -OH peaks disappear on adding deuterium oxide (D_2O). By comparing the original spectra and the deuteriated spectra, the -OH peak can easily be picked out. The -OH peak is usually a singlet, where the neighbouring protons do not have any effect and has no effect on neighbouring groups too. (–NH) peak also exhibit the same properties.[4]

Spin-spin coupling: The phenomenon of splitting of the proton signal to more than one signal depending on the neighboring protons is termed spin-spin coupling. Both the nuclei exhibit the same amount of perturbance (same coupling constant). This spin-coupling is transmitted through the connecting bonds, however, the interacting nuclei must be bonded in relatively close proximity as in the case of vicinal and geminal locations, or oriented in certain optimal and rigid configurations. The proximity of other "n" H atoms on neighbouring carbon atoms causes the signals to be split into "n+1" lines. If there is a single neighbour (except hydrogen atoms attached to the same carbon, which are termed equivalent hydrogen), the neighbouring nucleus can either align with or against the magnetic field and the signal splits to two (doublet) and if there is two chemically different proton neighbors, the signal splits to three (triplet) with intensity ratio 1:2:1. Here the central line is twice the intensity of the outer lines. The central lines of the splitting pattern in all cases are stronger than those on the periphery. The intensity ratio of these lines is given by the numbers in Pascal's triangle. However, if a proton has two or more types of neighbouring protons, the coupling gives more complex pattern, often with overlapping .[5]

- Singlet: Next carbon has no hydrogen attached
- Doublet: Next carbon has a CH group
- Triplet: Next carbon has a CH_2 group
- Quartet: Next carbon has a CH_3 group

The coupling constant, J (Hz) is a measure of the interaction between a pair of protons. The size of the coupling (coupling constant) is an

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indication of the nature of the neighbouring protons.

Equivalent hydrogen atoms: Hydrogen atoms attached to the same carbon atom are said to be equivalent. Equivalent hydrogen atoms have no effect on each other and thus one hydrogen atom in a CH_2 group doesn't cause any splitting in the spectrum of the other one.

Information from ^{1}H NMR

- Number of H atoms from the area of the peaks.
- Nature of hydrogen atoms from the chemical shift value.
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- Neighbouring hydrogen atoms from spin-spin coupling

^{13}C NMR Spectra

1.1% of elemental carbon is ^{13}C isotope, which has a spin I = 1/2. In 13C NMR also, the chemical shifts are presented with reference to TMS. The electrons in the C-Si bonds are closer to the carbons and the carbon nuclei are thus most shielded from the external magnetic field that demands a greater external magnetic field for the resonance to occur. ^{13}C NMR appears predominantly in the range 0-200 ppm downfield from TMS. A peak at a chemical shift of 60 downfield of TMS require a magnetic field 60 millionths less than the field needed by TMS to produce resonance. The chemical shift of the carbon increases if you attach an atom like oxygen to it. The electronegative oxygen pulls electrons away from the carbon nucleus leaving it more exposed to any external magnetic field. Thus in contrary to TMS, it requires a smaller external magnetic field to bring the nucleus into the resonance condition. The smaller the magnetic field needed, the higher the chemical shift.[7]

II. INSTRUMENTATION

The basic features of the NMR instrumentation are a magnet, a radiofrequency source and a detection system to measure the radiofrequency energy transition.

Magnet: To achieve frequencies greater than 100 MHz, super conducting magnets are required. The solenoid, wound from alloys based on niobium, is immersed in a bath of liquid Helium contained within an efficient cryostat. A set of field gradient coils is mounted inside the bore of the magnet and inside them sits the NMR probe.

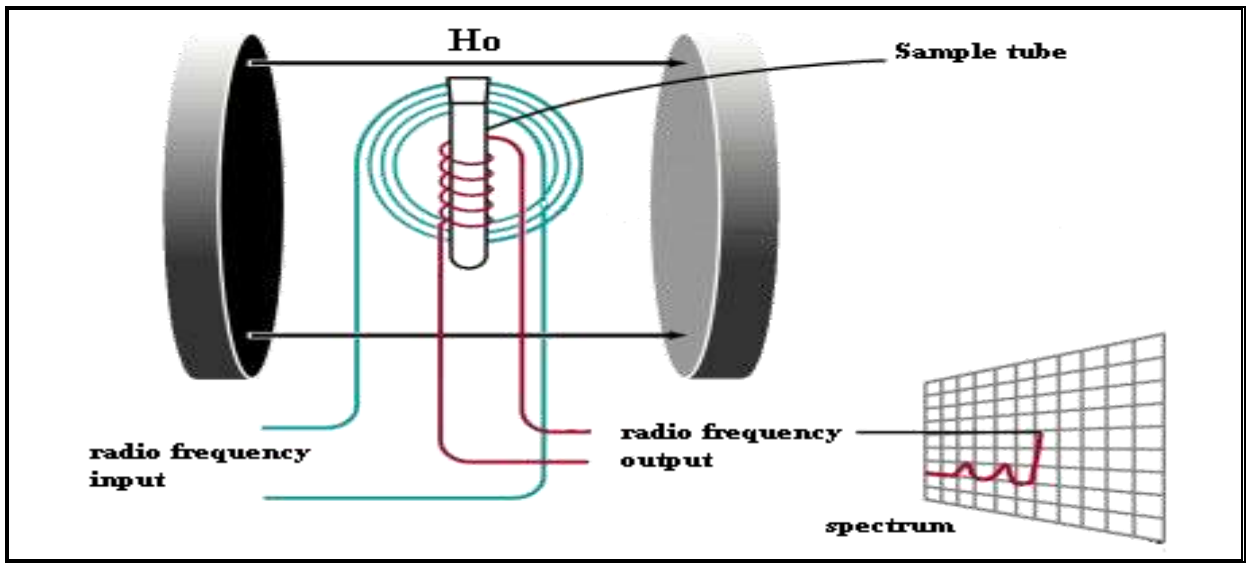

Fig 1.4: Schematic representation of NMR spectroscopy

Sample: NMR spectra are obtained from solutions of samples placed in high precision glass cylindrical NMR tubes. The gradient coils, probe and sample are at room temperature. The sample is spun (typically at 20 Hz) about its vertical axis by an air turbine in order to minimize any irregularities in the sample. With the most sophisticated instruments, with capillary NMR probes that can hold 5µL sample, a full range of 1D and 2D NMR spectra can now be acquired in 24-48 h with samples of 30 µg.

NMR solvents: Usually solvents without protons (aprotic) that interfere with the sample spectra with

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at least 10% solubility are preferred. The most frequently used NMR solvents for polar compounds are hexadeutero dimethyl sulfoxide (DMSO-d_6) and tetra deutero methanol (CD_3OD). For the analysis of relatively non polar compounds, solvents such as deuterated acetone, deuterated chloroform ($CDCl_3$), carbon tetrachloride (CCl_4), and pentadeutero pyridine are used .[12]

Shift reagents in NMR spectroscopy: Chiral lanthanide shift reagents are used to find the optical enantiomers by NMR.

Destortionless Enhancement Through Polarisation Transfer (DEPT): Sequence of pulses with various delay times are used to create the DEPT spectra where -CH_3 and CH peaks appear as normal and -CH_2 peaks appear inverted (downward). Quaternary carbons are not seen in the DEPT spectra.[11]

Two Dimensional NMR Methods : Since the early 1980s, with the advancements in NMR spectrometers with their increasingly powerful magnetic strength and the developments in computer programmes that enables to perform multiple experiments within very short time, 2D NMR methodology has become an extraordinary useful tool in the structure elucidation of organic compounds..

Correlation Spectroscopy (COSY): ^{1}H-^{1}H COSY is one of the oldest 2D NMR methods. The experiments generate NMR spectra in which ^{1}H chemical shifts along two axes are correlated with each other. Values on the diagonal in these spectra correspond to chemical shifts that would have been shown in a 1dimensional ^{1}H NMR experiment. The 1D ^{1}H NMR spectra may be placed as projections along the top and left parts. It is the off diagonal cross peaks that give new information. A cross peak observed above the diagonal will also be found below the diagonal. The spectrum correlates the different protons that are coupled to each other. Thus the neighbouring protons are correlated and are revealed as cross peaks in the spectrum. In double-quantum filtered COSY (^{1}H–^{1}H DQF-COSY) experiment non-coupled proton signals are eliminated and also the strong solvent signal and the often very strong H_2O signal are also eliminated.[7]

HMBC: Heteronuclear **M**ultiple **B**ond **C**orrelation is an experiment that identifies proton nuclei with carbon nuclei that are separated by more than one bond. The pulse sequence utilizes zero and double quantum coherence between J-coupled protons and carbons to label each proton with the frequency of a remote carbon in the F1 dimension of a two dimensional experiment[10]. The experiment is closely related to HMQC and uses the same principles to convert transverse magnetization into zero and double quantum coherence with the exception that the delay is matched to the inverse of the long range coupling constant $^nJ_{CH}$ and there is a filter to suppress cross peaks arising from one-bond proton-carbon interactions. The one-bond filter has a delay that is matched to the inverse of $^1J_{CH}$. In the pulse sequence that is currently implemented, the one-bond filter is optimized by entering an average $^1J_{CH}$, usually 14O Hz, but if there are no aromatic or alkene groups, then 125 Hz may be a better choice. The delay corresponding to the multiple bond J coupling $^nJ_{CH}$ (taumb) is entered directly in seconds (a good first choice is 0.04 sec, but if the long range couplings are small then 0.06 sec is optimum).[8]

HMQC: The 2D HMQC (Heteronuclear Multiple-Quantum Correlation) experiment permits to obtain a 2D heteronuclear chemical shift correlation map between directly-bonded 1H and X-heteronuclei (commonly, 13C and 15N). It is widely used because it is based on proton-detection, offering high sensitivity when compared with the conventional carbon-detected 2D HETCOR experiment. Similar results are obtained using the 2D HSQC experiment.[9]

III. APPLICATIONS

1.H NMR spectroscopy has been used for bacterial identi栽cation and quanti栽cation and for metabolic pathways studies. Several studies have been conducted for the diagnosis of the bacteria that cause urinary tract infections (UTI). [10-15]

2.A rapid and quantitative 1H nuclear magnetic resonance (NMR) method was developed to analyse histamine in cheeses. The procedure is simple because the acid extract is analyzed directly, without any need for further filtration, derivatization, or other manipulation. The NMR method was successfully applied to different types of cheese, ranging from soft to hard .[16-20]

3. The application of 1H nuclear magnetic resonance (NMR) spectroscopy to the measurement of conjugated linoleic acid (CLA) content in the lipid fraction of dairy products is both a novel and inviting alternative to traditional methods such as gas chromatography (GC), which can require time-consuming sample derivatization. 1H NMR analysis approach has potential application in the dairy industry as a screening technique for total CLA concentrations in large numbers of cheese

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samples and in the screening of CLA content in other dairy products .

4.NMR can be used for foodomics because of ease of quantification and identification, short time and low costs needed for analysis and high number of metabolites that can be measured through a single-pass. Because of highest sensitivity of NMR focus on hydrogen is prefered for foodomics studies.[18-22]

5.NMR spectroscopy is a Spectroscopy technique used by chemists and biochemists to investigate the properties of organic molecules, although it is applicable to any kind of sample that contains nuclei possessing spin.

For example, the NMR can quantitatively analyze mixtures containing known compounds. NMR can either be used to match against spectral libraries or to infer the basic structure directly for unknown compounds.

Once the basic structure is known, NMR can be used to determine molecular conformation in solutions as well as in studying physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion.

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