

What is NMR?

- Nuclear Magnetic Resonance

• Nuclear Magnetic Resonance

You may have heard the term NMR – nuclear magnetic resonance – but how much do you actually know about it? NMR is a valuable analytical technique for the laboratory scientist, while a similar, although not absolutely identical, technique, magnetic resonance imaging (MRI), has become an indispensable medical diagnostics tool.

In modern hospitals, magnetic resonance imaging is a familiar diagnostics tool. Anybody who has undergone an MRI scan will already have seen the amazingly detailed images it can generate of the inside of the body, helping doctors to make an informed diagnosis without the need for invasive – and costly – exploratory surgical procedures, for example arthroscopic examination of the knee. NMR, on the other hand, is probably still something of a mystery to most people outside of the scientific laboratory.

NMR was first described by Isidor Rabi as far back as 1938, and has grown to become a well-established, powerful physical tool for the investigation of matter through the measurement of nuclear magnetic interactions. The benefits of the technique as a probe to study ordinary matter really began to be appreciated in 1946, when Harvard University's Edward Mills Purcell detected the first solid-state NMR signal in 1 kg of paraffin wax. At almost exactly the same time, Felix Bloch

at Stanford University successfully performed the first liquid-state NMR experiment in water; both achievements were later recognized with the joint award of the 1952 Nobel Prize in Physics. Incredibly, no fewer than eight Nobel Laureates from around the globe have been honored in physics, chemistry and medicine for the discovery, development and application of NMR!

Today, a plethora of NMR experimental methods provide access to a wealth of in-depth information about the structure and dynamics of unbelievably complex molecules, and the versatility of the technique has allowed it to be employed in countless applications across diverse scientific fields, among them physics, chemistry, biology, biochemistry, materials science, food, geology, pharmaceutical research and medicine. With NMR methods and associated technologies continuing to be developed in universities and industry, the future possibilities are endless.



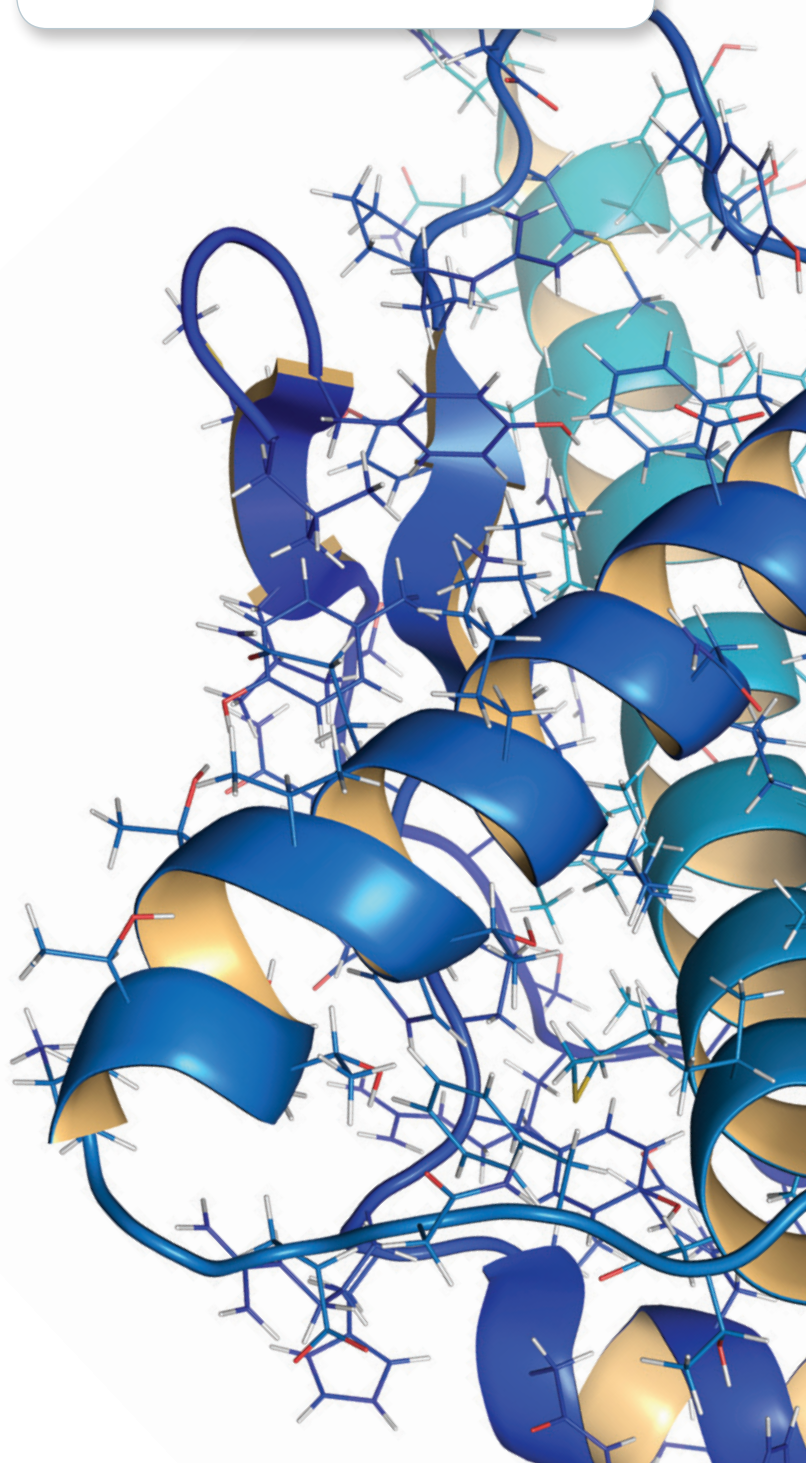
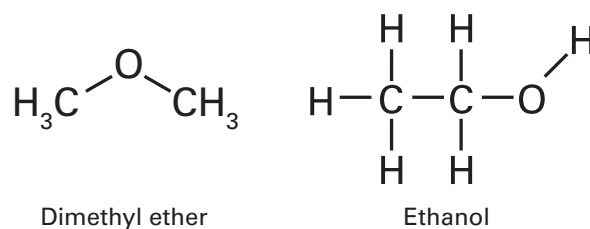
• Why do we need NMR?

Molecules are essentially arrangements of atoms, with varying structures depending on which atoms – and how many – are present and the way in which they are bonded to each other. But why do we want to know about molecular structure?

The structure of a molecule strongly influences a substance's physical and chemical properties and, as a result, how it reacts with other molecules or affects living organisms. For example, ethanol – also known as absolute alcohol – and dimethyl ether are composed of the same type and number of atoms; two carbon, six hydrogen and one oxygen. However, the structures, and therefore the properties, are quite different. Ethanol is a liquid, while dimethyl ether is a poisonous gas!

Ethanol and dimethyl ether clearly demonstrate one reason that chemical structure is so important, but this knowledge is also crucial in the field of organic synthesis to successfully create a synthetic equivalent of an existing substance, or to synthesize novel compounds. An in-depth understanding of molecular structure is particularly important in the chemical and pharmaceutical industries for the production of materials such as nylon and plastic, and for drug discovery. For scientists, NMR is a key tool that provides this vital information.

NMR can also be used to determine the structure of proteins – very large, biologically important molecules that regulate virtually all of the body's functions and the biochemical processes that are essential to life. For example, the protein insulin regulates blood sugar levels; another protein, hemoglobin, carries oxygen in the blood around the body; and antibodies – or immunoglobulins – enable the immune system to identify and neutralize foreign compounds such as bacteria and viruses. Such molecules and the processes associated with them must be studied in detail and a thorough understanding acquired if new medicines or therapies are to be developed. This requires knowledge of not only the molecular structure, but also the dynamics of proteins. NMR is the only analytical method with the capability to provide both structural and dynamic information of biological molecules in their physiological environment with atomic resolution.

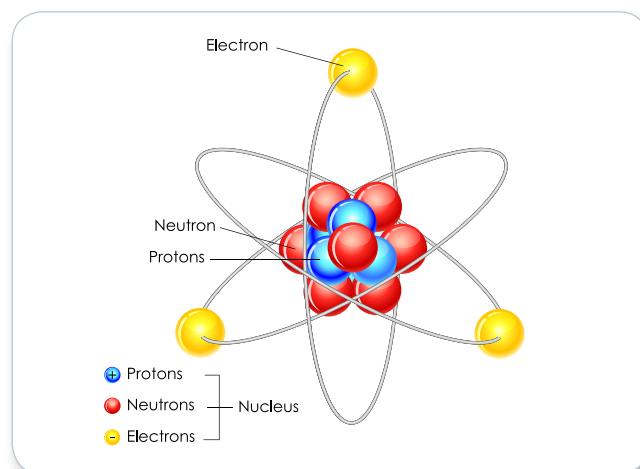


• Atoms, nuclei and molecules

To understand NMR, we need to first consider the relationship between atoms, nuclei and molecules.

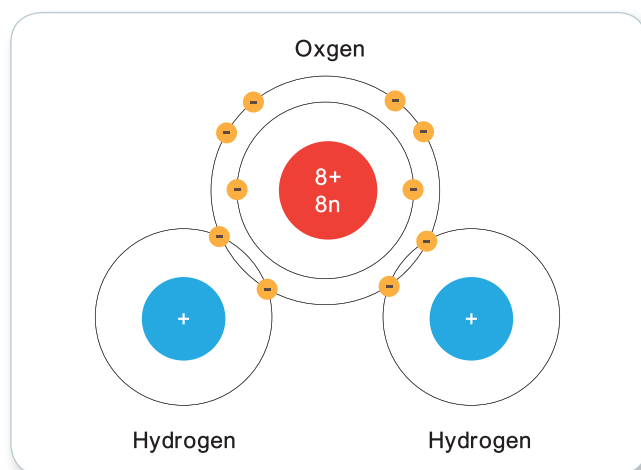
To date, 118 elements have been identified, including hydrogen, carbon and oxygen. Elements are pure chemical substances consisting of a single type of atom distinguished by its atomic number. Although the Ancient Greeks assumed atoms – named after the Greek word for indivisible, atomos – are the smallest units of matter, they can actually be subdivided into even smaller particles. Each atom consists of a positively charged nucleus containing protons (positively charged) and neutrons (uncharged), surrounded by a cloud of negatively charged electrons.

Molecules – collections of atoms bonded together in a unique molecular structure – exist in a wide range of shapes and sizes, from small compounds such as water through to much larger compounds consisting of hundreds of thousands of atoms. In each case, the molecular structure determines the physical and chemical properties of the compound. To gain an idea of scale, consider a molecule of water, a simple compound consisting of two hydrogen atoms bonded to one oxygen atom. A single raindrop contains 4×10^{21} molecules of water. If a water molecule were the size of a football, then a raindrop would be a similar size to the earth. It's a bit like looking for the proverbial needle in a haystack. Molecules, even very large ones, are so minute that they cannot be seen even with the most powerful conventional microscopes; other methods are required to establish molecular structure.



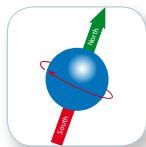
NMR is one of the best-suited methods for determining molecular structure. When a molecule is exposed to an external magnetic field, each atom will feel the effect of a marginally different, modified field, depending on the magnetic shielding effects of the neighboring electric charges – the nuclei and electrons.

This means that the magnetic field experienced by each atom is a local property of the molecule, and is dependent on its geometry. NMR has the capability to measure the response of an atom to the local magnetic field – modern instruments are sensitive to local magnetic field variations as small as one part in a billion – from which information can be determined about the molecular structure; even the structures of very large, complex molecules, such as proteins, can be determined with incredible accuracy. This is particularly important in the field of drug research, where contributions from Professor Kurt Wüthrich from the ETH Zürich were recognized by the award of the 2002 Nobel Prize for Chemistry.

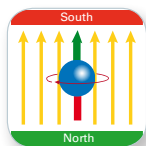


• How does NMR work?

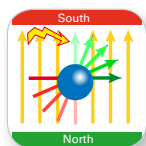
NMR takes advantage of the influence of a strong magnetic field. Many atomic nuclei possess a physical property known as spin. Put simply, this is the rotation of a nucleus about its own axis. Rotating, electrically charged nuclei generate a magnetic field and therefore carry a magnetic moment – a measure of an object's tendency to align with a magnetic field – represented graphically by the green and red arrows. This can be thought of as a tiny bar magnet rotating around a magnetic axis like a spinning top. Similarly to compass needles, the nuclei can be manipulated by means of magnetic fields. Typically, macroscopic magnetization does not occur with an ensemble of atoms, as each of these small 'magnets' is randomly oriented, pointing in completely different directions. This means that the magnetic fields generated cancel each other out. However, nuclear magnetic moments can be aligned by exposure to a sufficiently strong magnetic field. The resulting macroscopic ensemble magnetization is what makes NMR possible.



An NMR experiment involves placing a sample of the substance to be examined in a strong static magnetic field. This forces the nuclear magnetic moments to align parallel to the applied field – represented by the yellow arrows – acquiring macroscopic magnetization. The sample is then said to be polarized, or magnetized. While the magnetic fields in modern NMR spectrometers can be as large as 24 Tesla, the total fraction of aligned spins is of the order of just one in 10,000, and these few nuclei must provide sufficient information about the molecules under investigation. However, as the nuclear magnetic moments are very weak, they cause virtually no interference with the molecular degrees of freedom. The significance of this is that the molecular structure is not altered by NMR spectroscopy.

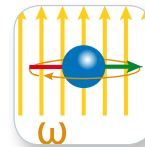


It is a remarkable physics fact that a broad range of different resonance frequencies can all be carried by a single radio wave pulse, which can be as short as a microsecond; this is the concept behind Fourier transform NMR (FT-NMR). The sample is irradiated with a short electromagnetic pulse, characterized by a broad spectrum of well-defined radio waves, or resonance frequencies. This has the effect of forcing the nuclear magnetic moments from their equilibrium position parallel to the magnetic field and into a perpendicular plane.

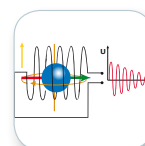


Each of the various nuclei in a molecule will respond to a specific resonance frequency, and this provides information about the type of atoms present in the molecule and their positions.

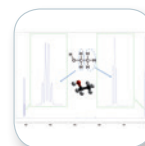
The deflected nuclear magnetic moments start a rotational motion, called a precession, around the static magnetic field (yellow arrows). The frequency of this precession, ω , depends mainly on the strength of the magnetic field and the type of atom and, to a very small but detectable degree, the atom's position in the molecule. The characteristic precession frequencies of the nuclei in a molecule contain valuable information that can be used to deduce the structure of the molecule itself. A means of measuring these frequencies is required: the NMR spectrometer.



In an NMR spectrometer, the sample is placed in a small coil of wire. The rotating (precessing) magnetic moments induce an alternating voltage in the coil which can be likened to a bicycle dynamo; the NMR signal. This NMR signal is often the overlay of many oscillating electric signals, with the frequency of each oscillating signal corresponding to the precession frequency of the magnetic moment that generates it. As the magnetic moments spontaneously and progressively return to their equilibrium state parallel to the vertical axis, the amplitude of the observed voltage decays. The tiny initial sample magnetization means that the NMR signal is extremely weak; by analogy, if the power of the input radio frequency used to stimulate the nuclei was the size of Mount Everest, the output signal measured in an NMR experiment would be 10,000 times smaller than the diameter of a human hair. Extremely sophisticated electronics are required to detect the signal.



Finally, Fourier transform – a mathematical manipulation which can unravel all the individual frequencies – allows the NMR signal to be displayed as an NMR spectrum, where the characteristic frequencies of the precessing nuclear magnetic moments are shown as distinct peaks known as resonance lines. For example, the NMR spectrum of ethanol shows two distinct families of peaks. Their positions, or frequencies, are determined by the atomic structure of the molecule, enabling the molecular structure of ethanol to be determined.



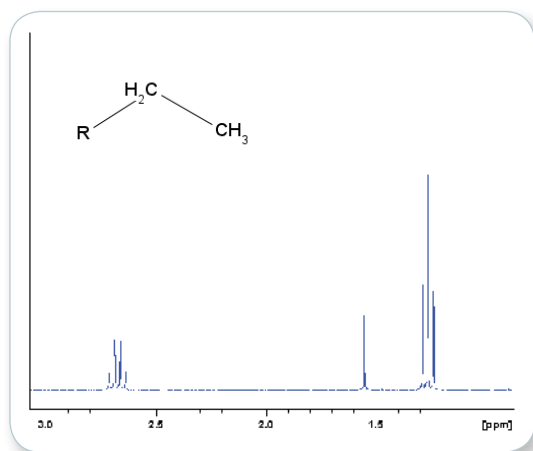
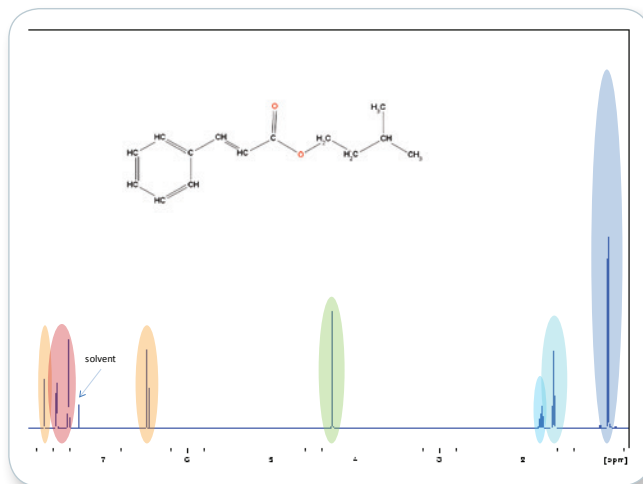
• The NMR spectrum: Chemical shift and spin-spin coupling

Shift

In addition to the field of the magnet, the exact resonance frequency of individual atoms is affected by the chemical environment. These frequency differences are generally in the range of parts per million of the basic resonance frequency of any given nucleus. These differences are also depending on the strength of the magnetic field. To allow comparison of spectra from different instruments a frequency independent scale the Chemical Shift has been defined. It is expressed in parts per million (ppm), according to the equation:

$$\delta \text{ (ppm)} = (\text{frequency of signal (Hz)} - \text{frequency of reference (Hz)}) / \text{frequency of reference (MHz)}$$

Compounds used as chemical shift references have been defined for all NMR active nuclei. For example the chemical shift of tetramethylsilane is assigned to zero ppm in ^1H and ^{13}C spectra.



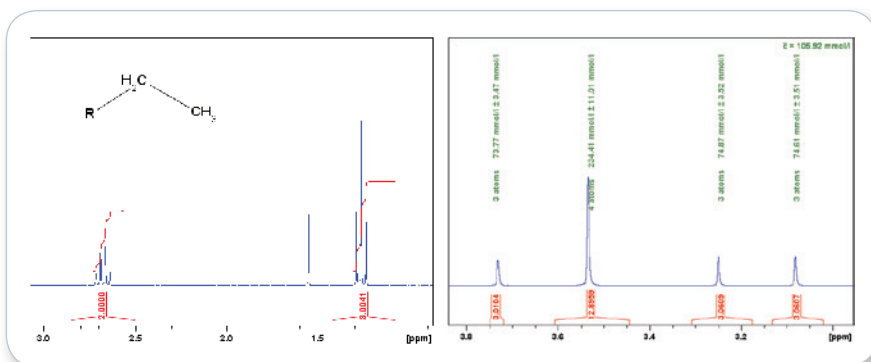
Coupling

As well as interacting with the magnetic field, nuclei interact with each other through the chemical bonds of the molecule, generating the fine structure in the NMR signal. This is known as coupling, and is extremely useful for characterizing neighboring atoms in a molecule. Each spin can be affected by spins a few bonds away according to the general rule $2nI+1$, where n is the number of spins and I is the spin quantum number. For spin = $\frac{1}{2}$ nuclei such as protons this is simplified to $n+1$. One neighboring spin leads to a splitting of the resonance into two lines, two spins result in three lines etc. The intensity of the lines follows the coefficients of Pascal's triangle.

Quantitation

NMR is a primary ratio method of analysis. The area of each signal is directly proportional to the number of atoms responsible for said signal. Signal areas are determined by integration or deconvolution of signals.

Quantitation of different components in a mixture can also be obtained with internal or external references allowing the direct determination of concentrations.



● NMR instrumentation

Early NMR experiments were carried out using a technique known as continuous wave (CW). A constant magnetic field was maintained, and the radio signal swept through the appropriate frequency range. This sequentially brought each nucleus into resonance. As the experiment progressed, the spectrum was drawn on a chart recorder in real time. This meant that resolution could only be improved by slowing down the frequency sweep, and the corresponding pen movement.

In the 1970s, continuous wave was replaced by Fourier transform NMR, described above. FTNMR – discovered by Professor Richard Ernst from the ETH Zürich, who received the Nobel Prize for Chemistry in 1991 for his efforts – resulted in a considerably faster and more sensitive technique on which hundreds of NMR experiments have since been based.

FT-NMR offers the advantages of:

- Signal averaging; repeated measurements to improve signal-to-noise ratios
- Resolution; the free induction decay (FID) – a composite of all the NMR signals – can be sampled with a large number of points to ensure the fine structure is resolved
- Versatility; FT spectroscopy is not limited to single pulse experiments, enabling manipulation of the various spins in a given molecule.

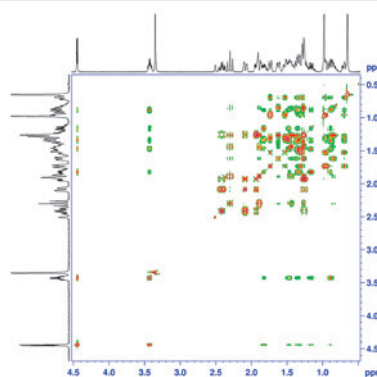
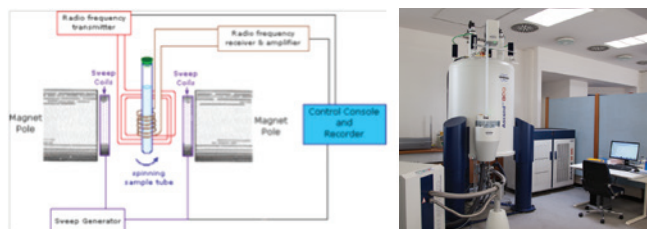
The most essential component of an NMR spectrometer is the magnet, which generates the field in which a sample's nuclear magnetic moments are aligned and magnetization created. Only a very small fraction of the nuclear spins in a sample can be effectively aligned to the magnetic field, which limits the degree of magnetization that can be achieved; as magnetization is approximately proportional to the intensity of the magnetic field, it is crucial to apply the strongest possible field.

Today's NMR spectrometers use superconducting magnets capable of producing magnetic fields of up to 24 Tesla – almost a million times greater than the magnetic

field of the Earth. These magnets are immersed in a cryostat-controlled liquid helium bath, operating at temperatures in the region of 2 to 4 K, which is about -270 °C. A system of coils built into the spectrometer's probe acts as a physical interface between the sample and the magnet, and generates radio wave pulses. The sample, either liquid or solid, is placed into the probe, where it is at the center of the radio wave coils and the electronics required for signal detection. The electronics of modern probes – known as cryoprobes – allow cooling to between 10 and 20 K using an external cooling unit. This reduces electronic noise, helping to increase sensitivity and enable the detection of small signals that would otherwise be invisible. The spectrometer also contains a pre-amplifier to boost the initial signal, as well as a control console that houses the sophisticated electronics required to generate the radio wave pulses and detect the NMR signal. Data acquisition and processing are software controlled.

Developing new experimental opportunities

FT-NMR has opened the door to exciting new experimental opportunities. FT-NMR is considerably faster and more sensitive than its predecessor, continuous wave, and hundreds of NMR experiments have been developed based on this technique. One particularly powerful development is two dimensional (2D) NMR, which allows the structure of even large molecules, with perhaps thousands of atoms, to be resolved, typically with acquisition times between a few minutes and a few hours, depending on the instrumentation and quality required. One example is 2D NMR of a steroid hormone, a complex molecule with many strongly overlapping resonance lines, where the technique has proved very effective.



2D NMR spectrum of approximately 200 micrograms of a steroid hormone

Summary

This article outlines the theory behind NMR spectroscopy, providing background knowledge for scientists wishing to learn about the technique. The powerful NMR technology has been applied to many different applications, among them chemical analysis, structural biology, drug development and materials science, as well as medicine, and has proved invaluable in the laboratory, while its clinical counterpart, MRI, has become indispensable in the hospital environment. Interest in NMR has never been greater.

