Nuclear Magnetic Resonance Spectroscopy Applications: A Review

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Abstract

Today, NMR has become a sophisticated and powerful analytical technology that has found a variety of applications in many disciplines of scientific research, medicine, and various industries[Cintil *et al,2017*]. NMR spectroscopy is the use of NMR phenomena to study the physical, chemical, and biological properties of matter. Chemists use it to determine molecular identity and structure. Medical practitioners employ magnetic resonance imaging (MRI), a multidimensional NMR imaging technique, for diagnostic purposes[Raghvendra *et al*, 2017].

Introduction

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique used in quality control and reserach for determining the content and purity of a sample as well as its molecular structure. For example, NMR can quantitatively analyze mixtures containing known compounds. For unknown compounds, NMR can either be used to match against spectral libraries or to infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as studying physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion [Ignác *et al.2017*]. 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). 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. 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 precise resonant frequency of the energy transition is dependent on the effective magnetic field at the nucleus. This field is affected by electron shielding which is in turn dependent on the chemical environment. As a result, information about the nucleus' chemical environment can be derived from its resonant frequency. In general, the more electronegative the nucleus is, the higher the resonant frequency. Other factors such as ring currents (anisotropy) and bond strain affect the frequency shift. It is customary to adopt tetramethylsilane (TMS) as the proton reference frequency. This is because the precise resonant frequency shift of each nucleus depends on the magnetic field used[Bryan *et al.,2016]*. The frequency is not easy to remember (for example, the frequency of benzene might be 400.132869 MHz) so it was decided to define chemical shift as follows to yield a more convenient number such as 7.17 ppm.

$$\delta = (\nu - \nu_0)/\nu_0$$

The chemical shift, using this equation, is not dependent on the magnetic field and it is convenient to express it in ppm where (for proton) TMS is set to v_0 thereby giving it a chemical shift of zero. For other nuclei, v_0 is defined as Xsi v_{TMS}, is the frequency ratio of the nucleus (*e. g.*, 25.145020% for ^{13}C). [C.J.L. Silwood *et al.*, 2002]

In the case of the ^{1}H NMR spectrum of ethyl benzene, the methyl (CH_3) group is the most electron withdrawing (electronegative) and therefore resonates at the lowest chemical shift. The aromatic phenyl group is the most electron donating (electropositive) so has the highest chemical shift. The methylene (CH_2) falls somewhere in the middle. However, if the chemical shift of the aromatics were due to electropositivity alone, then they would resonate between four and five ppm. The increased chemical shift is due to the delocalized ring current of the phenyl group.

In order to achieve the desired results, a variety of NMR techniques are available. The basics of NMR are described here. NMR is the branch of spectroscopy in which radio frequency wave induce transitions between magnetic energy levels of a nuclei of a molecule. The magnetic energy levels are created by keeping the nuclei in a magnetic field.

Without the magnetic field the spin states of nuclei are degenerate i.e. possess the same energy, and energy level transition is not possible. When a magnetic field is applied, the separate levels and radio frequency radiation can cause transitions between these energy levels. [J.C. Lindon *et al.*, 2015]

It is an powerful tool which investigates the nuclear structure. It is an analytical chemistry technique that determines the shape, structure, of a molecule and purity of the given sample. It is an qualitative and quantitative analysis.

It works on the principle of nuclei spin and all nuclei are electrically charged.

Instrumentation

Continuous Wave NMR Instruments

The apparatus consists of the components:-

- Sample Holder
  The chemically inert holder is used to hold the sample which is durable and transparent to RF radiations.
- Permanent Magnet
  The magnet used is permanent or electromagnetic which has homogenous field i.e. doesnot change from point to point and the strength of the field is also high.
- Magnetic Coils
  To overcome the problems regarding to handle the large magnetic field , the small variable magnetic field is superimposed on the main field by using pair of Helmholtz coils in the pole faces of the permanent magnet.
- Sweep Generator
  To bring about the resonance, the frequency of rf field should be changed so that it becomes equal to the resonance frequency.
- Radio Frequency Generator
  Radio frequency oscillator is used to generate the radio frequency radiations.

- Radio Frequency Reciever
  It detects the absorption and dispersion of the radio frequency.
- Read out system
  The weak signal of absorption signal received from radio frequency receiver which is amplified.

Fourier Transform NMR Instruments

The magnitude of the energy changes involved in NMR spectroscopy are small. This means that sensitivity is a major limitation. One way to increase sensitivity would be to record many spectra, and then add them together; because noise is random, it adds as the square root of the number of spectra recorded. For example, if one hundred spectra of a compound were recorded and summed, then the noise would increase by a factor of ten, but the signal would increase in magnitude by a factor of one hundred - giving a large increase in sensitivity. However, if this is done using a continuous wave instrument, the time needed to collect the spectra is very large (one scan takes two to eight minutes).

In FT-NMR, all frequencies in a spectrum are irradiated simultaneously with a radio frequency pulse. Following the pulse, the nuclei return to thermal equilibrium. A time domain emission signal is recorded by the instrument as the nuclei relax. A frequency domain spectrum is obtained by Fourier transformation.

The pulse

If a signal of frequency, F, is turned on and then off again very rapidly, then the result is an output consisting of many frequencies centred about F with a bandwidth of $1/t$, where t is the duration of the pulse. This means that radiation is produced of all frequencies in the range $F \pm 1/t$. If t is very small, then a large range of frequencies will be produced simultaneously, and all target nuclei in a sample will be excited.

Application

Nuclear magnetic resonance has been used in forensics in various ways. Some of the applications are:-

- Nmr studies the metabolic activities of bio fluids (metabonomics)
  It studies the endogenous metabolic processes through various types of bio fluids by using control organisms and abnormal one. The metabonomics is defined as the quantitative measurement of metabolic responses having different parameters of living things.[J.C. Lindon *et al.*, 2015]
- NMR for the determination of purity of drugs(pure and illicit)
  The proton is used for identifying purity of the drug. It can easily detect complex heroin, methamphetamine, MDMA, and cocaine samples.[P. Hays, 2005]
- NMR spectroscopy for determining Intramyocellular lipid content relation with insulin
  Relation between intramuscular lipid content and insulin resistance shown by muscle biopsy by using Proton NMR spectroscopy. The intramyocellular lipid content was independent of BMI, age and fasting. The NMR is good indicator of whole body

insulin sensitivity sensitivity in non-diabetic, non-obese persons. [M. Krssak *et al.*, 1999]

- NMR for analysis of human saliva
  High resolution NMR techniques are used for saliva analysis by making salivary biomolecules index and identifying organic acids and malodorous amines. Therefore it can be concluded that NMR spectroscopy can analyse the multicomponent of human saliva. [C.J.L. Silwood *et al.*, 2002]
- NMR for pharmaceutical research and analysis
  The solid state NMR(SSNMR) spectroscopy is used to study pharmaceutical solids.[R.T. Berendt *et al.*, 2006]
- NMR for poisoning cases
  NMR is used to analyse a xenobiotics and their metabolites in biological fluids. Xenobiotics can be identified and quantitated without any sample preparation. [M. Imbenotte *et al.*, 2003]
- NMR for MDMA in ecstasy tablets
  NMR spectra of MDMA and other illicit tablets or ecstasy has been identified by using carbon isotope NMR spectroscopy. [G. Lee *et al.* 1999]
- NMR for intrinsic signal-to-noise ratio
  The fundamental limit for NMR imaging is set by an intrinsic signal- to- noise ratio (SNR) for a particular combination of rf antenna and imaging subjects. [W. A. *Edelstein et al.1986*]

Conclusion

NMR is widely used these days. Mostly isotopes are used for NMR spectroscopy. NMR can be used in pharmaceuticals and forensic. In forensics it can be used for identification of bio fluids, saliva, drugs for identification purposes. In addition, NMR provides unique and important molecular motional and interaction profiles containing pivotal information on protein function.

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