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STUDY ON NUCLEAR MAGNETIC RESONANACE SPECTRPSCOPY& ITS SIGNIFICANCE IN PHARMA: ARTICLE

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ABSTRACT

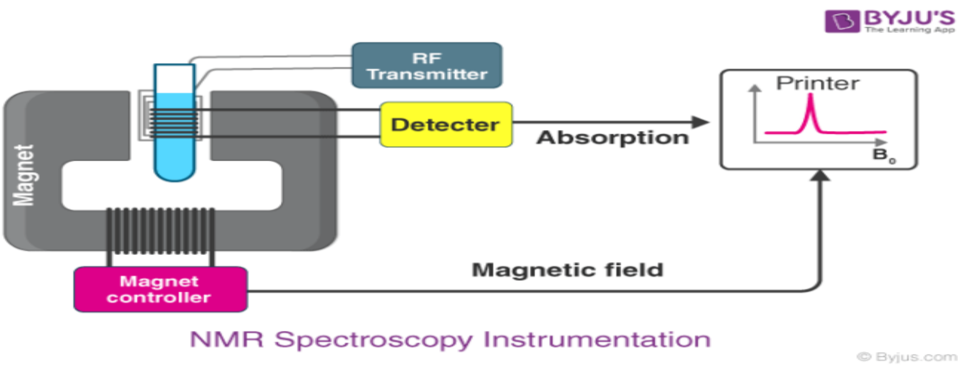
Nuclear magnetic resonance (NMR) describes the response of nuclei to an applied magnetic field. The NMR responses from downhole logs (e.g. amplitude, decay time) are analysed to determine lithology-independent estimates of porosity, saturations and pore system characteristics. This chapter describes the NMR measurements on core that are used to calibrate the log responses and to improve the characterisation of the fluid content in core material, and provide a unique look at the interaction of pore fluids within reservoir rock fabric. The theory and application of the NMR response in porous rocks are described, and the sample preparation methods, test equipment, measurement parameters, test procedures, data interpretation techniques and data reporting requirements for the principal NMR tests on core are detailed. The advantages and drawbacks/issues are described, and quality control checks and diagnostics are summarised.

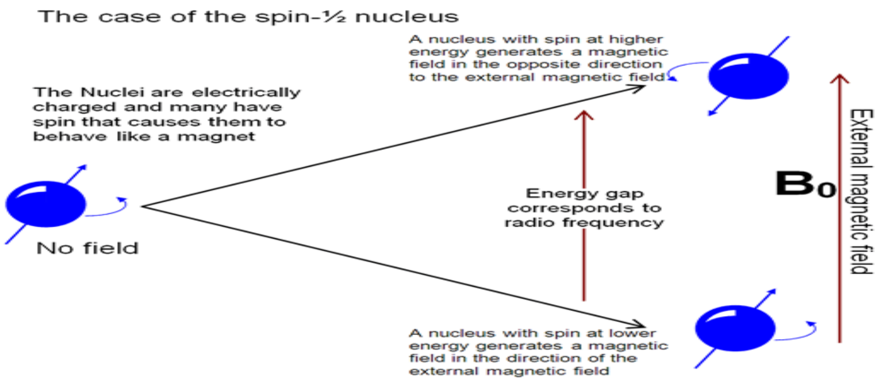
Nuclear Magnetic Resonance (NMR) spectroscopy has made a tremendous impact in many areas of chemistry, biology and medicine. In this report a student-oriented approach is presented, which enhances the ability of students to comprehend the basic concepts of NMR spectroscopy and the NMR spectra of various nuclei. The origin of chemical shifts, coupling constants, spin relaxation and the Nuclear Overhauser Effect (NOE) will be discussed and their relation to molecular structure will be provided.

1. INTRODUCTION

Nuclear Magnetic Resonance (NMR) is a nuclei (Nuclear) specific spectroscopy that has far reaching applications throughout the physical sciences and industry. NMR uses a large magnet (Magnetic) to probe the intrinsic spin properties of atomic nuclei. Like all spectroscopies, NMR uses a component of electromagnetic radiation (radio frequency waves) to promote transitions between nuclear energy levels (Resonance). Most chemists use NMR for structure determination of small molecules.

Commonly used NMR nuclei, protons (1H or hydrogen-1), and many other nuclei such as 13C, 15N, 31P. Many nuclei, such as deuterium (2H or hydrogen-2), have high spins and are therefore quadrupoles, providing NMR spectra, but with some characteristics that differ from the energy diagram.





2. PRINCIPLE OF NMR SPECTROSCOPY

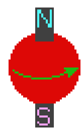
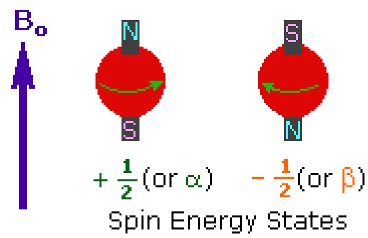
1. The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap).

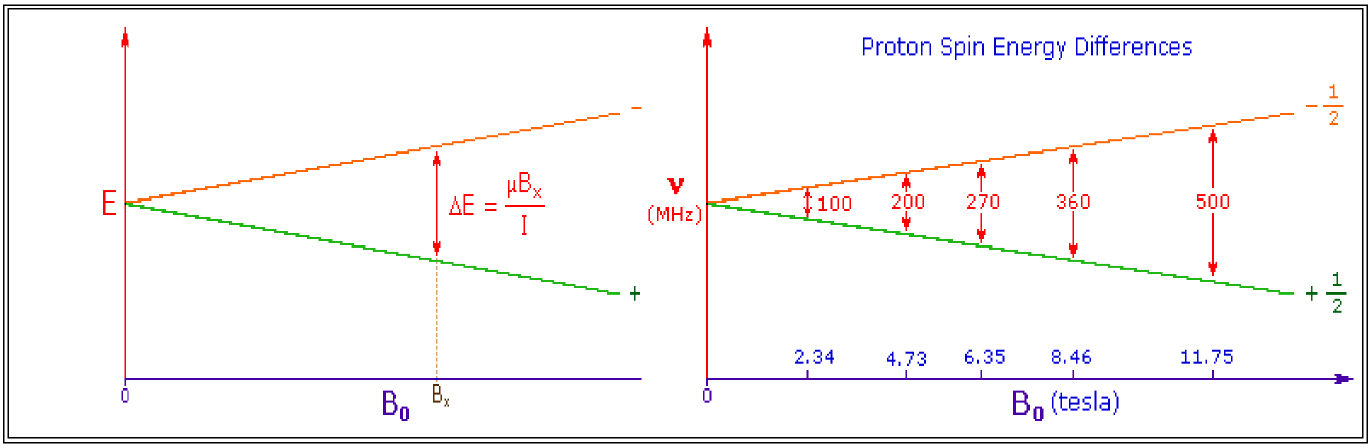
2. The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency.

3. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

The nuclei of many elemental isotopes have a characteristic spin (I). Some nuclei have integral spins (e.g. I = 1, 2, 3 ....), some have fractional spins (e.g. I = 1/2, 3/2, 5/2 ....), and a few have no spin, I = 0 (e.g. 12C, 16O, 32S, ...). Isotopes of particular interest and use to organic chemists are 1H, 13C, 19F and 31P, all of which have I = 1/2. Since the analysis of this spin state is fairly straightforward, our discussion of nmr will be limited to these and other I = 1/2 nuclei.

The following features lead to the nmr phenomenon:

1. A spinning charge generates a magnetic field, as shown by the animation on the right. The resulting spin-magnet has a magnetic moment (**μ**) proportional to the spin.	
2. In the presence of an external magnetic field (**B_0**), two spin states exist, **+1/2** and **-1/2**. **3.** The magnetic moment of the lower energy +1/2 state is aligned with the external field, but that of the higher energy -1/2 spin state is opposed to the external field. Note that the arrow representing the external field points North.	
4. The difference in energy between the two spin states is dependent on the external magnetic field strength, and is always very small. The following diagram illustrates that the two spin states have the same energy when the external field is zero, but diverge as the field increases. At a field equal to B_x a formula for the energy difference is given (remember I = 1/2 and μ is the magnetic moment of the nucleus in the field).	



Spin Properties of Nuclei:

Nuclear spin may be related to the nucleon composition of a nucleus in the following manner: Odd mass nuclei (i.e. those having an odd number of nucleons) have fractional spins.

Examples are I = 1/2 (^{1}H, ^{13}C, ^{19}F), I = 3/2 (^{11}B) & I = 5/2 (^{17}O).
Even mass nuclei composed of odd numbers of protons and neutrons have integral spins. Examples are I = 1 (^{2}H, ^{14}N).
Even mass nuclei composed of even numbers of protons and neutrons have zero spin (I = 0). Examples are ^{12}C, and ^{16}O.
Spin 1/2 nuclei have a spherical charge distribution, and their nmr behavior is the easiest to understand. Other spin nuclei have nonspherical charge distributions and may be analyzed as prolate or oblate spinning bodies. All nuclei with non-zero spins have magnetic moments (μ), but the nonspherical nuclei also have an electric quadrupole moment (eQ). Some characteristic properties of selected nuclei are given. * **μ** in units of nuclear magnetons = $5.05078 \cdot 10^{-27}$ JT^{-1}

† γ in units of 10^{7}rad T^{-1} sec^{-1}

Chemical shift in NMR:

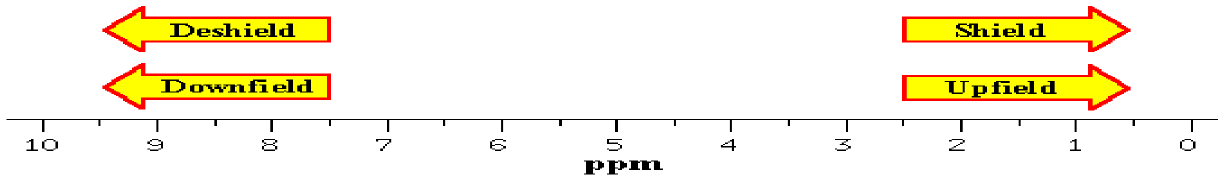
In nuclear magnetic resonance (NMR) spectroscopy, the chemical shift is the resonant frequency of an atomic nucleus relative to a standard in a magnetic field. Often the position and number of chemical shifts are diagnostic of the structure of a molecule.[1][2][3] Chemical shifts are also used to describe signals in other forms of spectroscopy such as photoemission spectroscopy.

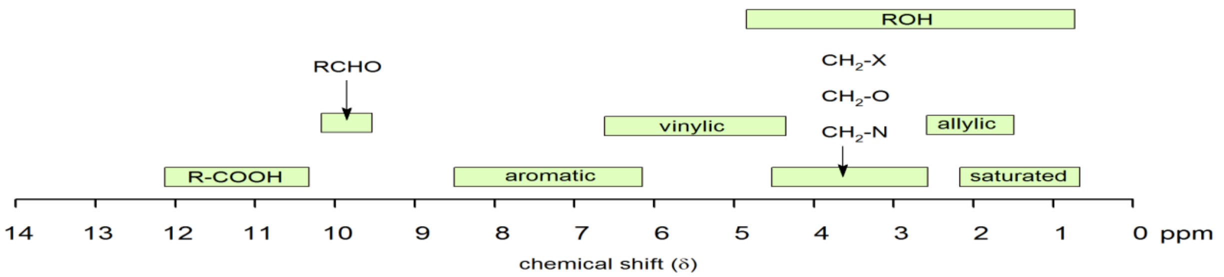
Some atomic nuclei possess a magnetic moment (nuclear spin), which gives rise to different energy levels and resonance frequencies in a magnetic field. The total magnetic field experienced by a nucleus includes local magnetic fields induced by currents of electrons in the molecular orbitals (note that electrons have a magnetic moment themselves). The electron distribution of the same type of nucleus (e.g. 1H, 13C, 15N) usually varies according to the local geometry (binding partners, bond lengths, angles between bonds, and so on), and with it the local magnetic field at each nucleus. This is reflected in the spin energy levels (and resonance frequencies). The variations of nuclear magnetic resonance frequencies of the same kind of nucleus, due to variations in the electron distribution, is called the chemical shift. The size of the chemical shift is given with respect to a reference frequency or reference sample (see also chemical shift referencing), usually a molecule with a barely distorted electron distribution.

Isotope	Natural % Abundance	Spin (I)	Magnetic Moment (μ)*	Magnetogyric Ratio (γ)†
^{1}H	99.9844	1/2	2.7927	26.753
^{2}H	0.0156	1	0.8574	4,107
^{11}B	81.17	3/2	2.6880	--
^{13}C	1.108	1/2	0.7022	6,728
^{17}O	0.037	5/2	-1.8930	-3,628
^{19}F	100.0	1/2	2.6273	25,179
^{29}Si	4.700	1/2	-0.5555	-5,319
^{31}P	100.0	1/2	1.1305	10,840

The **chemical shift** in absolute terms is defined by the frequency of the resonance expressed with reference to a standard compound which is *defined* to be at 0 ppm. The scale is made more manageable by expressing it in parts per million (**ppm**) and is **indepedent** of the spectrometer frequency.

$$\text{Chemical shift, } \delta = \frac{\text{frequency of signal} - \text{frequency of reference}}{\text{spectrometer frequency}} \times 10^{6}$$





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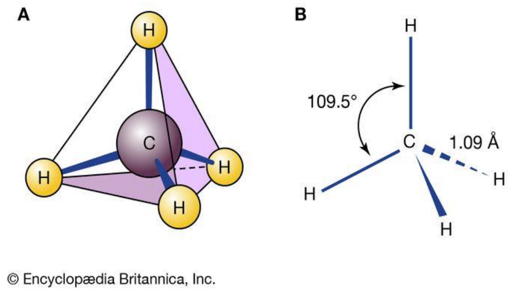
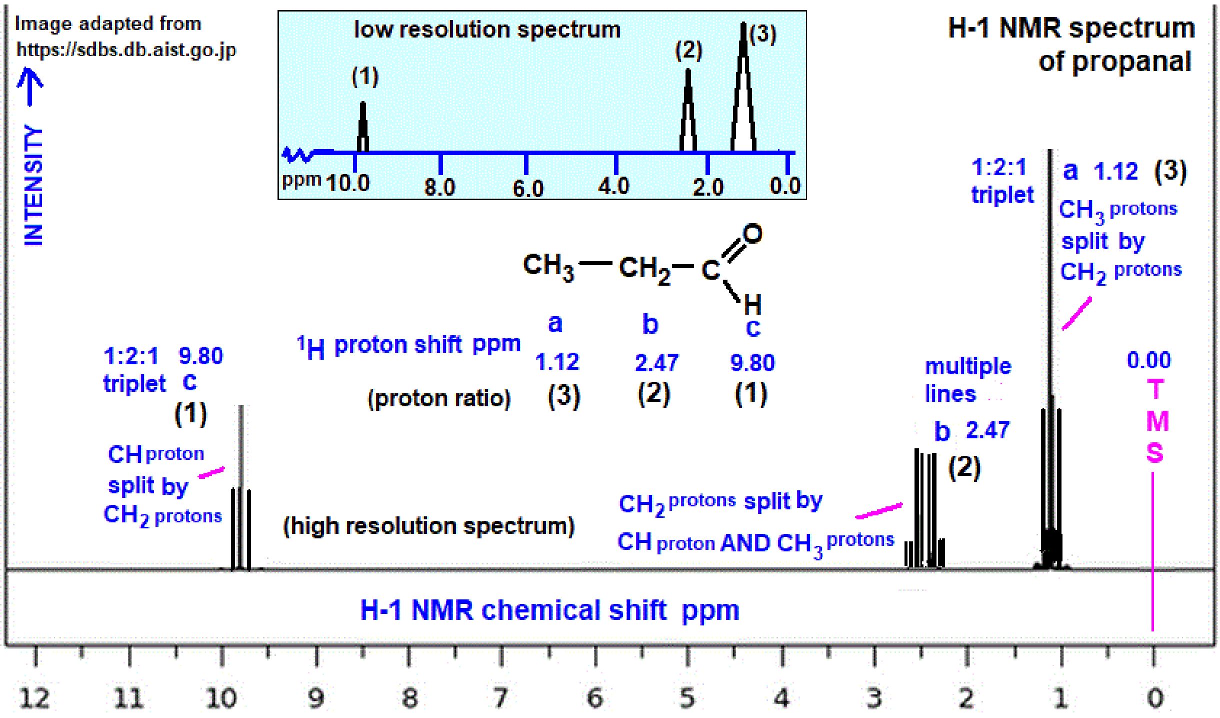
Proton nuclear magnetic resonance spectroscopy:

Proton nuclear magnetic resonance spectra can provide structural information about organic compounds such as bromoethane. This information is based on energy absorption by hydrogen atoms, which absorb different wavelengths of energy depending on their bonding environment.



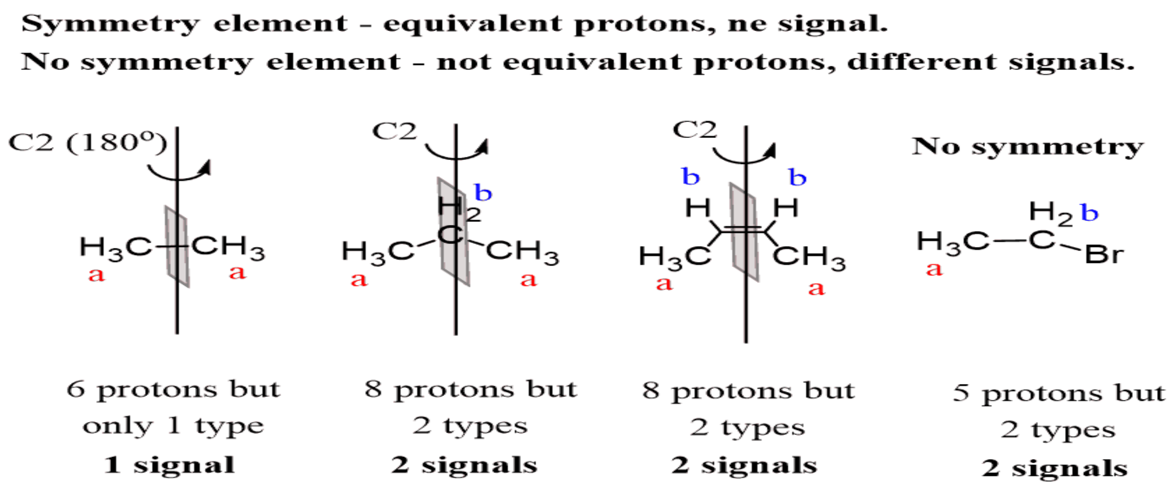
H-1 proton NMR spectroscopy - spectra index



TMS is an acronym for tetramethylsilane, formula Si(CH3) 4, whose protons have an arbitrary chemical shift of 0.0 ppm. This is the "norm" in 1H NMR spectroscopy, and all other proton shifts, known as chemical shifts, depend on the individual chemical (electronic) environments of the hydrogen atoms in a organic molecules - here propanal.

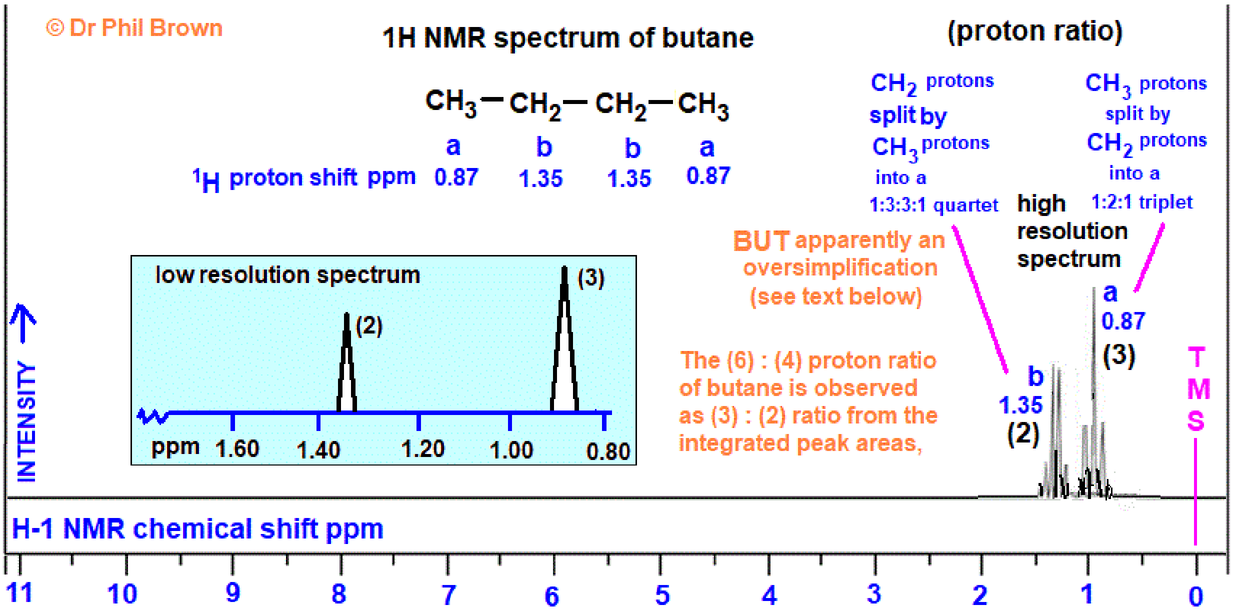
3. SIGNAL IN 1H NMR SPECTROSCOPY

NUMBER OF SIGNALS. The number of signal present in an NMR spectrum reflects the number of magnetically different protons. For our purposes, although not alwyas true, we will assum that magnetically different protons are also chemically different. Thus the number of signals reflects the number of chemically different protons or sets of protons. For example methanol has two different sets of protons (methyl and hydroxyl) and therefore is expected to show two signals in the proton NMR spectrum.



4. 13C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

The most abundant isotope of carbon is C-12 (nuclear spin, I = 0) and this is NMR inactive. However, the natural abundance of C-13 or 13C is 1.1% and has I = 1/2 like a proton. Therefore, C-13 nuclei are NMR active. Unfortunately, the resonances of 13C nuclei are more difficult to observe than those of protons (1H) due to low magnetogyric ratio (γ) (it is 1/4th of the proton) and low natural abundance. Therefore, 13C nuclei are very less sensitive and overall sensitivity is about 1 5700 of the proton. The advantage of 13C NMR over 1H NMR is that proton NMR gives indirect information about carbon skeleton of the molecule whereas, 13C NMR gives direct information because each carbon (if they are not equivalent) of the molecule gives its own signal whether it has any attached hydrogen or not.



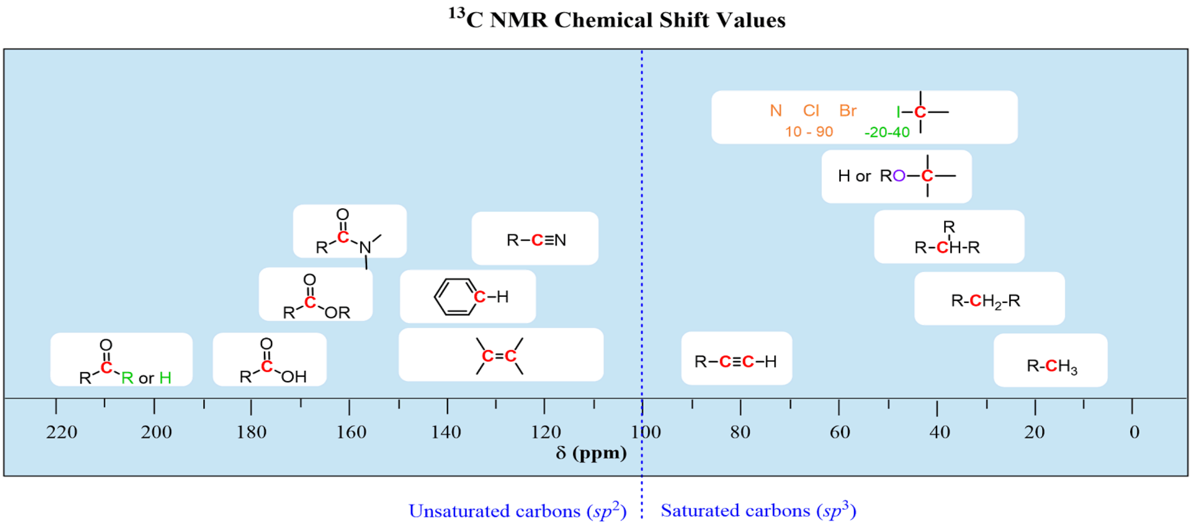
TMS is an acronym for tetramethylsilane, formula Si(CH3) 4, which has 13C atoms with an arbitrary chemical shift of 0.0 ppm. This is the "norm" in the 13C NMR spectrum and all other 13C resonances, known as the chemical shift, which is measured relative to the TMS and depends on the individual chemical (electronic) environments of the particles. 13C atom in an organic molecule - butane here

Butane C_4H_{10} $CH_3-CH_2-CH_2-CH_3$

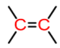
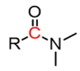
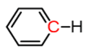
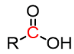
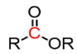
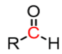
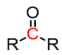
5. SIGNAL IN 13C NMR SPECTROSCOPY

Just like with 1H NMR, chemical shift equivalence applies to 13C NMR. A spectrum produced by a 13C NMR experiment may not always display a 1:1 ratio of signals to individual carbons atoms. When two or more carbon atoms in a molecule have chemically equivalent nuclei, instead of producing two or more signals on a 13C NMR spectrum, they will produce one signal at a specific chemical shift that will represent those equivalent carbons. In order for atoms to be chemically equivalent, their nuclei must be interchangeable through the performance of symmetry operations (planes of symmetry) or rapid intramolecular processes (bond rotation or tautomerization). Carbon atoms with chemical equivalence may be homotopic (identical) or enantiotopic (equivalent in achiral solvents).

For the purposes of this topic, we will be dealing with proton-decoupled 13C NMR in order work with spectra consisting of single line signals rather than multiplets corresponding to specific carbon atoms.



Approximate Values of Chemical Shifts for 13C NMR

$(CH_3)_4Si$	0	* I–C	-20 - 40		100 - 150		155 - 185
$R\text{-}CH_3$	8 - 30	Br–C	25 - 65		110 - 170	 	165 - 185
$R\text{-}CH_2\text{-}R$	15 - 55	Cl–C	35 - 80	C=N	150 - 170	 	190 - 220
R_3CH	20 - 60	O–C	40 - 80	C≡N	110 - 140		
R_4C	30 - 50	N–C	30 - 65				

To start visualize this concept, let's start with the basic example of methane. Methane contains one carbon atom. A 13C NMR experiment will produce one signal on a spectrum at a specific chemical shift, corresponding to the one carbon in methane.

6. RECENT DEVELOPMENTS IN SOLUTION NUCLEAR MAGNETIC RESONANCE (NMR)-BASED MOLECULAR BIOLOGY

Clinical significance - the awesome power of NMR

Visualizing post-translational modifications, conformations, and interaction surfaces of protein structures at atomic resolution underpins the development of novel therapeutics to combat disease. As computational resources expand, in silico calculations coupled with experimentally-derived structures and functional assays has led to an explosion in structure based drug design (SBDD) with several compounds in clinical trials. It is increasingly clear that 'hidden' transition-state structures along activation trajectories can be harnessed to develop novel classes of allosteric inhibitors. The goal of this mini-review is to empower the clinical researcher with a general knowledge of the strengths and weaknesses of nuclear magnetic resonance (NMR) spectroscopy in molecular medicine. Although NMR can determine protein structures at atomic resolution, its unrivaled strength lies in sensing subtle changes in a nuclei's chemical environment as a result of intrinsic conformational dynamics, solution conditions, and binding interactions. These can be recorded at atomic resolution, without explicit structure determination, and then incorporated with static structures or molecular dynamics simulations to produce a complete biological picture.

The niche of NMR in modern structural biology

Static structural representations subconciously train scientists to envision a rigid protein architecture. However, macromolecues are exceptionally acrobatic possessing fast, local fluctuations and slow, concerted structural motions

Therefore, a more accurate depiciton is a series of stuctures where the kinetics, thermodynamics, and function of each conformation varies significantly within a statistical ensemble. Understanding how these dynamic molecules behave in solution with atomic resolution is essential for deciphering their roles in biological processes such as ligand binding and protein protein interactions.

7. APPLICATION AND ITS SIGNIFICANCE

1. Chemistry laboratories

Chemists rely on NMR Spectroscopy as a tool to chart the complex molecular structures of matter. The technique sees samples placed in a specialised NMR spectrometer, where it's exposed to a strong magnetic field. The gravitational pull generated by the field excites the nuclei of some atoms and creates resonant frequencies. These resonant frequencies are then tracked, measured and used to generate useful data.

2. Food quality control and research

Before hitting the consumer market, foods undergo strict quality control and research studies. NMR Spectroscopy is widely used across the industry to map protein structures, profile amino acids, identify carotenoids and quantify metabolites.

3. MRI scans

Most people are familiar with Magnetic Resonance Imaging (MRI) scans which use powerful magnetic fields and radio waves to reveal detailed images of the internal organs. Data is generated based on rates of absorption and energy emission recorded in the radiofrequency (RF) range. The medical imaging technique is founded in NMR Spectroscopy and uses the same basic principles

4. Identifying human disorders

When used alongside metabolomics data, NMR Spectroscopy is an invaluable tool for identifying human disorders. The cellular metabolism contains important biomarkers used to diagnose a wide range of conditions, including tuberculosis, pneumonia and malaria. NMR Spectroscopy allows researchers to identify these tell-tale biomarkers and treat patients accordingly. The technique can also be used to investigate Parkinson's disease, as well as cardiovascular diseases and a range of neuropsychiatric disorders such as bipolar, schizophrenia, major depression and autism-spectrum disorders.

5. Cancer diagnosis

Over the past few decades, NMR Spectroscopy has become a key technique for cancer diagnosis. The ability to analyse abnormal behaviour in the cellular metabolism allows scientists to detect the metabolite-based biomarkers associated with cancers.

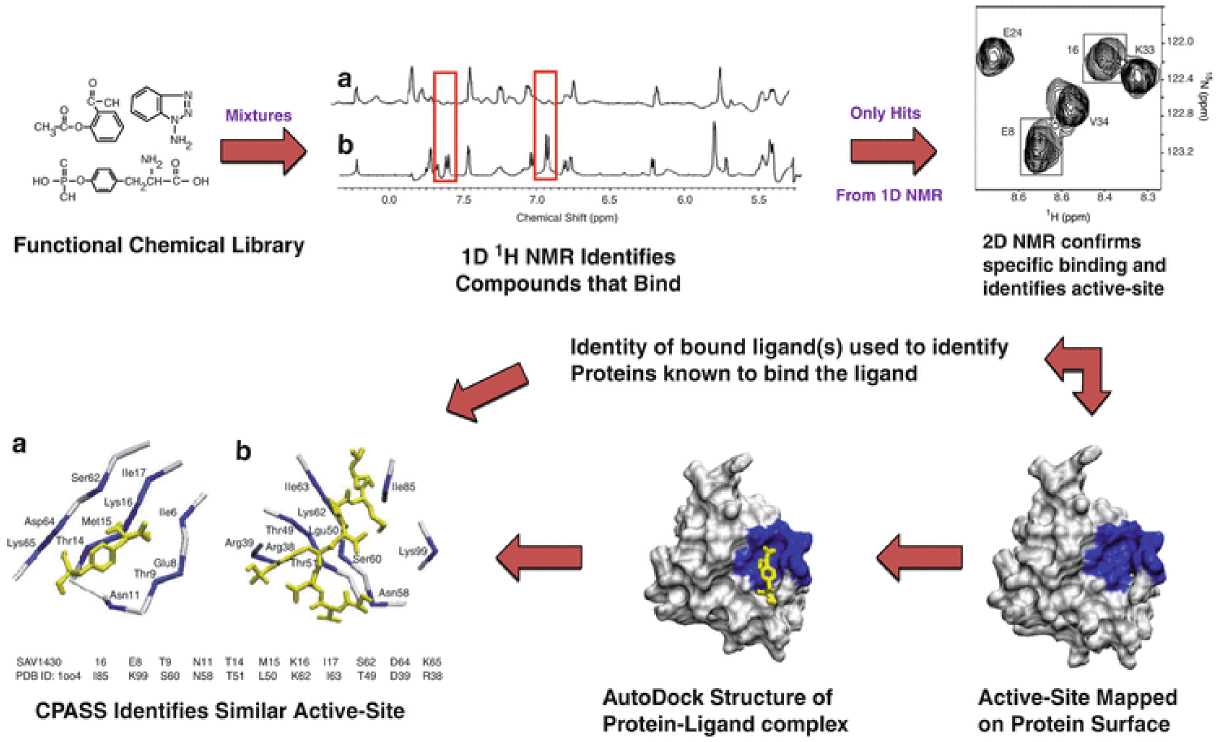
6. Environmental monitoring

It's not just the medical field where NMR Spectroscopy is considered a valuable tool, with the technique also used for environmental monitoring. Spectrometers are used to detect and characterise contaminants in air, soil and water samples as well as monitor the metabolic responses of organisms exposed to these contaminants.

7. Drug discovery and development

From trialling new cancer therapies to perfecting nutritional supplements, NMR Spectroscopy is a mainstay in the drug discovery and development arena.

Dr Robin J. Blagg, a representative from UK-based manufacturing and research company Oxford Instruments, offers more information on the latest NMR Spectroscopy techniques and instruments in 'X-Nuclei NMR Spectroscopy'.



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