

A STUDY OF NMR SPECTROSCOPY

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INTRODUCTION

Nuclear magnetic resonance spectroscopy, most commonly known as **NMR spectroscopy**, is the name given to a technique which exploits the magnetic properties of certain nuclei. This phenomenon and its origins are detailed in a separate section on nuclear magnetic resonance. The most important applications for the organic chemist are proton NMR and carbon-13 NMR spectroscopy. In principle, NMR is applicable to any nucleus possessing spin.

Many types of information can be obtained from an NMR spectrum. Much like using infrared spectroscopy to identify functional groups, analysis of a 1D NMR spectrum provides information on the number and type of chemical entities in a molecule.

The impact of NMR spectroscopy on the natural sciences has been substantial. It can, among other things, be used to study mixtures of analytes, to understand dynamic effects such as change in temperature and reaction mechanisms and is an invaluable tool in understanding protein and nucleic acid structure and function. It can be applied to a wide variety of samples, both in the solution and the solid state.

Nuclear Magnetic Resonance spectroscopy is a powerful and theoretically complex analytical tool. It is important to remember that, with NMR, we are performing experiments on the **nuclei** of atoms, not the electrons. The chemical environment of specific nuclei is deduced from information obtained about the nuclei.

ISOTOPES

Many chemical elements can be used for NMR analysis.

- ^1H , the most commonly used, very useful. Highly abundant, the most sensitive nucleus apart from tritium. Narrow chemical shift, but sharp signals. In particular, the ^1H signal is that used in magnetic resonance imaging.
- ^2H , commonly used in the form of deuterated solvents to avoid interference of solvents in measurement of ^1H . Often used in determining the behavior of lipids in lipid membranes and other solids or liquid crystals as it is a relatively non-perturbing label which can selectively replace ^1H .
- ^3He , very sensitive. Low percentage in natural helium, has to be enriched. Used mainly in studies of endohedral fullerenes.

- ^{13}C , commonly used. Low percentage in natural carbon, therefore spectrum acquisition takes a long time. Frequently used for labeling of compounds in synthetic and metabolic studies. Has low sensitivity and wide chemical shift, yields sharp signals. Low percentage makes it useful by preventing spin-spin couplings and makes the spectrum appear less crowded.
- ^{15}N , relatively commonly used. Can be used for labeling compounds. Nucleus very insensitive but yields sharp signals. Low percentage in natural nitrogen together with low sensitivity requires high concentrations or expensive isotope enrichment.
- ^{14}N , medium sensitivity nucleus with wide chemical shift. Its large quadrupole moment interferes in acquisition of high-resolution spectra, limiting usefulness to smaller molecules.
- ^{19}F , relatively commonly measured. Sensitive, yields sharp signals, has wide chemical shift.
- ^{31}P , 100% of natural phosphorus. Medium sensitivity, wide chemical shift range, yields sharp lines. Used in biochemical studies.
- ^{17}O , low sensitivity and very low natural abundance.
- ^{10}B , lower sensitivity than ^{11}B . Use quartz tubes, as borosilicate glass interferes with measurement.
- ^{11}B , more sensitive than ^{10}B , yields sharper signals. Use quartz tubes, as borosilicate glass interferes with measurement.
- ^{35}Cl and ^{37}Cl , broad signal. ^{35}Cl significantly more sensitive, preferred over ^{37}Cl despite its slightly broader signal. Organic chlorides yield very broad signals, use limited to inorganic and ionic chlorides and very small organic molecules.
- ^{43}Ca , used in biochemistry to study calcium binding to DNA, proteins, etc. Moderately sensitive, very low natural abundance.
- ^{195}Pt , used in studies of catalysts and complexes.
- Other nuclei, usually used in the studies of their complexes and chemical binding, or to detect presence of the element: ^6Li , ^7Li , ^9Be , ^{19}F , ^{21}Ne , ^{23}Na , ^{25}Mg , ^{27}Al , ^{29}Si , ^{31}P , ^{33}S , ^{39}K , ^{40}K , ^{41}K , ^{45}Sc , ^{47}Ti , ^{49}Ti , ^{50}V , ^{51}V , ^{53}Cr , ^{55}Mn , ^{57}Fe , ^{59}Co , ^{61}Ni , ^{63}Cu , ^{65}Cu , ^{67}Zn , ^{69}Ga , ^{71}Ga , ^{73}Ge , ^{77}Se , ^{81}Br , ^{87}Rb , ^{87}Sr , ^{95}Mo , ^{109}Ag , ^{113}Cd , ^{125}Te , ^{127}I , ^{133}Cs , ^{135}Ba , ^{137}Ba , ^{139}La , ^{183}W , ^{199}Hg .

BASIC PRINCIPLE

In NMR, electromagnetic (EM) radiation is used to "flip" the alignment of nuclear spins from the low energy, spin aligned state to the higher energy spin opposed state.

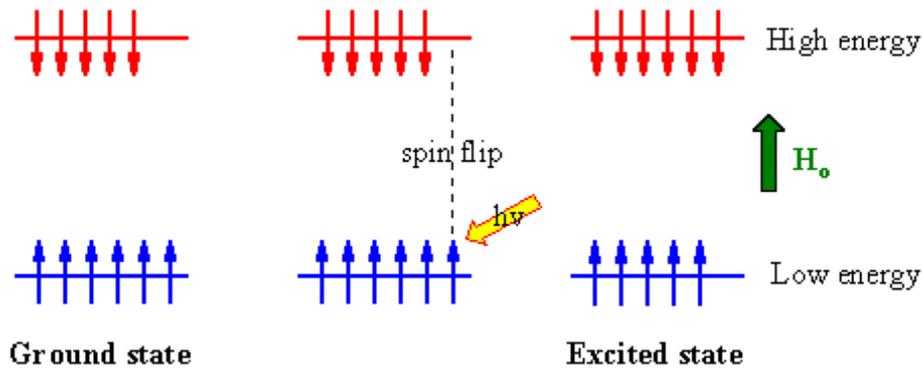


Figure : 1

The energy required for this transition depends on the strength of the applied magnetic field (see below). The energy difference between the spin states is quite small and corresponds to the radiofrequency range of the EM spectrum.

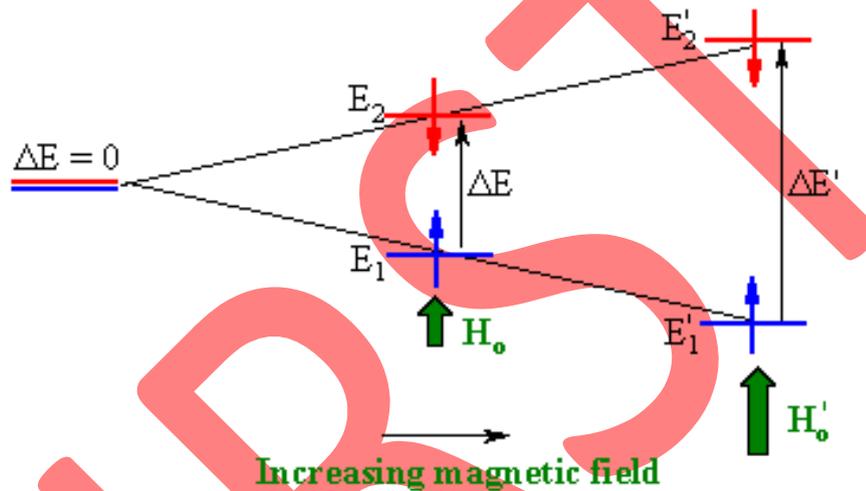


Figure : 2

As this diagram shows, the energy required to cause the spin-flip, ΔE , depends on the magnetic field strength at the nucleus. With no applied field, there is no energy difference between the spin states, but as the field increases, so does the separation of energies of the spin states and therefore so does the frequency required to cause the spin-flip.

$$\Delta E' > \Delta E \text{ since } H_0' > H_0$$

The basic arrangement of an NMR spectrometer is shown to the left. The sample is positioned in the magnetic field and excited via pulsations in the radio frequency input circuit. The realigned magnetic fields induce a radio signal in the output circuit which is used to generate the output signal. Fourier analysis of the complex output produces the actual spectrum. The pulse is repeated as many times as necessary to allow the signals to be identified from the background noise.

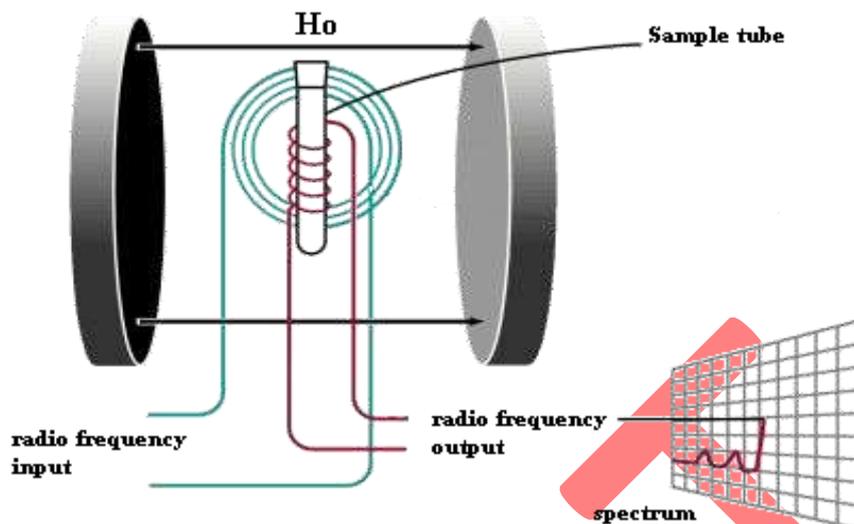


Figure : 3

DISCOVERY OF NMR

Nuclear magnetic resonance was first described and measured in molecular beams by Isidor Rabi in 1938. Eight years later, in 1946, Felix Bloch and Edward Mills Purcell refined the technique for use on liquids and solids, for which they shared the Nobel Prize in physics in 1952.

Purcell had worked on the development and application of RADAR during World War II at Massachusetts Institute of Technology's Radiation Laboratory. His work during that project on the production and detection of radiofrequency energy, and on the absorption of such energy by matter, preceded his discovery of NMR.

They noticed that magnetic nuclei, like ^1H and ^{31}P , could absorb RF energy when placed in a magnetic field of a strength specific to the identity of the nuclei. When this absorption occurs, the nucleus is described as being *in resonance*. Interestingly, for analytical scientists, different atoms within a molecule *resonate* at different frequencies at a given field strength. The observation of the resonance frequencies of a molecule allows a user to discover structural information about the molecule.

The development of nuclear magnetic resonance as a technique of analytical chemistry and biochemistry parallels the development of electromagnetic technology and its introduction into civilian use.

NMR SPECTROMETERS

Two types of NMR spectrometers are commonly encountered. They are:

- (a) Continuous wave (CW) NMR spectrometer
- (b) Fourier transform (FT) NMR spectrometer
- (a) Sweep (CW) NMR spectrometer.

The following points may be noted: -

- In the continuous wave (CW) spectrometers the spectra can be recorded either with field sweep or frequency sweep.
- Keeping the frequency constant, while the magnetic field is varied (swept) is technically easier than holding the magnetic field constant and varying the frequency.
- The sample (0.5 mg) is dissolved in a solvent like CDCl_3 (0.5 ml, deuterated chloroform) or CCl_2 with no protons to enable only the protons of the sample to be observed.
- The solution is placed in a long thin glass tube which is placed between the poles of a magnet (fig.). The sample tube is rotated about its long axis to average the molecules with respect to their position in the magnetic field. This increases the resolution of the spectrum.
- When the magnetic field is varied, and when it reaches the correct strength for a given proton (or a given set of protons). The nuclei absorb energy and resonance occurs. This absorption causes a tiny electrical current to flow in the receiver coil surrounding the sample and displays it as a signal (a peak or series of peaks).

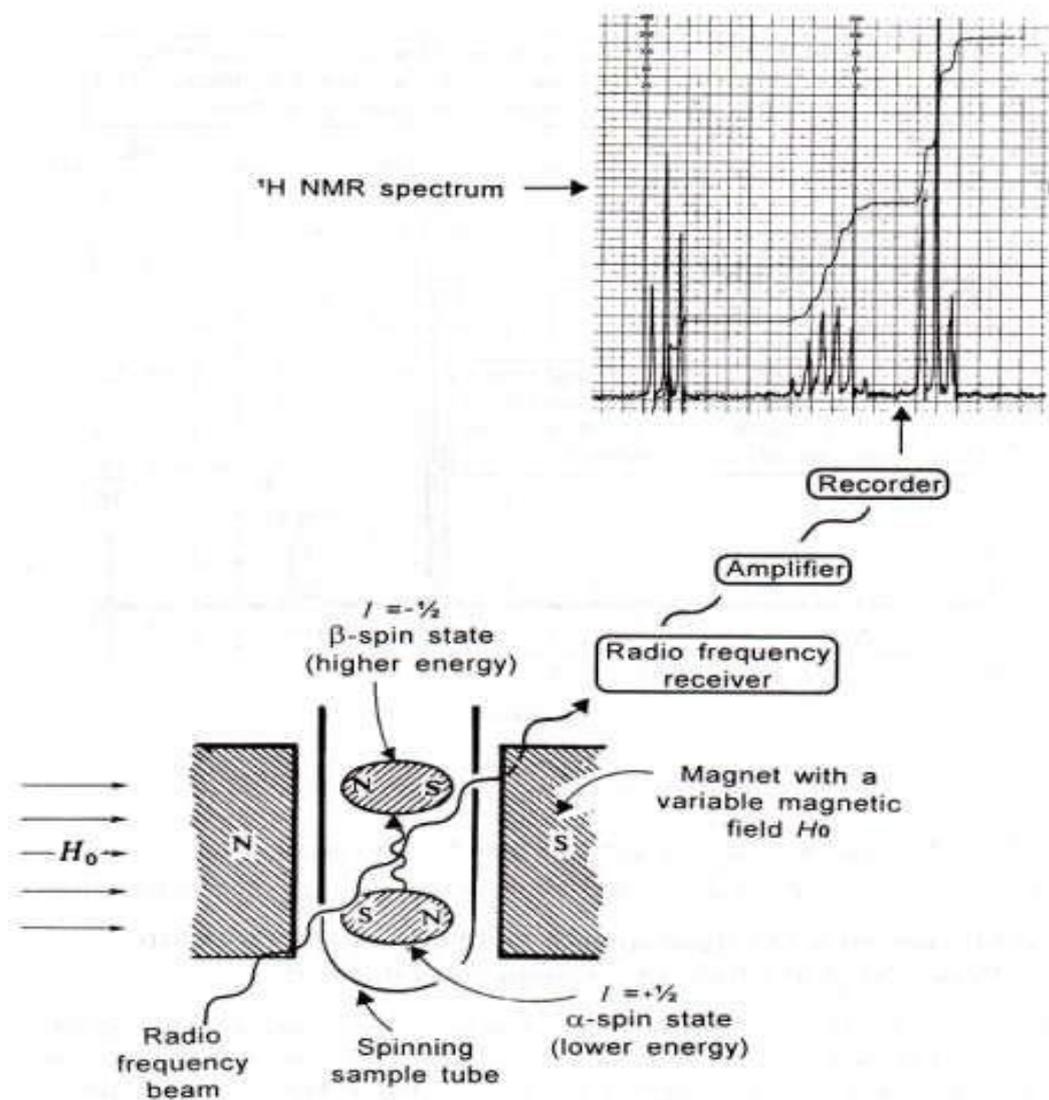


Figure : 1.10

(b) Fourier transform (FT) NMR spectrometer.

One has learnt that in the CW spectrometers H_0 could be held constant, and ν varied, or ν could be kept constant and H_0 changed. The latter option is more common. The modern Fourier transfer (FT) spectrometers operate with a pulse technique when a rf pulse of short duration excites all the nuclei simultaneously and all the signals are collected at the same time with a computer. The individual method allows several hundred runs to be collected within seconds. The data are mathematically converted (a Fourier transform) to a spectrum.

The advantages of FT NMR spectra over those obtained by CW (continuous wave) spectrometer are many and these are:

- FT-NMR is much faster (seconds instead of minutes) and more sensitive.
- FT-NMR can be obtained with less than 5 mg of compound.
- The signals stand out clearly with almost no electronic background noise.

FT NMR spectrum looks like a spectrum obtained from a CW spectrometer. The higher the operating frequency e.g., 300 MHz (compared with 60 MHz) better the resolution, and thus easier the interpretation. However, spectrometers operating on e.g., 300 MHz are very expensive and are used to get the spectra of compounds which are difficult to interpret when taken at lower operating frequencies.

APPLICATION OF NMR

Medicine

The use of nuclear magnetic resonance best known to the general public is in magnetic resonance imaging for medical diagnosis, however, it is also widely used in chemical studies, notably in NMR spectroscopy such as proton NMR and carbon-13 NMR. Biochemical information can also be obtained from living tissue (e.g human brain tumours) with the technique known as in vivo magnetic resonance spectroscopy.

Chemistry

By studying the peaks of nuclear magnetic resonance spectra, skilled chemists can determine the structure of many compounds. It can be a very selective technique, distinguishing among many atoms within a molecule or collection of molecules of the same type but which differ only in terms of their local chemical environment.

By studying T_2^* information a chemist can determine the identity of a compound by comparing the observed nuclear precession frequencies to known frequencies. Further structural data can be elucidated by observing *spin-spin coupling*, a process by which the precession frequency of a nucleus can be influenced by the magnetization transfer from nearby nuclei. Spin-spin coupling is most commonly observed in NMR involving common isotopes, such as Hydrogen-1 (HNMR).

T_2 information can give information about dynamics and molecular motion.

Because the nuclear magnetic resonance *timescale* is rather slow, compared to other spectroscopic methods, changing the temperature of a T_2^* experiment can also give information about fast reactions, such as the Cope rearrangement or about structural dynamics, such as ring-flipping in cyclohexane. At low enough temperatures, a distinction can be made between the axial and equatorial hydrogens in cyclohexane.

Non-destructive testing

Nuclear magnetic resonance is extremely useful for analyzing samples non-destructively. Radio waves and static magnetic fields easily penetrate many types of matter and anything that is not inherently ferromagnetic. For example, various expensive biological samples, such as nucleic acids, including RNA and DNA, or proteins, can be studied using nuclear magnetic resonance for weeks or months before using destructive biochemical experiments. This also makes nuclear magnetic resonance a good choice for analyzing dangerous samples.

Data acquisition in the petroleum industry

Another use for nuclear magnetic resonance is data acquisition in the petroleum industry for petroleum and natural gas exploration and recovery. A borehole is drilled into rock and sedimentary strata into which nuclear magnetic resonance logging equipment is lowered. Nuclear magnetic resonance analysis of these boreholes is used to measure rock porosity, estimate permeability from pore size distribution and identify pore fluids (water, oil and gas). These instruments are typically low field NMR spectrometers.

Process control

NMR has now entered the arena of real-time process control and process optimization in oil refineries and petrochemical plants. Two different types of NMR analysis are utilized to provide real time analysis of feeds and products in order to control and optimize unit operations. Time-domain NMR (TD-NMR) spectrometers operating at low field (2-20 MHz for ^1H) yield free induction decay data that can be used to determine absolute hydrogen content values, rheological information, and component composition. These spectrometers are used in mining, polymer production, cosmetics and food manufacturing as well as coal analysis. High resolution FT-NMR spectrometers operating in the 60 MHz range with shielded permanent magnet systems yield high resolution ^1H NMR spectra of refinery and petrochemical streams. The variation observed in these spectra with changing physical and chemical properties is modelled utilizing chemometrics to yield predictions on unknown samples. The prediction results are provided to control systems via analogue or digital outputs from the spectrometer.

Earth's field NMR

In the Earth's magnetic field, NMR frequencies are in the audio frequency range. EFNMR is typically stimulated by applying a relatively strong dc magnetic field pulse to the sample and, following the pulse, analysing the resulting low frequency alternating magnetic field that occurs in

the earth's magnetic field due to free induction decay (FID). These effects are exploited in some types of magnetometers, EFNMR spectrometers, and MRI imagers[[2]]. Their inexpensive portable nature makes these instruments valuable for field use and for teaching.

Magnetometers

Various magnetometers use NMR effects to measure magnetic fields, including proton precession magnetometers (PPM) (also known as proton magnetometers), and Over Hauser magnetometers. See also Earth's field NMR.

TYPES OF NMR SPECTROSCOPY

Two types of NMR spectroscopy in common use today are notably ^1H (proton, i.e., ^1H NMR) and ^{13}C (carbon-13, i.e., ^{13}C NMR).

^1H NMR Spectroscopy

The NMR Spectroscopy studied for the absorption of most abundant natural isotope of Hydrogen, ^1H is called proton magnetic resonance (PMR) Spectroscopy. **Proton NMR** (also **Hydrogen-1 NMR**, or **^1H NMR**) is the application of nuclear magnetic resonance in NMR spectroscopy with respect to hydrogen nuclei within the molecules of a substance, in order to determine the structure of its molecules.

Simple NMR spectra are recorded in solution, and solvent protons must not be allowed to interfere. Therefore a large range of **deuterated** solvents exist especially for NMR such as deuterated **chloroform** CDCl_3 and **deuterated dimethyl sulfoxide** $(\text{CD}_3)_2\text{SO}$. These solvents contain small quantities of undeuterated solvent which may give rise to a signal; CHCl_3 is seen as a single peak at 7.27 ppm. Water may be present as a contaminant; this gives a broad peak whose chemical shift varies greatly with solvent; it occurs around 1.6 ppm in CDCl_3 . Spectra are usually recorded against tetramethyl silane as the internal standard, set as zero.

Proton NMR spectra are characterized by chemical shifts in the range +12 to -4 ppm and by spin-spin coupling between protons. The integration curve for each proton reflects the abundance of the individual protons.

Simple molecules have simple spectra. The spectrum of **ethyl chloride** consists of a triplet at 1.5 ppm and a quartet at 3.5 ppm in a 3:2 ratio. The spectrum of **benzene** consists of a single peak at 7.2 ppm due to the diamagnetic ring current.

Together with Carbon-13 NMR proton NMR is a powerful tool in structure elucidation in **chemistry**.

¹³C NMR Spectroscopy

In organic chemistry the most significant magnetic nucleus other than the proton is ¹³C which has a net nuclear spin equal to half. It has a low natural abundance (1.11%) and is inherently less sensitive than the proton because of its lower magnetogyric ratio (γ).

The first NMR observations regarding ¹³C nuclei were reported in 1957. These experiments concluded that the direct observation of carbon nuclei had greater utility over the equivalent proton studies. However, the extreme difficulty in conducting the experiments together with the poor spectral resolution and the requirement of working with highly soluble substances with low molecular weights severely restricted the early applications of ¹³C-NMR. The first great success of experimental ¹³C-NMR was achieved as early as 1965 but the advancement of ¹³C-NMR spectroscopy to the status of practical analytical research tool for organic chemists became available only after 1970 and now days ¹³C-NMR is used routinely to complement PMR spectroscopy.

¹³C NMR is particularly Important in the analysis of large, biochemically significant molecules since the ¹³C-NMR spectra can be much simpler than the corresponding proton spectra.

It is useful to compare and contrast H-NMR and C-NMR as there are certain differences and similarities:

- ¹³C has only about 1.1% natural abundance (of carbon atoms)
- ¹²C does not exhibit NMR behaviour (I=0)
- ¹³C nucleus is also a spin 1/2 nucleus
- ¹³C nucleus is about 400 times less sensitive than H nucleus to the NMR phenomena
- Due to the low abundance, we do not usually see ¹³C-¹³C coupling
- Chemical shift range is normally 0 to 220 ppm
- Chemical shifts are also measured with respect to tetramethylsilane, (CH₃)₄Si (*i.e.* TMS)
- Similar factors affect the chemical shifts in ¹³C as seen for H-NMR
- Long relaxation times (excited state to ground state) mean no integrations
- "Normal" ¹³C spectra are "broadband, proton decoupled" so the peaks show as single lines
- Number of peaks indicates the number of types of C

The general implications of these points are that ¹³C-NMR spectra take longer to acquire than H-NMR, though they tend to look simpler. Accidental overlap of peaks is much less common than for H-NMR which makes it easier to determine how many types of C are present.

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