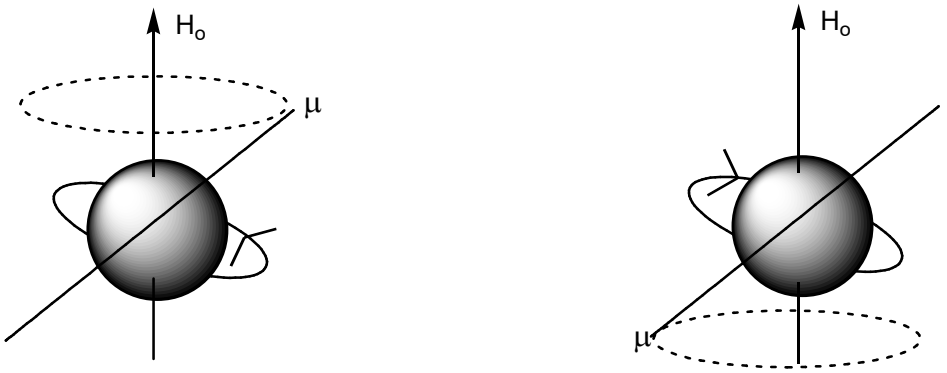
NUCLEAR MAGNETIC RESONANCE (NMR)

Nuclear magnetic resonance (**NMR**) is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties and the frequency range is (60–1000 MHz). .

All isotopes that contain an odd number of protons and/or neutrons (see Isotope) have an intrinsic magnetic moment and angular momentum, in other words a nonzero spin, while all nuclides with even numbers of both have a total spin of zero.

Certain nuclei, such as the hydrogen nucleus (but not carbon-12 or oxygen-16), have a nuclear spin. The spinning nucleus generates a small magnetic field, called "μ". When placed in a strong external magnetic field, called H_o, the nucleus can exist in two distinct spin states: a low energy state A, in which μ is aligned with the external magnetic field, H_o, and a high energy state B, in which μ is opposed to the external magnetic field, H_o (figure 1). Alignment of μ with H_o is the more stable and lower energy state.



In NMR, transitions from the more stable alignment, A, (with the field) to the less stable alignment, B, (against the field) occur when the nucleus absorbs electromagnetic energy that is

exactly equal to the energy separation between the states (ΔE). This amount of energy is usually found in the radiofrequency range. The condition for absorption of energy is called the condition of resonance. It can be calculated as the following:

$$\Delta E = \frac{\gamma h}{2\pi} H = h\upsilon$$

h = Planck's constant

H = the strength of the applied magnetic field, H_o, at the nucleus

γ = the gyromagnetic ratio (a constant that is characteristic of a particular nucleus)

υ = the frequency of the electromagnetic energy absorbed that causes the change in spin states

There are three features of NMR spectra that we will focus on: the number and size of signals, the chemical shift, and spin-spin coupling.

The most commonly studied nuclei are 1H and 13C, although nuclei from isotopes of many other elements
(e.g. 2H, 6Li, 10B, 11B, 14N, 15N, 17O, 19F, 23Na, 29Si, 31P, 35Cl, 113Cd, 129Xe, 195Pt)
have been studied by high-field NMR spectroscopy as well.

A key feature of NMR is that the resonance frequency of a particular substance is directly proportional to the strength of the applied magnetic field.

The principle of NMR usually involves two sequential steps:

- The alignment (polarization) of the magnetic nuclear spins in an applied, constant magnetic field $\mathbf{B}_0$.
- The perturbation of this alignment of the nuclear spins by employing an electro-magnetic, usually radio frequency (RF) pulse. The required perturbing frequency is dependent upon the static magnetic field ($\mathbf{H}_0$) and the nuclei of observation.

The two fields are usually chosen to be perpendicular to each other as this maximizes the NMR signal strength. The resulting response by the total magnetization (**M**) of the nuclear spins is the phenomenon that is exploited in NMR spectroscopy and magnetic resonance imaging. Both use intense applied magnetic fields ($\mathbf{H}_0$) in order to achieve dispersion and very high stability to deliver spectral resolution, the details of which are described by chemical shifts, the Zeeman effect, and Knight shifts (in metals).

CHEMICAL SHIFT: it is the resonant frequency of a 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.

Chemical shift δ is usually expressed in parts per million (ppm) by frequency, because it is calculated from:

$$\delta = \frac{\text{v sample- v reference}}{\text{v reference}}$$

Where ν_{sample} is the absolute resonance frequency of the sample and ν_{ref} is the absolute resonance frequency of a standard reference compound, measured in the same applied magnetic field B_0. Since the numerator is usually expressed in hertz, and the denominator in megahertz, δ is expressed in ppm.

The detected frequencies (in Hz) for ^{1}H, ^{13}C, and ^{29}Si nuclei are usually referenced against TMS (tetramethylsilane) or DSS, which by the definition above have a chemical shift of zero if chosen as the reference. Other standard materials are used for setting the chemical shift for other nuclei.

Thus, an NMR signal observed at a frequency 300 Hz higher than the signal from TMS, where the TMS resonance frequency is 300 MHz. Although the absolute resonance frequency depends on the applied magnetic field, the chemical shift is independent of external magnetic field strength. On the other hand, the resolution of NMR will increase with applied magnetic field.

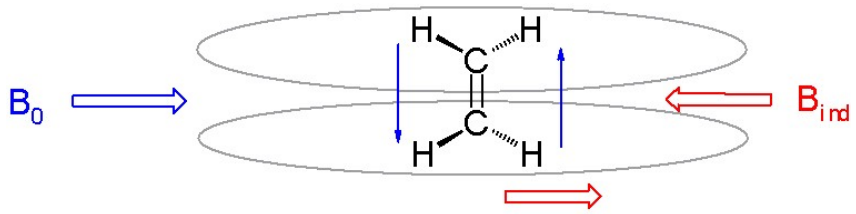
FACTOR AFFECTING CHEMICAL SHIFT

Important factors influencing chemical shift are electron density, electronegativity of neighboring groups and anisotropic induced magnetic field effects.

Electron density shields a nucleus from the external field. For example, in proton NMR the electron-poor tropylium ion has its protons downfield at 9.17 ppm, those of the electron-rich cyclooctatetraenyl anion move upfield to 6.75 ppm and its dianion even more upfield to 5.56 ppm.

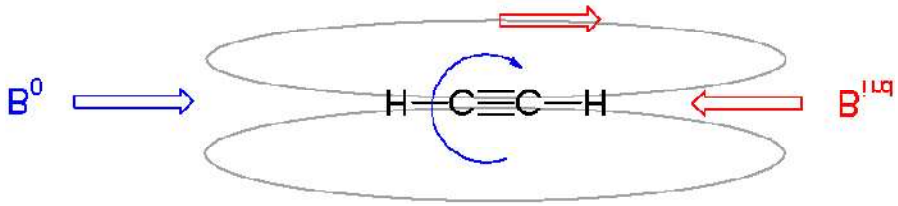
A nucleus in the vicinity of an electronegative atom experiences reduced electron density and the nucleus is therefore deshielded. In proton NMR of methyl halides (CH_3X) the chemical shift of the methyl protons increase in the order I < Br < Cl < F from 2.16 ppm to 4.26 ppm reflecting this trend. In carbon NMR the chemical shift of the carbon nuclei increase in the same order from around −10 ppm to 70 ppm. Also when the electronegative atom is removed further away the effect diminishes until it can be observed no longer.

Anisotropic induced magnetic field effects are the result of a local induced magnetic field experienced by a nucleus resulting from circulating electrons that can either be paramagnetic when it is parallel to the applied field or diamagnetic when it is opposed to it. It is observed in alkenes where the double bond is oriented perpendicular to the external field with pi electrons likewise circulating at right angles. The induced magnetic field lines are parallel to the external field at the location of the alkene protons which therefore shift downfield to a 4.5 ppm to 7.5 ppm range. The three-dimensional space where a diamagnetic shift is called the shielding zone with a cone-like shape aligned with the external field.



Induced magnetic field of alkenes in external magnetic fields, field lines in grey

The protons in aromatic compounds are shifted downfield even further with a signal for benzene at 7.73 ppm as a consequence of a diamagnetic ring current.

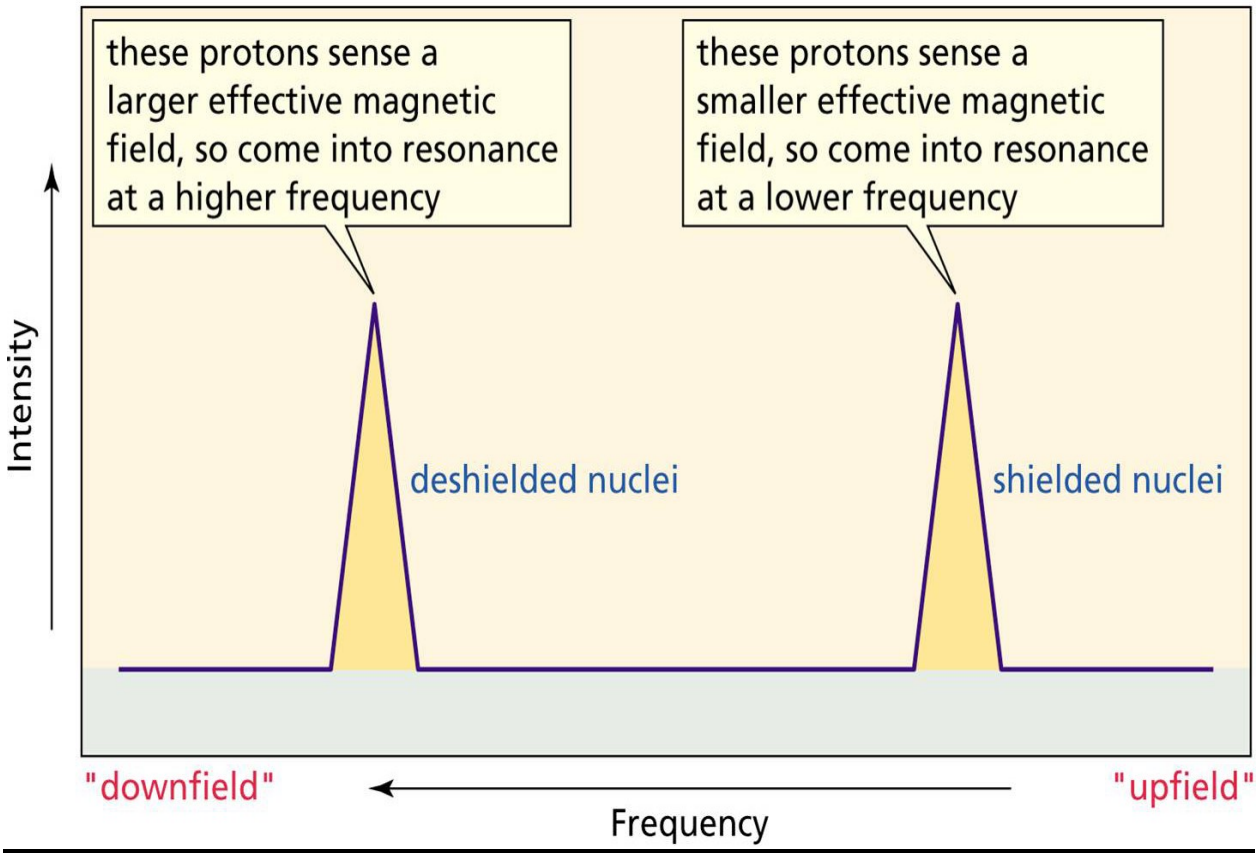


Alkyne protons by contrast resonate at high field in a 2–3 ppm range. For alkynes the most effective orientation is the external field in parallel with electrons circulation around the triple bond. In this way the acetylenic protons are located in the cone-shaped shielding zone hence the upfield shift.

SHIELDING (UPFIELD)

Shielded is defined as "a nucleus whose chemical shift has been decreased due to addition of electron density, magnetic induction, or other effects."Hydrogen atoms in the molecule feel different magnetic fields depending upon their location and the neighbor they directly attached to. The Nucleus feels weaker magnetic field. Shielding is a barrier made of inner-shell electrons and it decreases the nucleus' pull on the outer electrons. The Nucleus feels weaker magnetic field.

DESHIELDING (DOWNFIELD) Deshielding is the opposite of shielding. When we say that an atom is deshielded, we mean that "A nucleus whose chemical shift has been increased due to removal of electron density, magnetic induction, or other effects. The Nucleus feels stronger magnetic field.



SPIN-SPIN COUPLING: It is a magnetic interaction between individual nuclear spins transmitted by the bonding electrons through which the nuclear spins are indirectly connected.

Coupling contains information about bond distance and angles. Most importantly, *J*-coupling provides information on the connectivity of molecules. In NMR spectroscopy, it is responsible for the appearance of many signals in the NMR spectra of fairly simple molecules.

Magnetically equivalent nuclei possess the same resonance frequency and only one characteristic spin spin interaction with the nuclei of a neighboring group. The spin-spin coupling between magnetically equivalent nuclei does not appear in the spectrum. Nuclei with the same resonance frequency are called chemically equivalent or isochronous. Chemically equivalent nuclei will not

be magnetically equivalent if they have different couplings to other nuclei in the molecules. Magnetic equivalence causes great simplification in the resulting NMR spectra, but those cases where nuclei are chemically equivalent but not magnetically equivalent give complicated spectra in which second-order effects are prevalent.

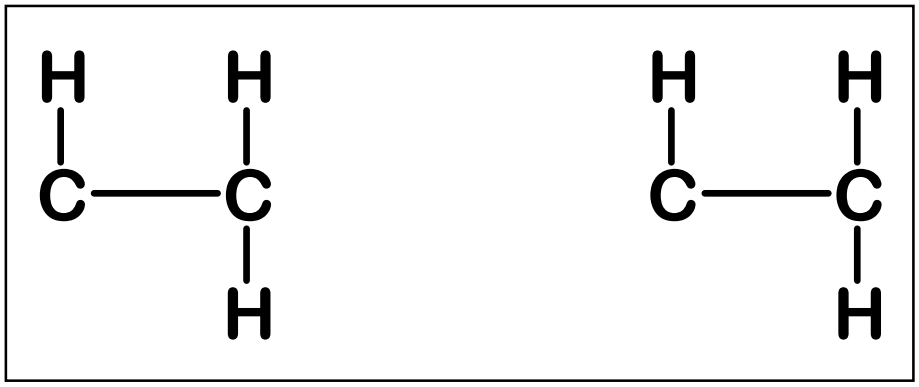
COUPLING CONSTANT (J)

The coupling (*J*)is a bond interaction, in which the spin of one nucleus perturbs (polarizes) the spins of the intervening electrons, and the energy levels of neighboring magnetic nuclei are in turn perturbed by the polarized electrons. This leads to a lowering of the energy of the neighboring nucleus when the perturbing nucleus has one spin, and a raising of the energy when it has the other spin. The *J* coupling is field-independent (i.e. *J* is constant at different external magnetic field strength), and is mutual (i.e. $J_{AX} = J_{XA}$). Because the effect is usually transmitted through the bonding electrons, the magnitude of *J* falls off rapidly as the number of intervening bonds increases. coupling constants can be either positive or negative, defined as follows: coupling constants are positive if the energy of A is lower when X has the opposite spin as A (αβ or βα), and negative if the energy of A is lower when X has the same spin as A (αα or ββ).

SPIN SPIN SPLITING: It is interaction with neighboring hydrogens nuclei from which multiplet peak is appear. and hydrogen will appear as a multiplet rather than as a single peak.

Singlet (s)	Quintet (quin)
Doublet (d)	Sixtet (six)
Triplet (t)	Septet (sept)
Quartet (q)	Multiplet (m)

[n + 1 RULE]



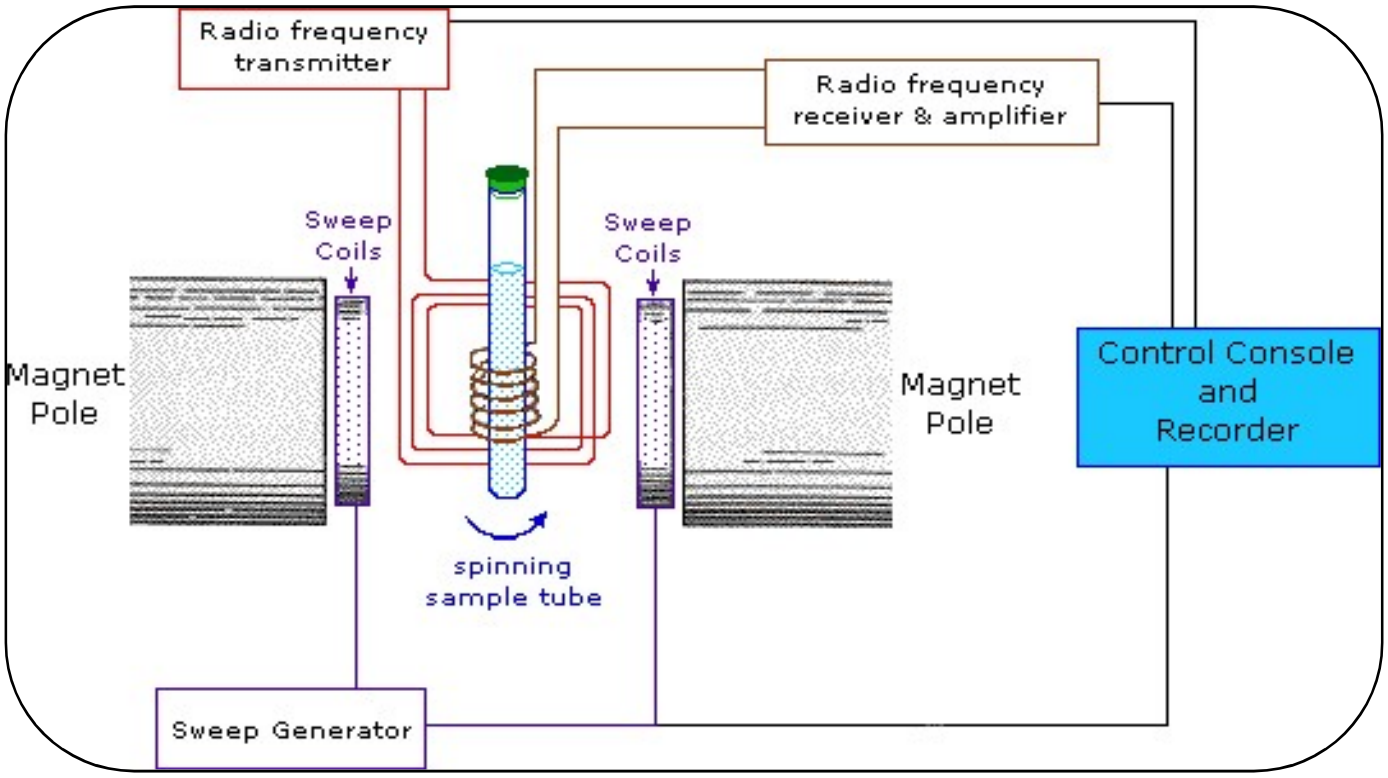
two neighbors

n+1 = 3 one neighbor n+1 = 2 doublet

triplet

where **n** is the number of **equivalent** protons on Adjacent carbon atoms

NMR INSTRUMENTION



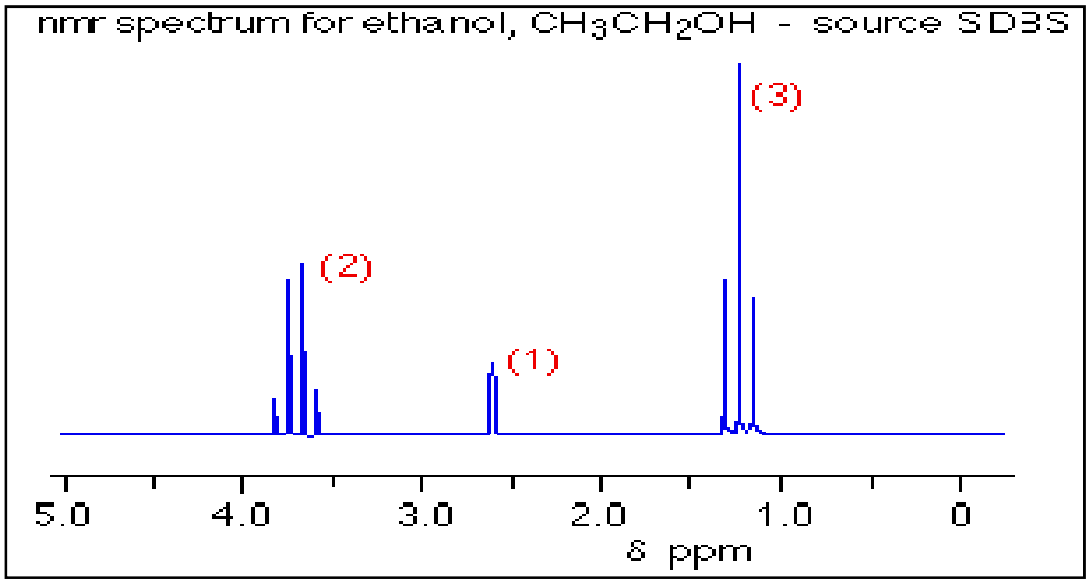
MAGNET - Normally superconducting. Some electromagnets and permanent magnets (EM-360, EM-390) still around.

FREQUENCY GENERATOR - Creates the alternating current (at ωo) that induces B1. Continuous wave or pulsed.

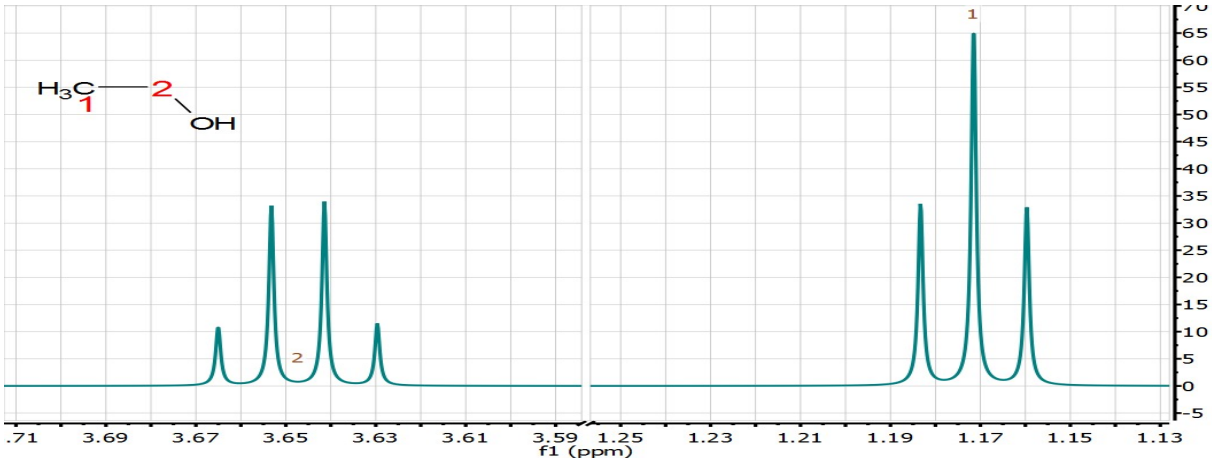
DETECTOR - Subtracts the base frequency (a constant frequency very close to ωo) to the output frequency. It is lower frequency and much easier to deal with.

RECORDER - XY plotter, oscilloscope, computer, etc.,

SIGNAL AND NMR SPECTRA OF ETHANOL

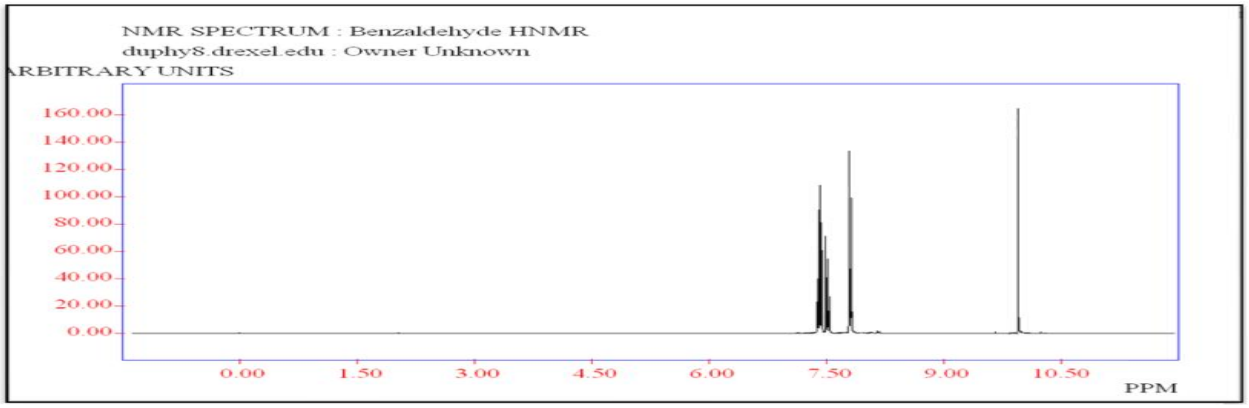


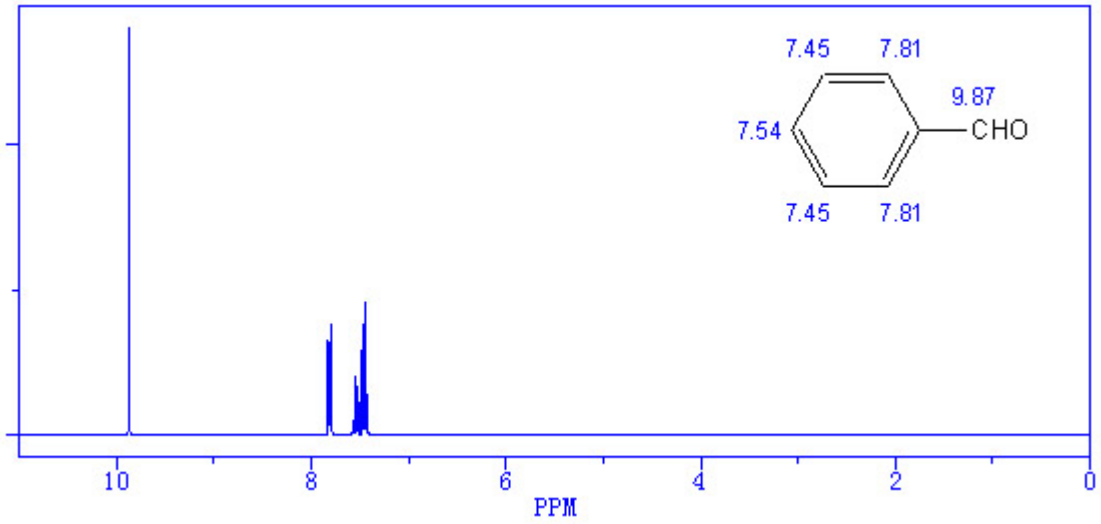
SIGNAL



SIGNAL IN ETHANOL

NMR SPECTRA OF BENZALDEHYDE





C-13 NMR

Carbon-13 nuclear magnetic resonance (most commonly known as **carbon-13 NMR** or **^{13}C NMR** or sometimes simply referred to as **carbon NMR**) is the application of nuclear to carbon. It is analogous to proton NMR (1H NMR) and allows the identification of carbon atoms in an organic molecule just as proton NMR identifies hydrogen atoms. As such ^{13}C NMR is an important tool in chemical structure elucidation in organic chemistry. ^{13}C NMR detects only the 13C isotope of carbon, whose natural abundance is only 1.1%, because the main carbon isotope, 12C, is not detectable by NMR since it has zero net spin.

^{13}C NMR has a number of complications that are not encountered in proton NMR. ^{13}C NMR is much less sensitive to carbon than ^{1}H NMR is to hydrogen since the major isotope of carbon, the ^{12}C isotope, has a spin quantum number of zero and so is not magnetically active and therefore not detectable by NMR. Only the much less common ^{13}C isotope, present naturally at 1.1% natural abundance, is magnetically active with a spin quantum number of 1/2 (like ^{1}H) and therefore detectable by NMR. Therefore, only the few ^{13}C nuclei present resonate in the magnetic field, although this can be overcome by isotopic enrichment of e.g. protein samples. In addition, the gyro magnetic ratio ($6.728284\ 10^{7}$rad $T^{-1}\ s^{-1}$) is only 1/4 that of ^{1}H, further reducing the sensitivity. The overall receptivity of ^{13}C is about 4 orders of magnitude lower than ^{1}H.[1]

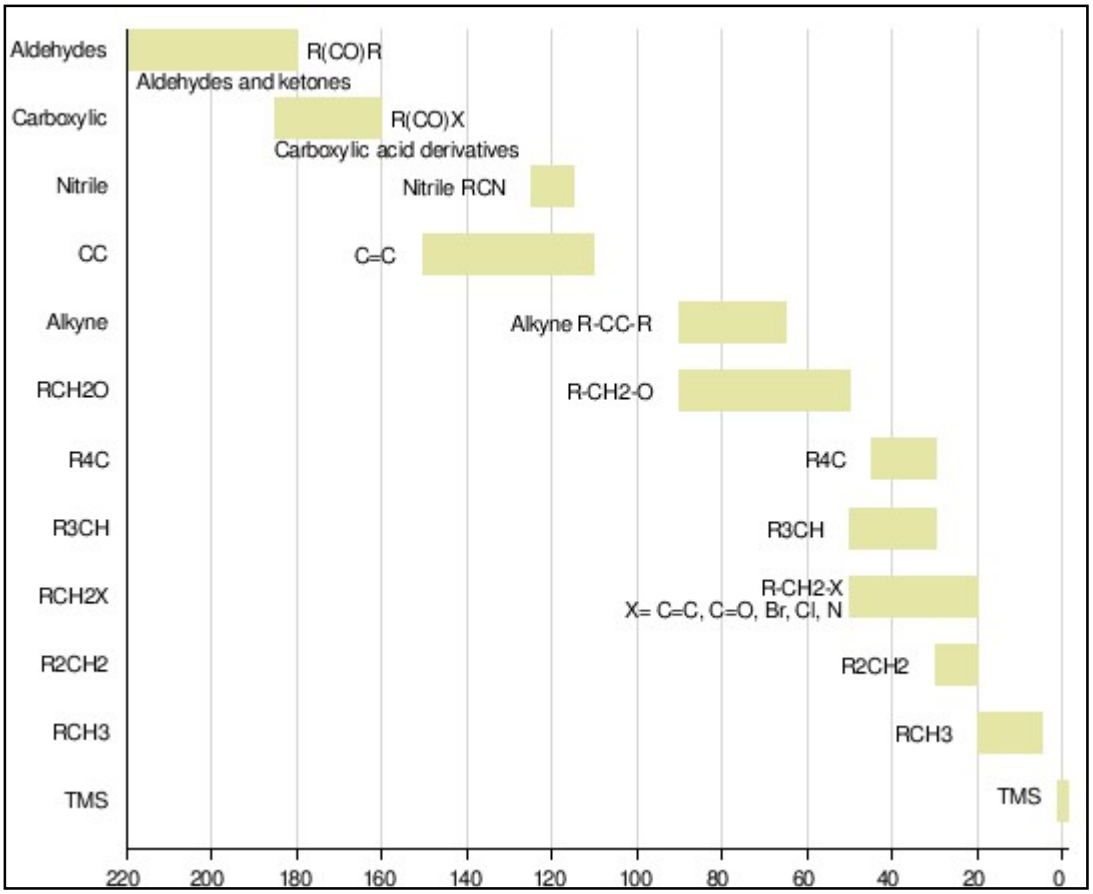
Another potential complication results from the presence of large one bond J-coupling constants between carbon and hydrogen (typically from 100 to 250 Hz). In order to suppress these couplings, which would otherwise complicate the spectra and further reduce sensitivity, carbon NMR spectra are proton decoupled to remove the signal splitting. Couplings between carbons can be ignored due to the low natural abundance of ^{13}C. Hence in contrast to typical proton NMR spectra which show multiplets for each proton position, carbon NMR spectra show a single peak for each chemically non-equivalent carbon atom.

In further contrast to ^{1}H NMR, the intensities of the signals are not normally proportional to the number of equivalent ^{13}C atoms and are instead strongly dependent on the number of

surrounding spins (typically ^{1}H). Spectra can be made more quantitative if necessary by allowing sufficient time for the nuclei to relax between repeat scans.

High field magnets with internal bores capable of accepting larger sample tubes (typically 10 mm in diameter for ^{13}C NMR versus 5 mm for ^{1}H NMR), the use of relaxation reagents,[2] for example $Cr(acac)_3$ (chromium (III) acetylacetonate, CAS number 21679-31-2), and appropriate pulse sequences have reduced the time needed to acquire quantitative spectra and have made quantitative carbon-13 NMR a commonly used technique in many industrial labs. Applications range from quantification of drug purity to determination of the composition of high molecular weight synthetic polymers.

^{13}C chemical shifts follow the same principles as those of ^{1}H, although the typical range of chemical shifts is much larger than for ^{1}H (by a factor of about 20). The chemical shift reference standard for ^{13}C is the carbons in tetramethylsilane (TMS), [3] whose chemical shift is considered to be 0.0 ppm.


Aldehydes
R(CO)R
Aldehydes and ketones
Carboxylic
R(CO)X
Carboxylic acid derivatives
Nitrile
Nitrile RCN
CC
C=C
Alkyne
Alkyne R-CC-R
RCH2O
R-CH2-O
R4C
R4C
R3CH
R3CH
RCH2X
R-CH2-X
X= C=C, C=O, Br, Cl, N
R2CH2
R2CH2
RCH3
RCH3
TMS
TMS
220
200
180
160
140
120
100
80
60
40
20
0

APPLICATION OF IR SPECTRA

Infrared spectroscopy is widely used in industry as well as in research. It is a simple and reliable technique for measurement, quality control and dynamic measurement. It is also employed in forensic analysis in civil and criminal analysis.

1. Identification of functional group and structure elucidation

Entire IR region is divided into group frequency region and fingerprint region. Range of group frequency is 4000-1500 cm^{-1} while that of finger print region is 1500-400 cm^{-1}.

In group frequency region, the peaks corresponding to different functional groups can be observed. According to corresponding peaks, functional group can be determined.

Each atom of the molecule is connected by bond and each bond requires different IR region so characteristic peaks are observed. This region of IR spectrum is called as finger print region of the molecule. It can be determined by characteristic peaks.

2. Identification of substances

IR spectroscopy is used to establish whether a given sample of an organic substance is identical with another or not. This is because large number of absorption bands is observed in the IR spectra of organic molecules and the probability that any two compounds will produce identical spectra is almost zero. So if two compounds have identical IR spectra then both of them must be samples of the same substances.

IR spectra of two enatiomeric compound are identical. So IR spectroscopy fails to distinguish between enantiomers.

For example, an IR spectrum of benzaldehyde is observed as follows.

C-H stretching of aromatic ring- 3080 cm^{-1}

C-H stretching of aldehyde- 2860 cm^{-1} and 2775 cm^{-1}

C=O stretching of an aromatic aldehyde- 1700 cm^{-1}

C=C stretching of an aromatic ring- 1595 cm^{-1}

C-H bending- 745 cm^{-1} and 685 cm^{-1}

No other compound then benzaldehyde produces same IR spectra as shown above.

3. Studying the progress of the reaction:

Progress of chemical reaction can be determined by examining the small portion of the reaction mixure withdrawn from time to time. The rate of disappearance of a characteristic absorption band of the reactant group and/or the rate of appearance of the characteristic absorption band of the product group due to formation of product is observed.

4. Detection of impurities

IR spectrum of the test sample to be determined is compared with the standard compound. If any additional peaks are observed in the IR spectrum, then it is due to impurities present in the compound.

5. Quantitative analysis

The quantity of the substance can be determined either in pure form or as a mixure of two or more compounds. In this, characteristic peak corresponding to the drug substance is chosen and log I0/It of peaks for standard and test sample is compared. This is called base line technique to determine the quantity of the substance.