

Unit 2: NMR Spectroscopy

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2.1 Principle and quantum numbers

Nuclear magnetic resonance spectroscopy studies nuclei that possess spin. A nucleus with spin behaves like a tiny magnet with a magnetic moment. Whether a nucleus has spin is decided by its spin quantum number I :

- Nuclei with both mass number and atomic number even, such as carbon-12 and oxygen-16, have $I = 0$ and are NMR inactive.
- Nuclei with odd mass number, such as hydrogen-1, carbon-13, fluorine-19 and phosphorus-31, have half integral spin ($I = 1/2$ for the common ones) and are NMR active.
- Nuclei with even mass number but odd atomic number, such as hydrogen-2 and nitrogen-14, have integral spin ($I = 1$).

Proton NMR (^1H NMR) is the workhorse because hydrogen-1 is present in almost every drug, has $I = 1/2$, high natural abundance and the highest sensitivity of common nuclei.

When a spinning nucleus is placed in an external magnetic field B_0 , it can align with the field (lower energy, alpha state) or against the field (higher energy, beta state). The nucleus precesses around the field axis at the **Larmor frequency**, which is directly proportional to the field strength and to the magnetogyric ratio of the nucleus. Resonance, the absorption of radiofrequency energy that flips the nucleus from alpha to beta, happens when the applied radiofrequency exactly equals the Larmor frequency. The tiny excess of nuclei in the lower state (only a few per million at ordinary field strengths) is what produces the signal, which is why NMR is an inherently insensitive technique compared with UV or mass spectroscopy.

2.2 Instrumentation of NMR spectrometer

The main parts are:

NMR Spectrometer

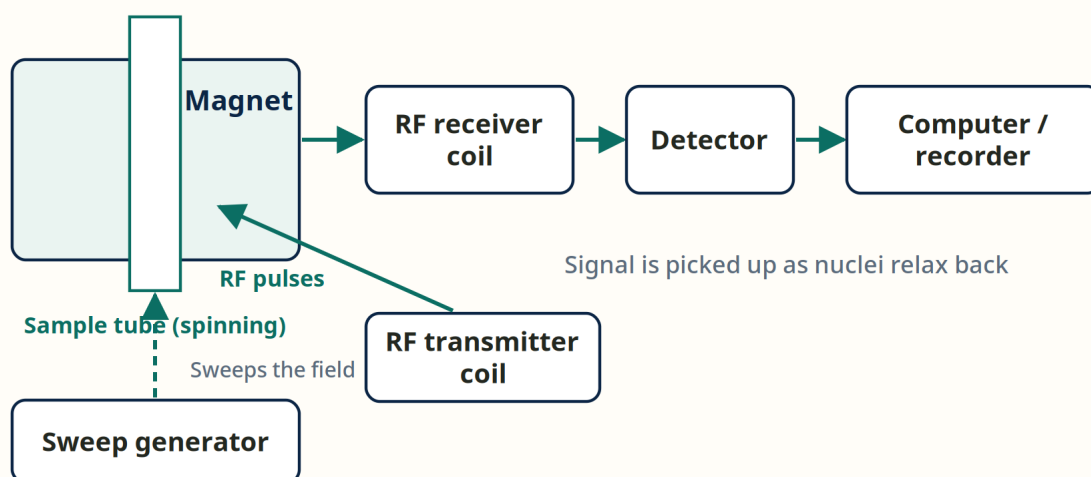


Figure: Main parts of an NMR spectrometer.

- **Magnet:** must give a very strong, perfectly homogeneous and stable field. Modern instruments use a superconducting magnet (niobium alloy coil cooled in liquid helium, with liquid nitrogen as an outer jacket) giving fields described by their proton frequency, for example 400 MHz or 600 MHz. Older instruments used permanent or electromagnets.
- **Radiofrequency transmitter:** generates the exact frequency needed to irradiate the sample, applied through a coil around the sample tube.
- **Sample holder:** a thin glass tube, usually 5 mm diameter, which is spun in the probe to average out field inhomogeneity in continuous wave instruments.
- **Detector and receiver coil:** picks up the weak signal emitted as nuclei relax back.
- **Sweep generator and recorder or computer:** in continuous wave (CW) NMR either the field or the frequency is swept slowly through the resonance conditions and a pen recorder draws the spectrum. In pulsed Fourier transform (FT) NMR all nuclei are excited together by a short powerful radiofrequency pulse, the free induction decay (FID) signal is collected, and a Fourier transform converts it into the normal frequency spectrum. FT NMR is the standard today because it is fast and allows signal averaging for dilute samples and for insensitive nuclei like carbon-13.
- **Internal standard:** tetramethylsilane (TMS) is added in a small amount. Its 12 equivalent protons give one sharp signal, it is chemically inert, miscible and volatile, and its signal appears at the extreme right of the spectrum where few other signals fall. Its position is defined as $\delta = 0$ ppm.

2.3 Solvent requirement in NMR

The solvent must dissolve the sample, must not exchange protons with the solute in a confusing way, and above all must not give signals of its own in the proton spectrum. Therefore solvents without protons, such as carbon tetrachloride and carbon disulphide, or fully deuterated solvents are used: deuterated chloroform (CDCl_3 , the most common), deuterated DMSO (DMSO-d_6 , good for polar drugs and shows exchangeable OH and NH protons), deuterated methanol and heavy water (D_2O). In D_2O the OH, NH, SH and COOH protons exchange with deuterium and their signals disappear; this D_2O exchange or D_2O shake is a standard test to identify exchangeable protons in a spectrum.

2.4 Relaxation process

After the radiofrequency pulse, excited nuclei must return to equilibrium before the next pulse, and the ways they lose energy are called relaxation:

- **Spin lattice relaxation (longitudinal, T₁):** the excess energy is transferred to the surrounding lattice (solvent and other molecules) as heat. It decides how quickly the experiment can be repeated.
- **Spin spin relaxation (transverse, T₂):** nuclei exchange energy among themselves, which does not change total energy but causes the signals to lose phase coherence. It decides the width of the lines: short T₂ means broad lines.

Solids and viscous samples relax by T₂ very fast and give broad lines, which is why routine NMR is done in free flowing solutions. Paramagnetic substances such as dissolved oxygen or metal ions shorten relaxation times drastically and broaden signals, so samples are sometimes degassed.

2.5 NMR signals: number, position, area and splitting

A proton NMR spectrum gives four kinds of information, and exams test all four:

- 1 **Number of signals** tells how many sets of chemically equivalent protons the molecule has. Protons in identical chemical environments are equivalent and absorb at the same position.
- 2 **Chemical shift (position of the signal)** tells the electronic environment of those protons (Section 2.6).
- 3 **Integration (area under the signal)** is proportional to the number of protons in that set. The integrator trace rises in steps whose heights are in the ratio of the proton counts.
- 4 **Splitting or multiplicity** tells how many protons sit on neighbouring atoms (Section 2.7).

2.6 Chemical shift

The electrons around a nucleus shield it from the external field, so the field actually felt is slightly less than B₀. Different environments shield to different extents, so signals appear at different positions. Chemical shift is expressed on the delta scale in parts per million (ppm):

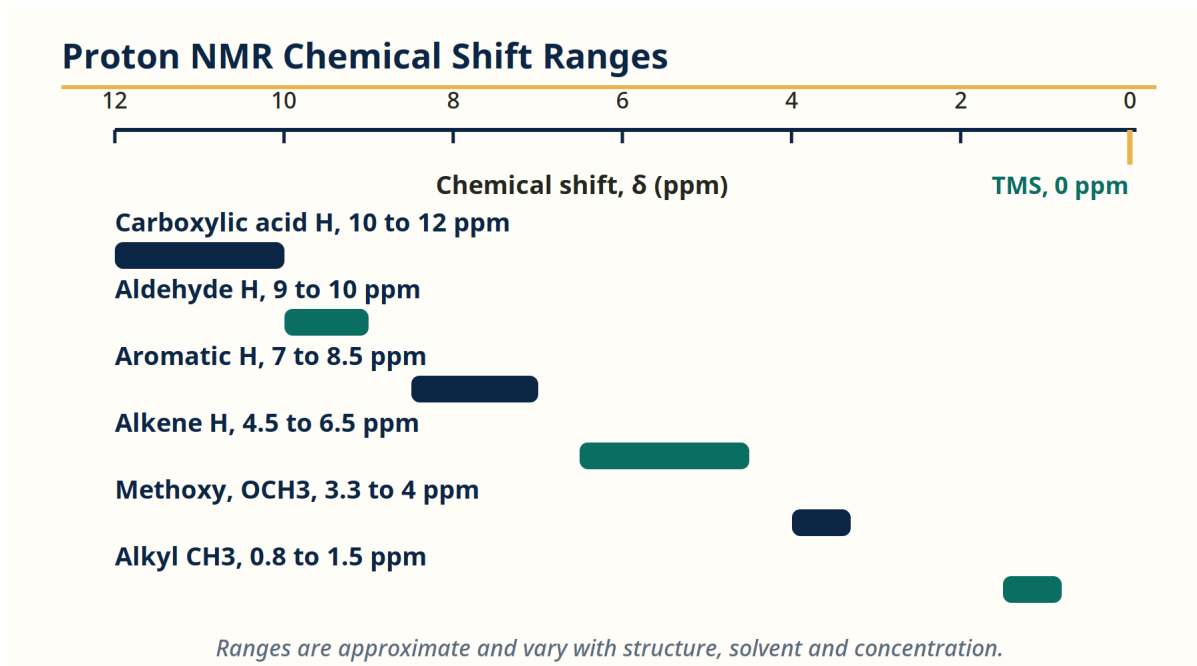


Figure: Approximate proton chemical shift ranges, TMS is the zero reference.

$\delta = (\text{frequency of sample signal minus frequency of TMS signal, in Hz}) \div (\text{operating frequency of the spectrometer, in Hz}) \times 10^6$

Expressing shift in ppm makes it independent of the instrument: a proton at delta 7.3 on a 400 MHz instrument is also at delta 7.3 on a 600 MHz instrument, only its distance in Hz changes. Movement to higher delta (left, downfield) is called deshielding, movement to lower delta (right, upfield) is called shielding.

Approximate proton chemical shift regions worth memorising:

Proton type	Typical delta (ppm)
Alkyl, CH ₃ and CH ₂ away from other groups	0.9 to 1.5
Allylic and benzylic protons	1.7 to 2.6
CH ₃ or CH ₂ attached to carbonyl (acetyl type)	2.0 to 2.5
Protons on carbon bearing O or N (OCH ₃ , OCH ₂ , NCH ₃)	3.3 to 4.0
Olefinic (alkene) protons	4.5 to 6.5
Aromatic protons	6.5 to 8.5
Aldehyde proton	9.5 to 10.0
Carboxylic acid proton	10.5 to 12.0
Alcohol and phenol OH, amine NH	Variable, about 0.5 to 5.5, concentration and solvent dependent

2.7 Factors influencing chemical shift

- **Electronegativity of the attached group:** an electronegative atom such as oxygen or chlorine pulls electrons away, deshields the proton and moves it downfield. Compare CH₃F (4.26), CH₃Cl (3.05), CH₃Br (2.68) and CH₃I (2.16 ppm), which follows the electronegativity order exactly.
- **Hybridisation:** sp² protons (alkene, aromatic, aldehyde) appear far downfield of sp³ protons; sp protons of terminal alkynes appear near 2 to 3 ppm because of a special anisotropy effect.
- **Anisotropy (neighbour anisotropic effect):** the pi electrons of double bonds, triple bonds, carbonyls and aromatic rings circulate under the field and create local fields that deshield protons in some zones and shield them in others. This explains the very downfield aldehyde proton (about 9.7 ppm), aromatic protons (about 7.3 ppm) and the unusually upfield alkyne proton.
- **Hydrogen bonding:** bonded OH and NH move downfield, and the shift increases with concentration and decreases on dilution or on heating, which distinguishes intermolecular from intramolecular hydrogen bonding.
- **Van der Waals deshielding:** in crowded molecules, a proton forced close to another bulky group is deshielded by electron cloud repulsion.
- **Solvent and temperature:** aromatic solvents such as benzene induce shifts, and exchangeable protons are the most sensitive to temperature.

2.8 Spin-spin coupling and coupling constant

A signal is split into a multiplet when the protons of that set have non equivalent protons on an adjacent atom. The neighbouring protons can align with or against the field, so the effective field at the observed nucleus takes several slightly different values. The rule is:

Multiplicity = (n + 1), where n is the number of equivalent neighbouring protons.

One neighbour gives a doublet, two give a triplet, three give a quartet. The relative intensities follow Pascal triangle: a triplet is 1:2:1 and a quartet is 1:3:3:1. Protons of the same set do not split each other, and in

most drug like molecules coupling is significant only up to three bonds (geminal 2J on the same carbon when its protons are non equivalent, vicinal 3J on adjacent carbons, and long range 4J which is usually small, as in allylic or aromatic meta coupling).

The **coupling constant J** is the distance in Hz between the lines of a multiplet. Unlike chemical shift in Hz, J is independent of the operating frequency and of field strength, which helps in confirming that two multiplets are coupled to each other: partners share the same J value. Typical values: geminal about 12 to 15 Hz in magnitude, vicinal about 6 to 8 Hz for freely rotating chains (and dependent on dihedral angle by the Karplus relationship, large near 0 and 180 degrees, small near 90 degrees), cis alkene about 10 Hz, trans alkene about 15 to 18 Hz, ortho aromatic about 8 Hz, meta about 2 Hz and para about 0 to 1 Hz.

2.9 Nuclear magnetic double resonance and related techniques

In **double resonance (spin decoupling)**, a second strong radiofrequency is applied at the resonance frequency of one nucleus while the spectrum is recorded. The irradiated protons are saturated and their coupling disappears from the spectrum, so a triplet may collapse to a singlet. This identifies which signals are coupled to which, and simplifies crowded spectra. The nuclear Overhauser effect (NOE), produced in a related double resonance experiment, increases the intensity of signals of nuclei that are close in space, and is used to establish stereochemistry and three dimensional closeness rather than through bond connection.

2.10 FT-NMR and ¹³C NMR: brief principles

FT-NMR: a short pulse containing all frequencies excites every nucleus of the chosen type at once. The resulting time domain signal, the FID, is the sum of decaying sine waves, and Fourier transformation converts it into the frequency domain spectrum. Advantages: the whole spectrum is obtained in seconds, many scans can be added to improve signal to noise (essential for dilute samples and weak nuclei), and quantitative and multi pulse experiments become possible.

¹³C NMR: carbon-13 has $I = 1/2$ but only about 1.1 percent natural abundance and a lower magnetogyric ratio, so it is roughly 6000 times less sensitive than proton NMR and needs FT operation with signal averaging. Its chemical shift range is wide, about 0 to 220 ppm, so overlap is rare and each chemically different carbon usually gives one line. In the routine broadband proton decoupled spectrum every carbon signal is a singlet, which makes counting carbons simple. Carbon types are located approximately as: alkyl carbons 5 to 45 ppm, carbons bearing O or N 50 to 90 ppm, alkene and aromatic carbons 100 to 150 ppm, and carbonyl carbons 160 to 220 ppm (esters and acids near 165 to 180, ketones near 195 to 220). Off resonance decoupling and DEPT experiments tell whether a carbon is CH₃, CH₂, CH or quaternary.

2.11 Applications of NMR spectroscopy

Complete structure elucidation of new drugs and impurities (with IR and mass data), confirmation of identity and purity, distinguishing isomers and tautomeric forms, quantitative NMR (qNMR) using an internal standard without needing a reference standard of the analyte itself, study of drug receptor and drug protein binding, determination of residual solvents in bulk drugs, and checking the ratio of components in a mixture from integration values.