SRI AKILANDESWARI WOMEN'S COLLEGE, WANDIWASH

NMR SPECTROSCOPY

CLASS : II PG PHYSICS

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Introduction:-

Nuclear Magnetic Resonance (NMR) is a spectroscopy technique which is based on the absorption of electromagnetic radiation in the **radio frequency region 4 to 900 MHz** by nuclei of the atoms.

Proton Nuclear magnetic resonance spectroscopy is one of the most powerful tools for elucidating the number of hydrogen or proton in the compound.

It is used to study a wide variety of nuclei:

- ^{1}H ^{15}N
- ^{19}F ^{19}F
- ^{13}C ^{31}P

Theory of NMR:-

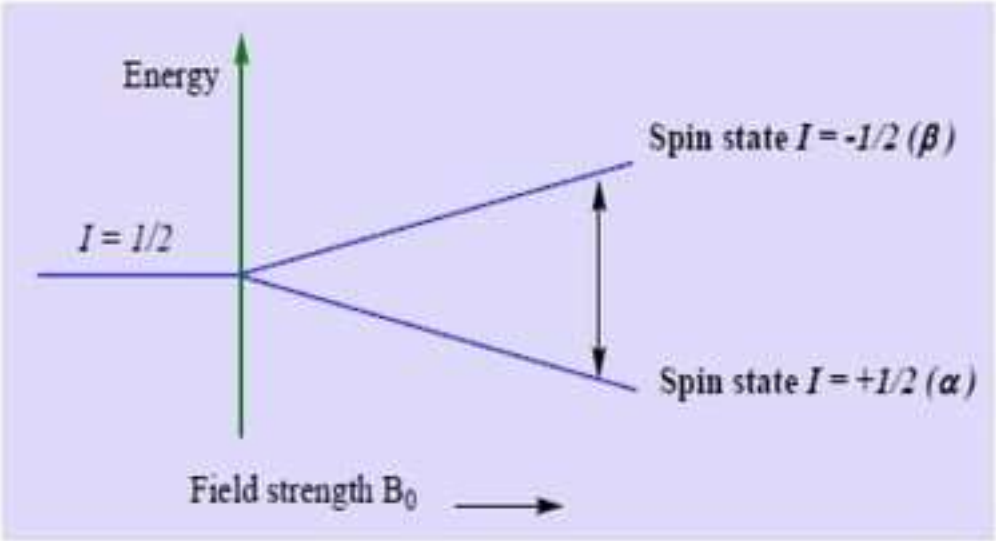
Spin quantum number (I) is related to the atomic and mass number of the nucleus.

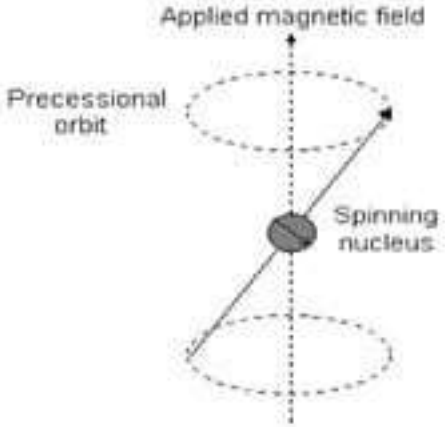
I	Atomic Mass	Atomic Number	Examples	
Half-integer	Odd	Odd	^{1}H (1/2)	NMR active
Half-integer	Odd	Even	^{13}C (1/2)	
Integer	Even	Odd	^{2}H (1)	
Zero	Even	Even	^{12}C (0)	Not NMR active

Elements with either odd mass or odd atomic number have the property of nuclear "spin".

If an external magnetic field is applied, the number of possible orientations calculated by **(2I+1).**

Hydrogen has spin quantum number **I=1/2** and possible orientation is (2*1/2+1=2) two **+1/2** and **-1/2.**



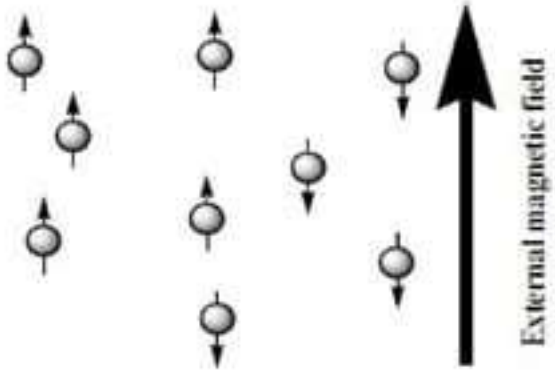


Principles of NMR

The theory behind NMR comes from the spin of a nucleus and it generates a magnetic field. Without an external applied magnetic field, the nuclear spins are random in directions.

No external magnetic field

But when an external magnetic field(B_o), is present the nuclei align themselves either with or against the field of the external magnet.

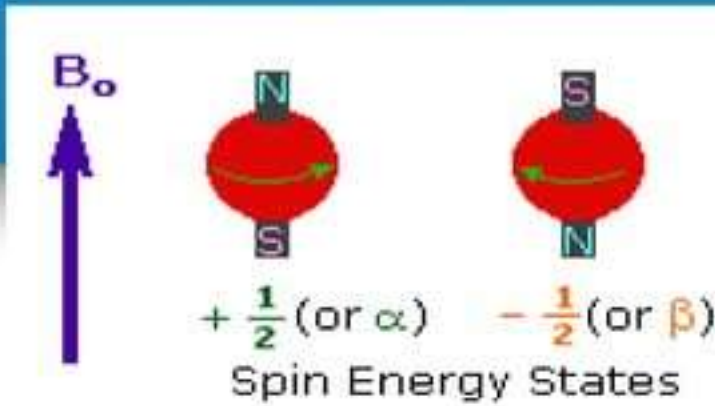


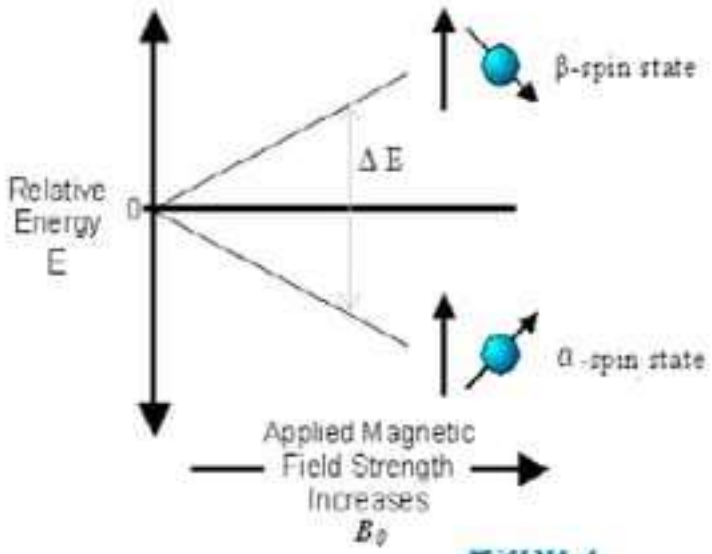
With external magnetic field

If an external magnetic field is applied, an energy transfer (ΔE) is possible between ground state to excited state.

when the spin returns to its ground state level, the absorbed radiofrequency energy is emitted at the same frequency level.

The emitted radiofrequency signal that give the NMR spectrum of the concerned nucleus.





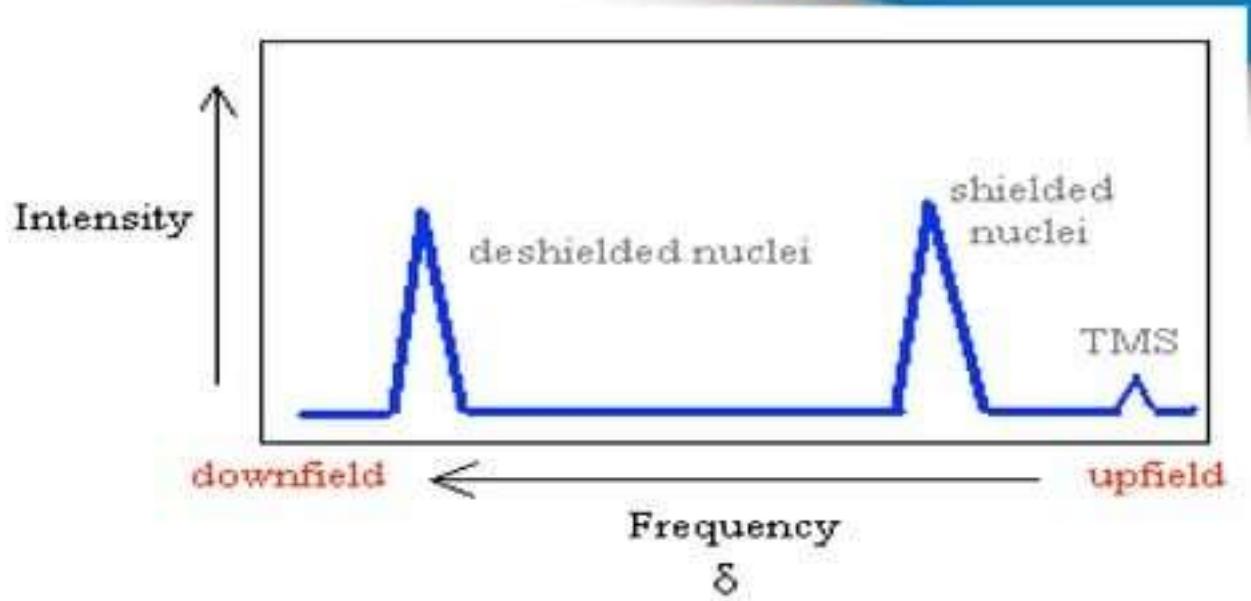
The emitted radio frequency is directly proportional to the strength of the applied field.

$$\nu = \frac{\gamma B_o}{2\Pi}$$

B_o = *External magnetic field experienced by proton*

γ = *Magnetogyric ratio (The ratio between the nuclear magnetic moment and angular moment)*

NMR spectrum

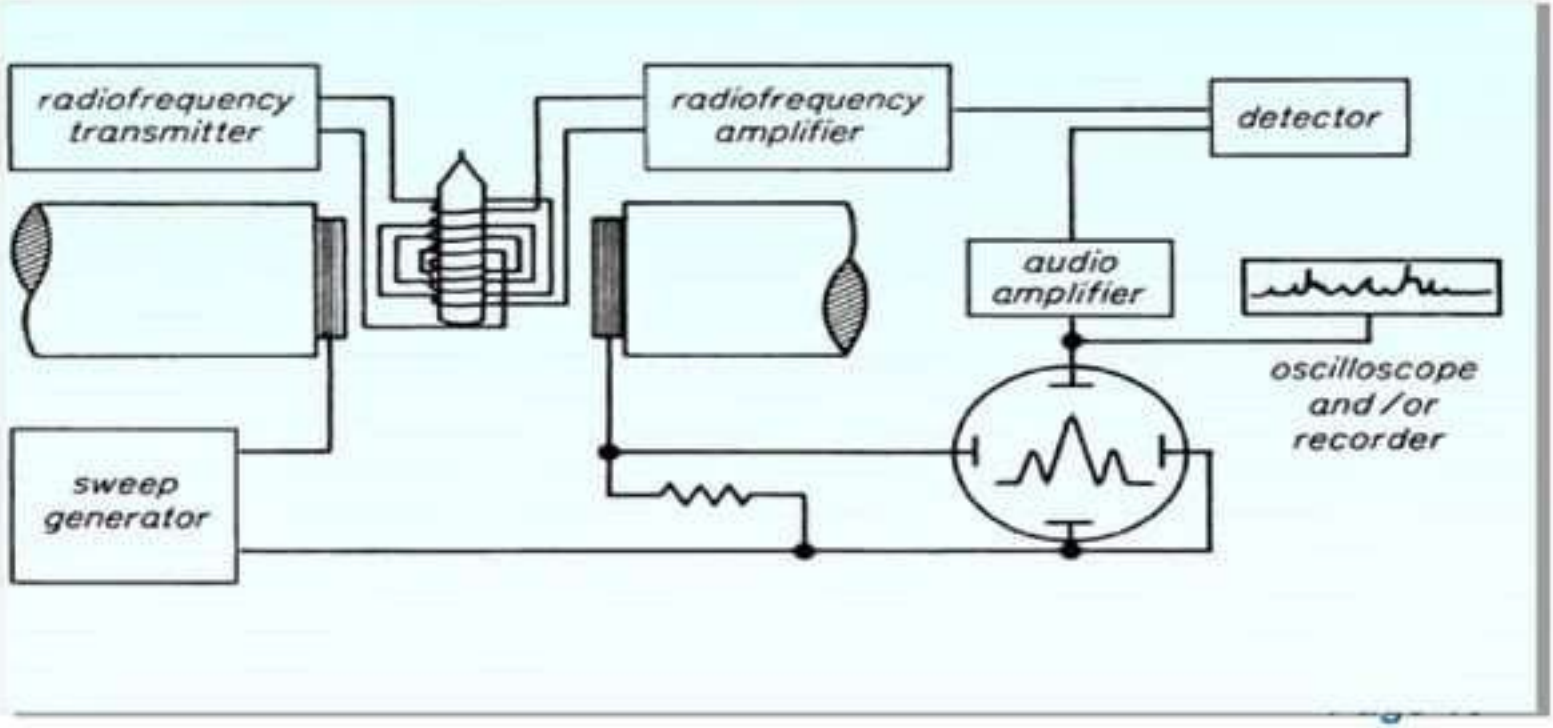


The NMR spectrum is a plot of intensity of NMR signals VS magnetic field (frequency) in reference to TMS

NMR instrumentation

1. Sample holder
2. Permanent magnet
3. Magnetic coils
4. Sweep generator
5. Radio frequency transmitter
6. Radio frequency receiver

7.Read out systems



1.Sample holder :- Glass tube with 8.5 cm long,0.3 cm in diameter

2.Permanent magnet :- It provides homogeneous magnetic field at 60-100 MHZ

3.Magnetic coils :- These coils induce magnetic field when current flows through them.

4.Sweep generator :- To produce the equal amount of magnetic field pass through the sample

5.Radio frequency transmitter :- A radio transmitter coil that produces a short powerful pulse of radio waves

6.Radiofrequency Receiver :- A radio receiver coil that detects radio frequencies emitted as nuclei relax to a lower energy level

7.Readout system :- A computer that analyses and record the data

Solvents used in NMR

The following solvents are normally used in which hydrogen replaced by deuterium.

CCl_4 - carbon tetrachloride

CS_2 - carbon disulfide

$CDCl_3$ - Deuteriochloroform

C_6D_6 - Hexa deuteriobenzene

D_2O - Deuterium oxide

Chemical shift

A **chemical shift** is defined as the difference in parts per million (ppm) between the resonance frequency of the observed proton and tetramethylsilane (TMS) hydrogens.

TMS is the most common reference compound in NMR, it is set at δ=0 ppm

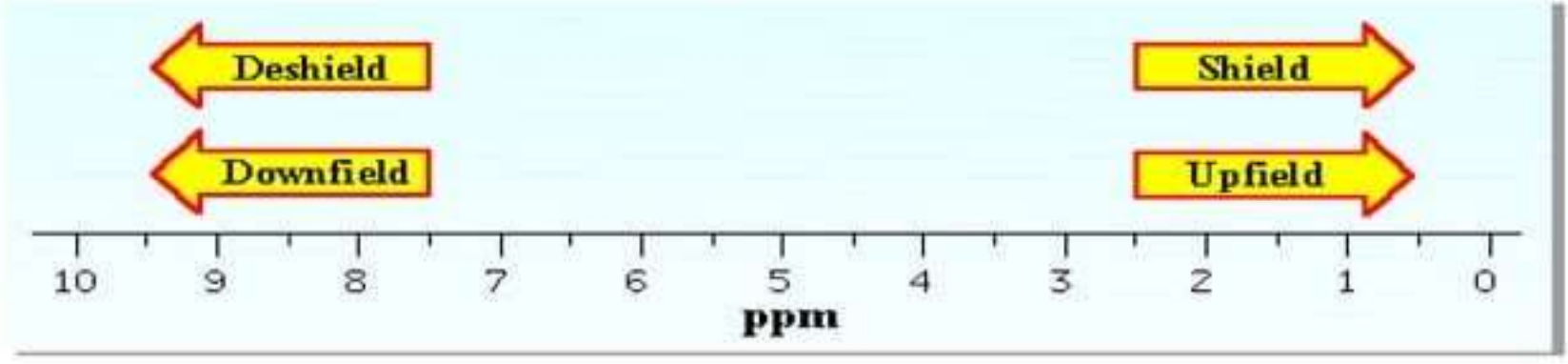
$$\text{Chemical shift, } \delta = \frac{\text{frequency of signal} - \text{frequency of reference}}{\text{spectrometer frequency}} \times 10^6$$

Shielding of protons:-

High electron density around a nucleus **shields** the nucleus from the external magnetic field and the signals are **upfield** in the NMR spectrum

Deshielding of protons:-

Lower electron density around a nucleus **deshields** the nucleus from the external magnetic field and the signals are **downfield** in the NMR spectrum



Factors affecting chemical shift:-

- Electronegative groups
- Magnetic anisotropy of π-systems
- Hydrogen bonding

Electronegative groups:-

Electronegative groups attached to the C-H system decrease the electron density around the protons, and there is less shielding (*i.e.*deshielding) and chemical shift increases

Compound	Chemical shift
CH_3I	2.16
CH_3Br	2.65
CH_3Cl	3.10
CH_3F	4.26

Magnetic anisotropy of π-systems:-

- The word "anisotropic" means "non-uniform". So magnetic anisotropy means that there is a "non-uniform magnetic field".
- Electrons in π systems (e.g. aromatics, alkenes, alkynes, carbonyls etc.) interact with the applied field which induces a magnetic field that causes the anisotropy.
- It causes both shielding and deshielding of protons.
- Example:-Benzene

Hydrogen bonding:-

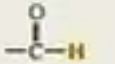
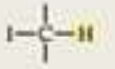
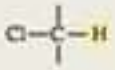
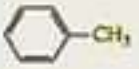
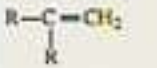
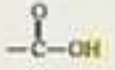
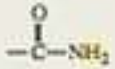
- Protons that are involved in hydrogen bonding are typically change the chemical shift values.
- The more hydrogen bonding, the more proton is deshielded and chemical shift value is higher.

Proton NMR

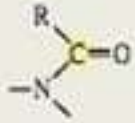
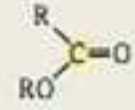
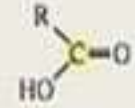
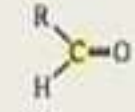
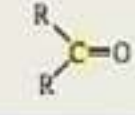
The most common for of NMR is based on the hydrogen-1 (^1H), nucleus or proton.

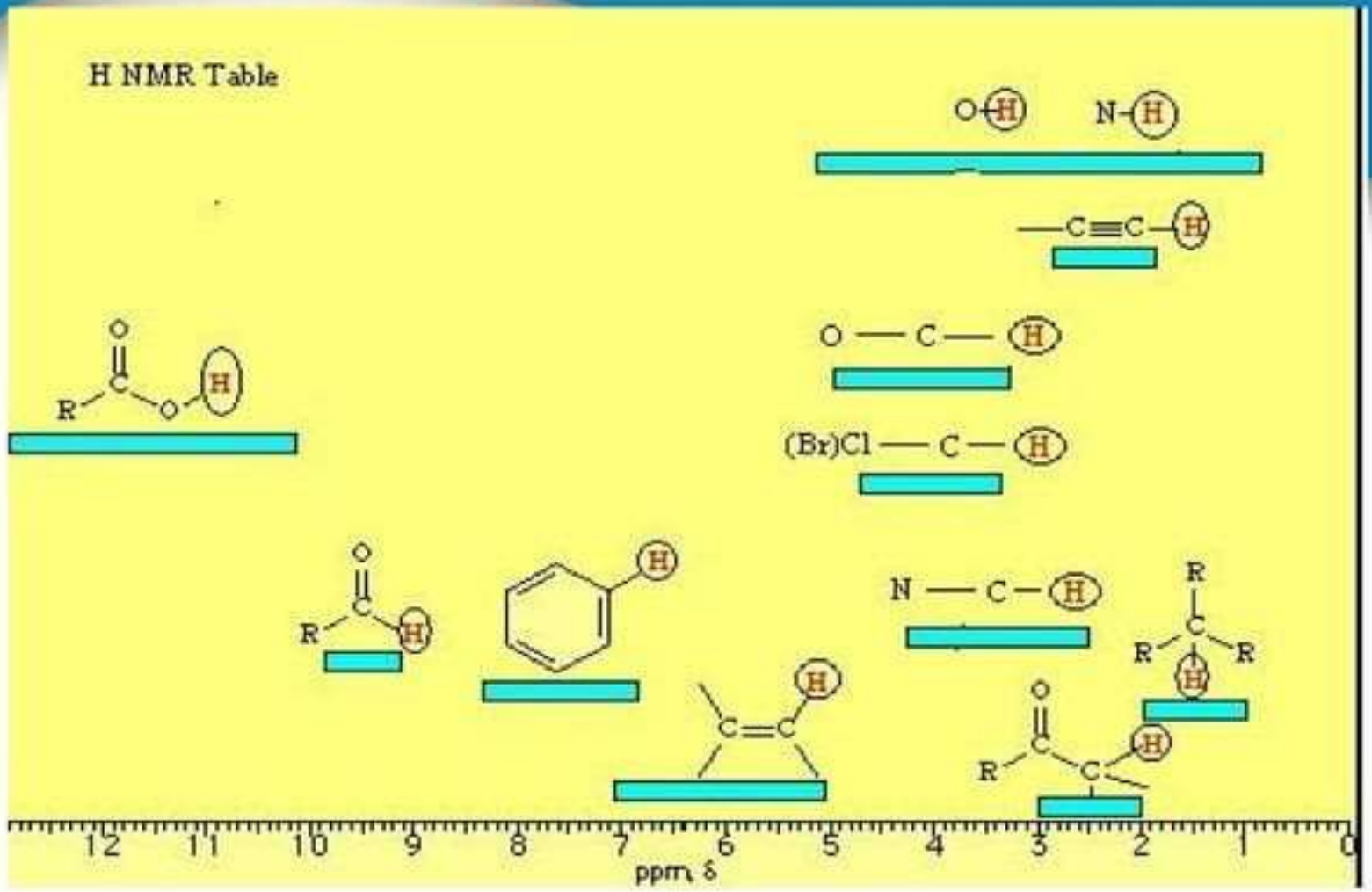
It can give information about the structure of any molecule containing hydrogen atoms.

^{1}H NMR chemical shift

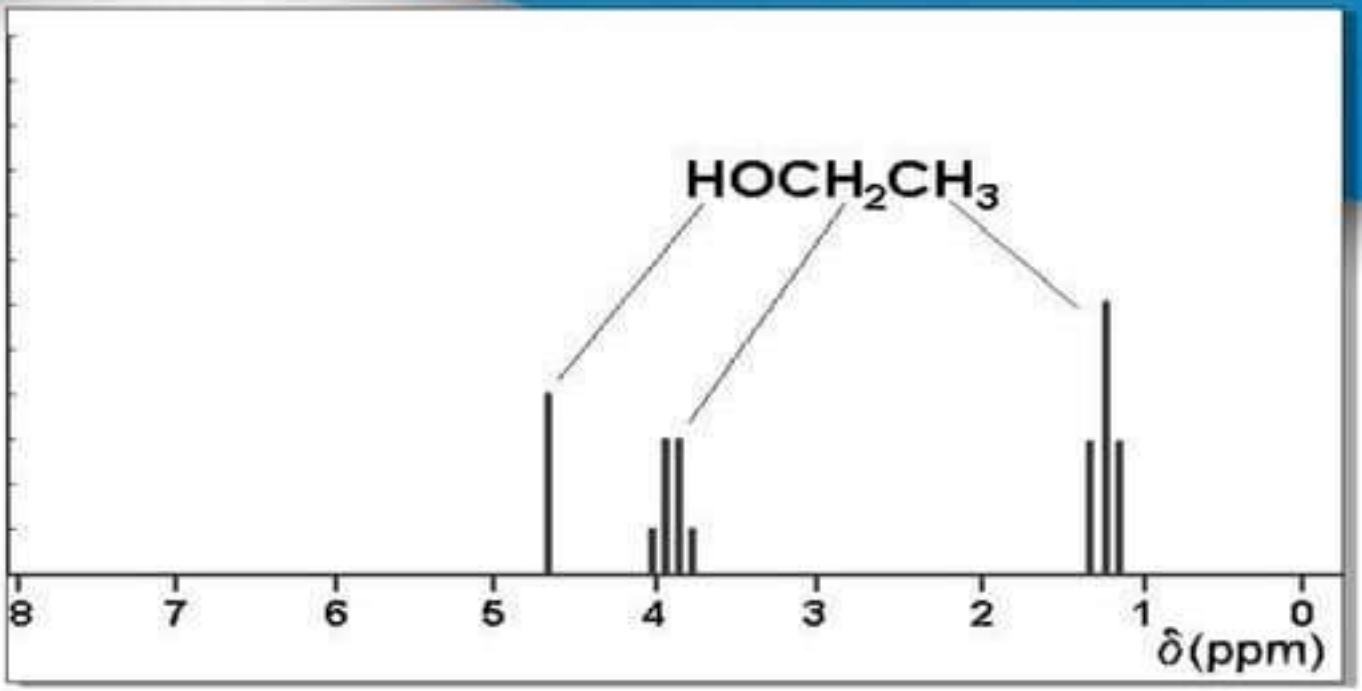
Type of proton	Approximate chemical shift (ppm)	Type of proton	Approximate chemical shift (ppm)
$(CH_3)_4Si$	0		6.5–8
$-CH_3$	0.9		
$-CH_2-$	1.3		9.0–10
	1.4		2.5–4
	1.7		
			2.5–4
	2.1		
			3–4
	2.3		
			4–4.5
$-C\equiv C-H$	2.4		
$R-O-CH_3$	3.3	RNH_2	Variable, 1.5–4
	4.7	ROH	Variable, 2–5
		ArOH	Variable, 4–7
	5.3		Variable, 10–12
			Variable, 5–6

*The values are approximate because they are affected by neighboring substituents.

Type of carbon	Approximate chemical shift (ppm)	Type of carbon	Approximate chemical shift (ppm)
$(CH_3)_4Si$	0	C—I	0–40
$R-CH_3$	8–35	C—Br	25–65
$R-CH_2-R$	15–50	C—Cl C—N C—O	35–80 40–60 50–80
	20–60		165–175
	30–40		165–175
$=C$	65–85		175–185
$-C$	100–150		190–200
	110–170		205–220


H NMR Table
O-H
N-H
—C≡C—H
O — C — H
(Br)Cl — C — H
N — C — H
R
C
O
H
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm, δ

Proton NMR spectra of Ethanol:-

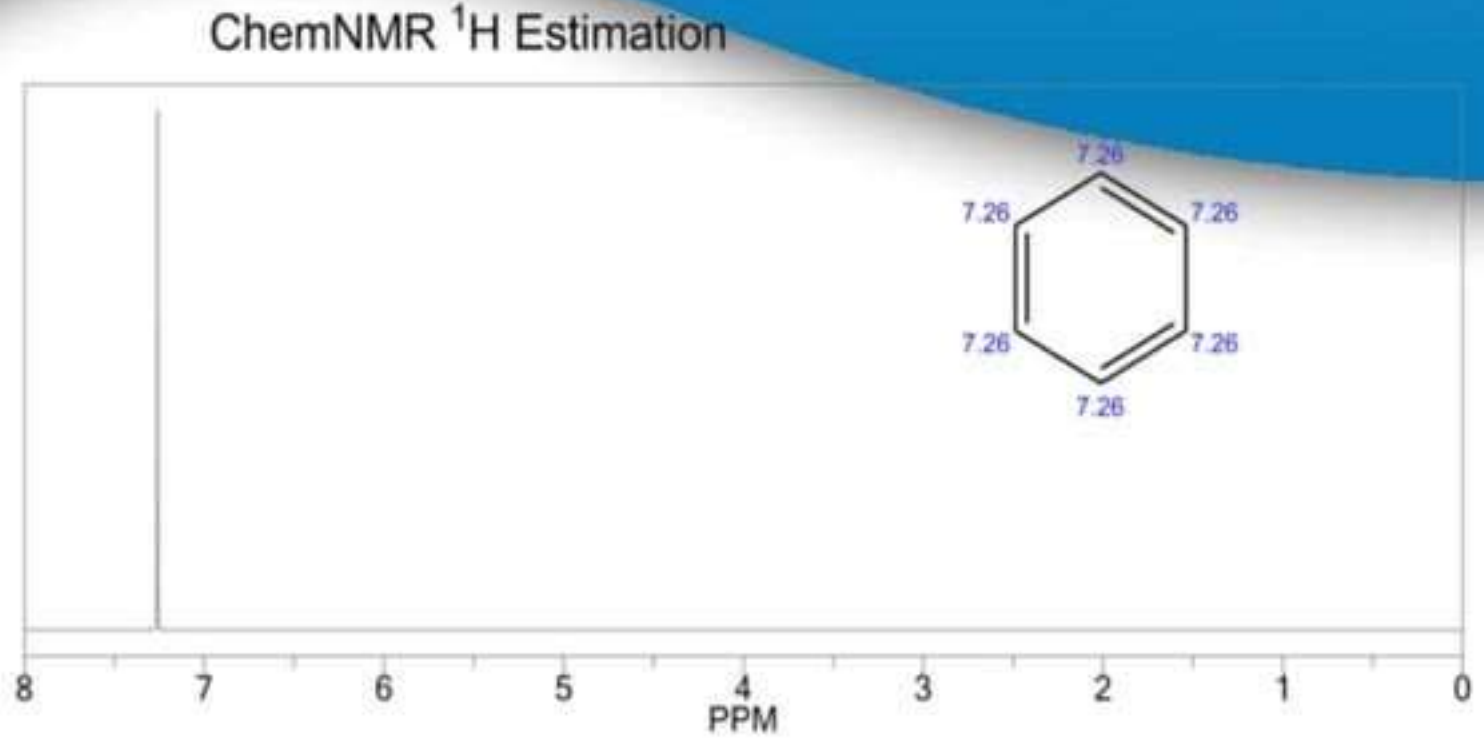


^{1}H spectrum of Ethanol:-

3 types of proton

CH_3CH_2OH

^{1}H NMR spectra of Benzene



- Aromatic Hydrogen shows peak in the chemical shift scale 6.5-8.0 ppm.
- From the above spectrum Benzene has same type of protons and it shows single peak at 7.26

Interpretation of ^{1}HNMR spectra:-

Number of signals	- Indicates how many "different kinds" of protons are present.
Position of signals	- Indicates something about (chemicalshift) magnetic (electronic) environment of protons
Relative intensity of signals	- Proportional to number of protons present
Splitting of signals (spin spin coupling)	- Indicates the number of nearby nuclei usually protons

n+1 rule:-

- The multiplicity of signal is calculated by using n+1 rule.
- This is one of the rule to predict the splitting of proton signals. This is considered by the nearby hydrogen nuclei.

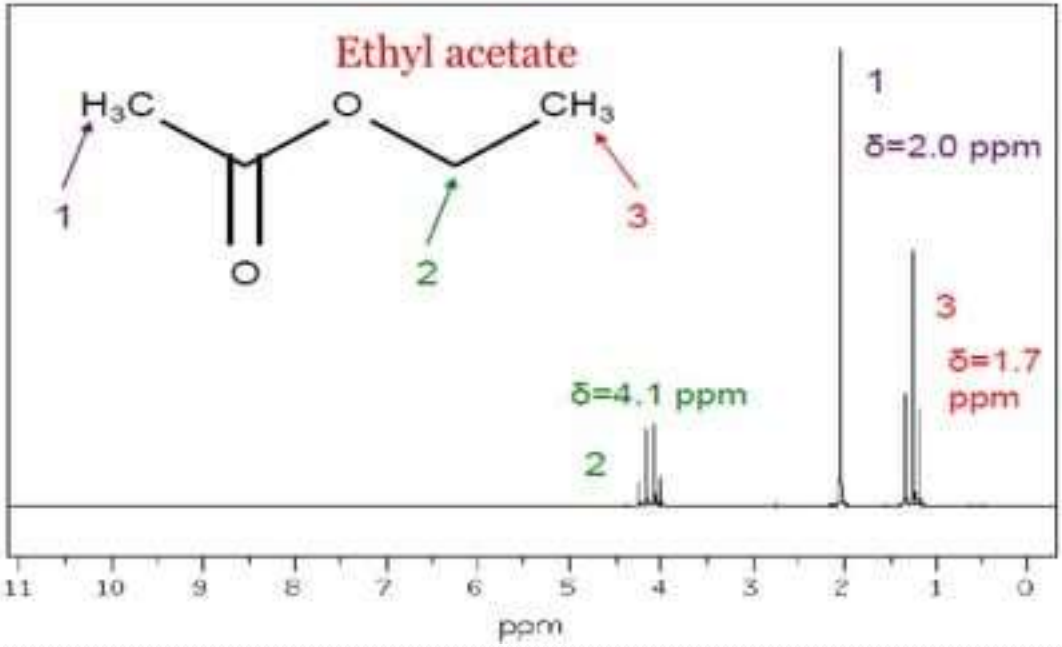
Therefore, n= Number of protons in nearby nuclei

- Zero H atom as neighbour n+1=0+1=1(singlet)
- One H atom as neighbour n+1=1+1 = 2(doublet)
- Two H atom as neighbour n+1=2+1 =3(triplet)

Spin-spin coupling (splitting)

The interaction between the spins of neighbouring nuclei in a molecule may cause the splitting of NMR spectrum.This is known as spin-spin coupling or splitting.

The splitting pattern is related to the number of equivalent H-atom at the nearby nuclei.



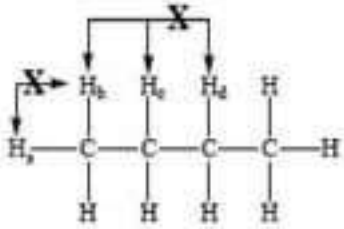
Spin-Spin Splitting patterns

1. Singlet. 3 equivalent protons. Not coupled to any neighboring protons.
2. Quartet. 2 equivalent protons. Split (1:3:3:1) because coupled to the 3 ^{1}Hs at the 2 position.
3. Triplet. 3 equivalent protons. Split (1:2:1) because coupled to the 2 ^{1}Hs at the 3 position.

Rules for spin-spin coupling:-

* Chemically equivalent protons do not show spin-spin coupling.

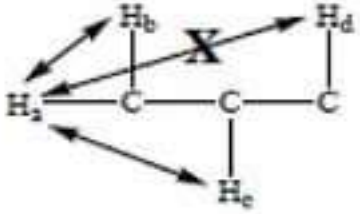
* Only nonequivalent protons couple.



H_b couples with H_c.
H_b and H_a do not couple because they are equivalent.
H_c and H_d do not couple because they are equivalent.

* Protons on adjacent carbons normally will couple.

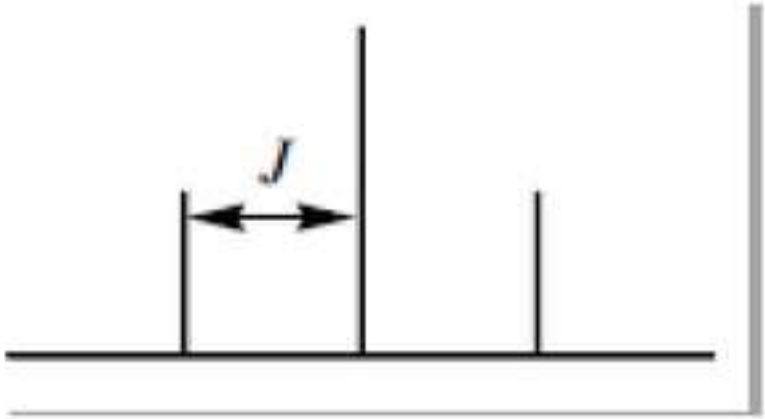
* Protons separated by four or more bonds will not couple.



H_a can couple with H_b
H_a can couple with H_c
H_a cannot couple with H_d

Coupling Constant

- The distance between the peaks in a given multiplet is a measure of the splitting effect known as coupling constant.
- It is denoted by symbol J, expressed in Hz.
- Coupling constants are a measure of the effectiveness of spin-spin coupling and very useful in ^{1}H NMR of complex structures.



2D NMR

Basics of 2D NMR Experiment:-

Basis: Interaction of nuclear spins (^1H with ^1H, ^1H with ^{13}C, etc.) plotted in two dimensions

- Normal spectra(1D NMR) are plots of intensity Vs frequency.
- In 2D NMR intensity is plotted as function of two frequencies called f_1 & f_2
- In general, 2D's can be divided into two types,

  Homonuclear

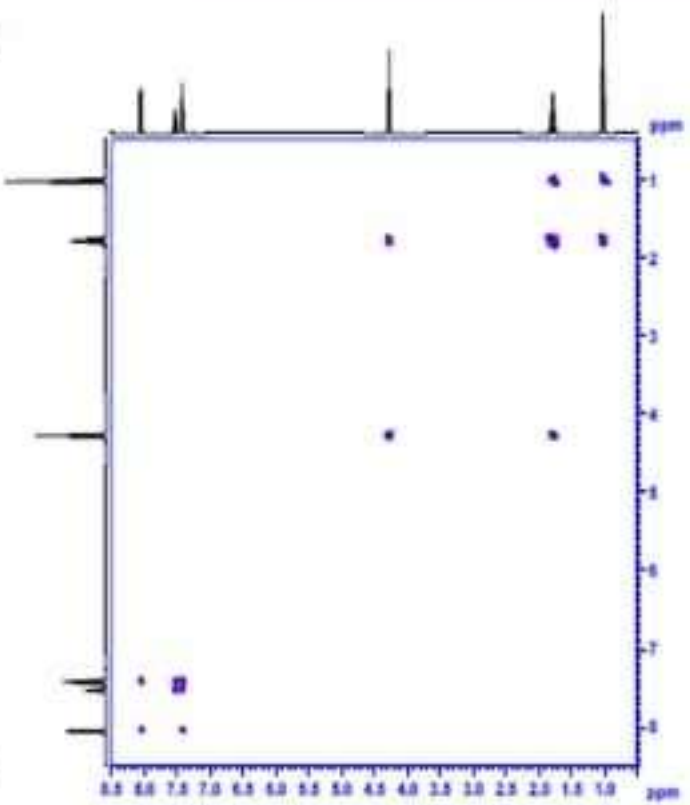
  Heteronuclear
- Each type can provide either through-bond

  (COSY-type) or through space (NOESY-type)

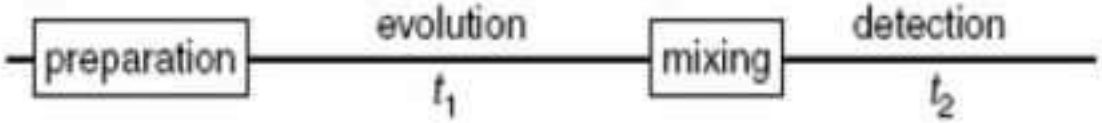
  coupling information

2D-Correlation spectra

- Correlates one spectrum with another
- May be the same or different nuclei
  - ^{1}H-^{1}H
  - ^{1}H-^{13}C
  - ^{13}C-^{13}C ^{1}H-^{15}N
  - May correlate differently
    - COSY – through bond
    - TOCSY – extended system
    - NOESY – through space



2-D NMR –Signal is recorded as a function of two time variables, *t1* and *t2*.



Pulse Sequence

•Rf pulses are generally applied during the preparation and mixing periods.

Applications of 2D NMR:-

- Simplifies analysis of more complex or ambiguous cases such as proteins.
- Obtain structural information not accessible by one-dimensional NMR methods.

Techniques include:

- Correlation Spectroscopy (COSY)
- Heteronuclear Correlation Spectroscopy (HETCOR)
- Heteronuclear Multiple-Quantum Coherence (HMQC)
- Nuclear Overhauser Effect Spectroscopy (NOESY)
- Incredible Natural Abundance Double Quantum Transfer Experiment (INADEQUATE)
- Many others

Routine 2-D NMR Experiments:-

COrrelationSpectroscopY(COSY) –Scalar Coupling

»Identifies all coupled spins systems.

Nuclear OverhauserEffect SpectroscopY(NOESY) –Dipolar Coupling

»Identifies neighboring spin systems (≤5 Å)

»Identifies chemical exchange.

Heteronuclear Multiple/Single Quantum Correlation (HMQC/HSQC) –Scalar Coupling

»Identifies coupling between heteronuclei(C-H, N-H)

COSY: Homonuclear correlated spectroscopy. Correlation between protons that are coupled to each other

TOCSY: Total Correlation Spectroscopy- Uses a spin-lock for coherence transfer. During the spin-lock all protons of a coupled system become "strongly coupled," leading to cross peaks between all resonances of a coupled system.

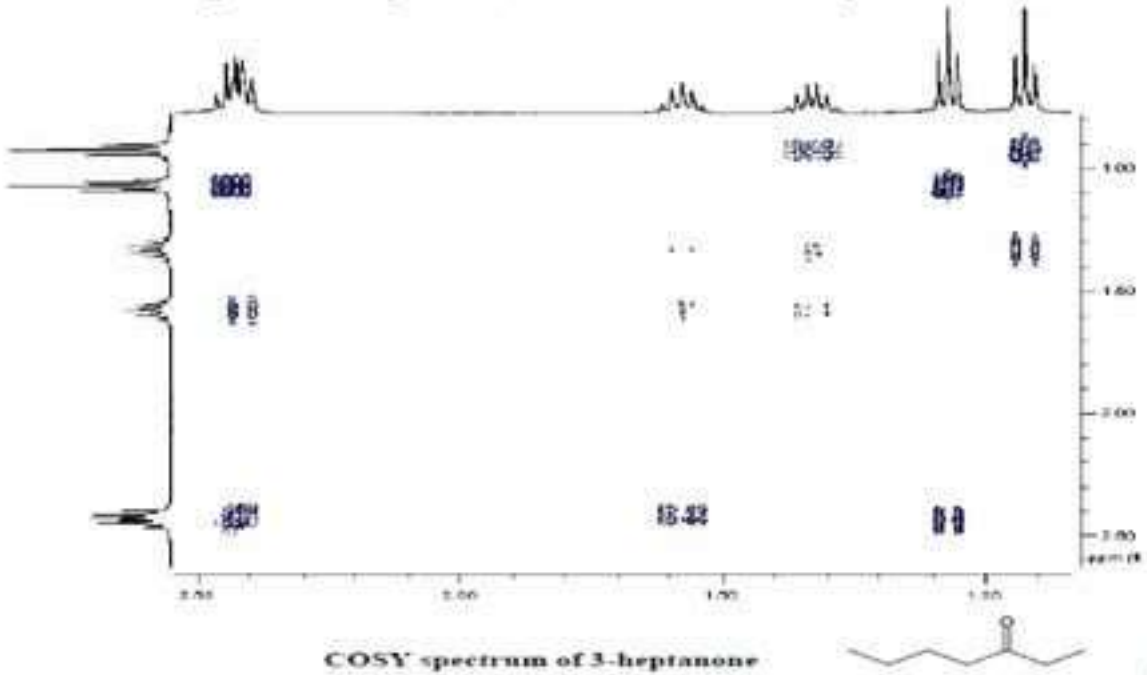
HETCOR: Heteronuclear correlation, usually between ^{1}H and ^{13}C resonances mediated by $J_{C\text{-}H}$.

NOESY, ROESY: Proton-proton correlation mediated by dipolar coupling (NOE effect). Correlation between protons that are close in space. This is the single most powerful NMR technique for determining the 3-dimensional structure of molecules from conformations of small molecules to the 3-dimensional structure of small proteins.

HOESY: Heteronuclear Overhauser Effect Spectroscopy. Correlation between protons and heteronuclei that are close in space.

COSY experiment

- Correlation Spectroscopy
- Commonly used for regiochemical assignment
- Cross-peaks appear if spin coupling is present
- Protons that are separated by 2 or 3 bonds are usually detected

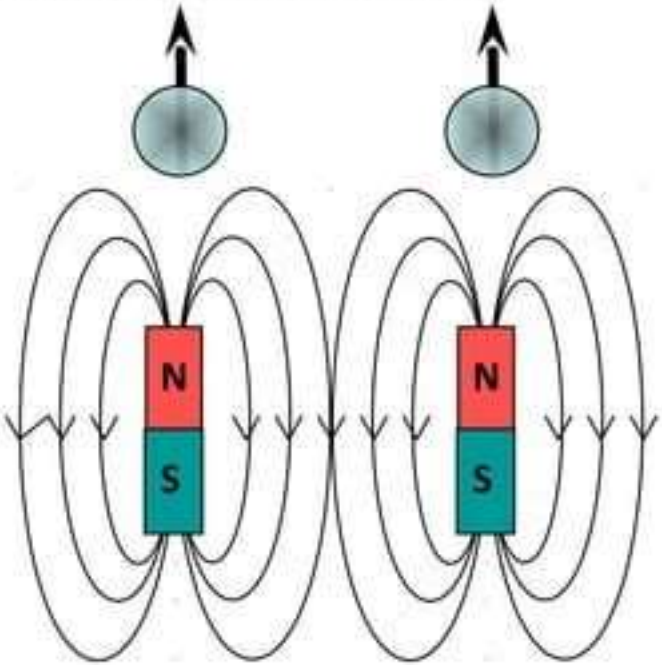


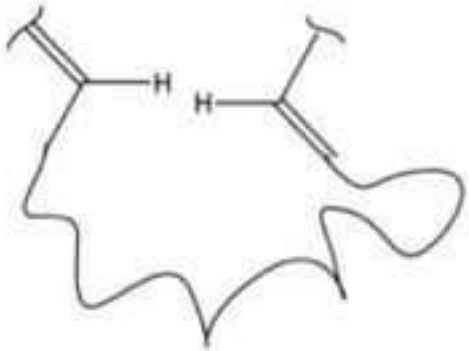
COSY spectrum of 3-heptanone

Nuclear Overhauser Effect

- NOE Caused by dipolar coupling between nuclei.

- The local field at one nucleus is affected by the presence of another nucleus. The result is a mutual modulation of resonance frequencies.



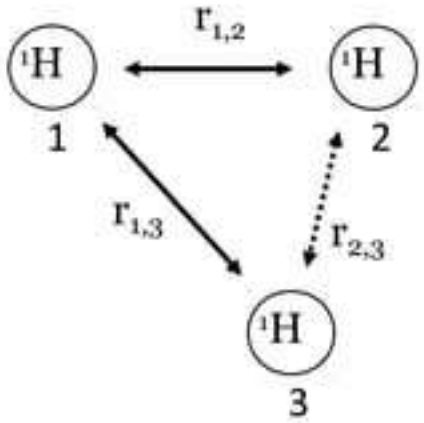


Nuclear Overhauser Effect

The intensity of the interaction is a function of the distance between the nuclei according to the following equation.

$$I = A\,(1/r^6)$$

I - intensity
A - scaling constant
r - internuclear distance



Arrows denote cross relaxation pathways
$r_{1,2}$ - distance between protons 1 and 2
$r_{2,3}$ - distance between protons 2 and 3

The NOE provides a link between an experimentally measurable quantity, I, and internuclear distance.
NOE is only observed up to 5 Å.

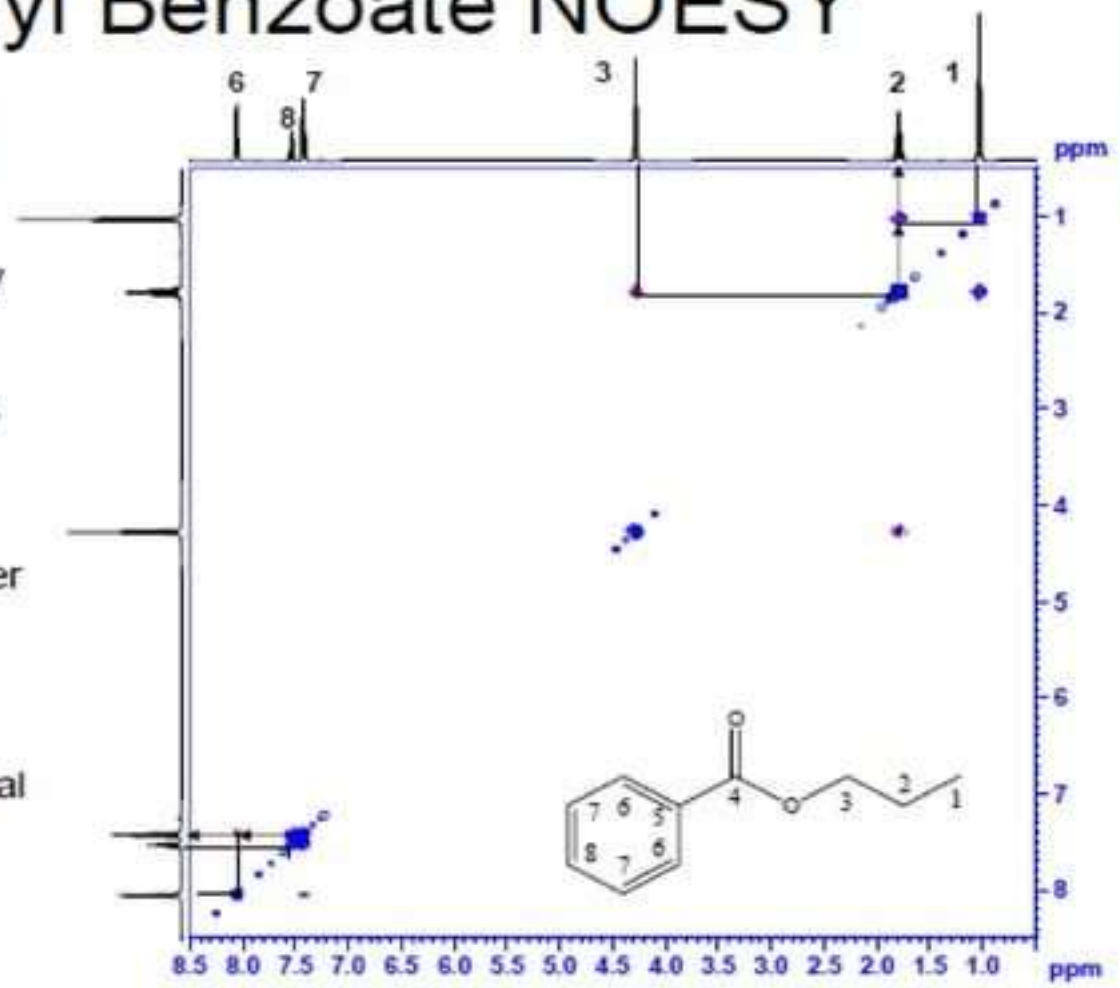
Propyl Benzoate NOESY

Nuclear Overhauser Effect Spectroscopy

Shows correlations to protons that are nearby in space to about 4 angstroms (most J coupling is filtered out).

Interpret by Noting which protons are close to one another (very useful for stereochemical analysis).

Protons that are geminal and vicinal will still appear because they are close.



GENERAL APPLICATIONS OF NMR SPECTROSCOPY

✓ NMR is used in biology to study the Biofluids, Cells, Per fused organs and biomacromolecules such as Nucleic acids(DNA, RNA), carbohydrates Proteins and peptides. And also Labeling studies in biochemistry.

✓ NMR is used in physics and physical chemistry to study High pressure Diffusion ,Liquid crystals ,liquid Crystal solutions , Membranes, Rigid solids.

✓ NMR is used in food science.

Contd...

✓ NMR is used in pharmaceutical science to study Pharmaceuticals and Drug metabolism.

✓ NMR is used in chemistry to ;

- Determine the Enantiomeric purity.
- Elucidate Chemical structure of organic and inorganic compounds.
- Macromolecules –ligand interaction.

^{1}H-NMR SPECTROSCOPY applications

^{1}H widely used for structure elucidation.

Inorganic solids- In organic compounds are investigated by solid state 1H-NMR.eg $CaSO_4 \cdot H_2O$.

Organic solids- Solid-state ^{1}H NMR constitutes a powerful approach to investigate the hydrogen-bonding and ionization states of small organic compounds.

- Direct correlation with hydrogen-bonding lengths could be demonstrated, e.g. for amino acid carboxyl groups.

Polymers and rubbers- Examine hydrogen bonding and acidity.

Peptides and proteins

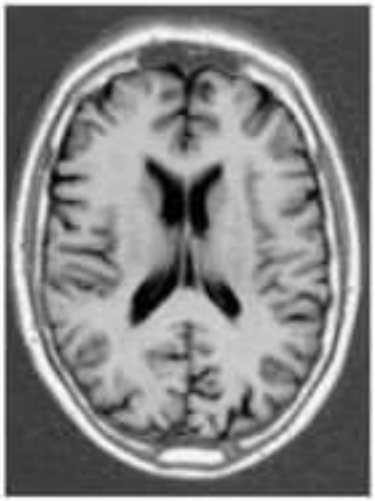
Clinical and scientific research

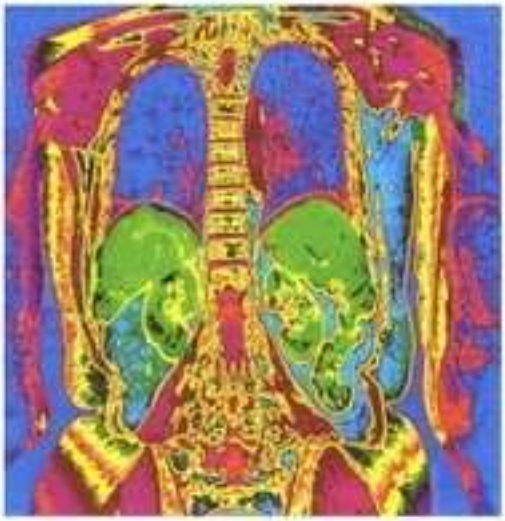
In vivo NMR studies-

- concerned with ^{1}H NMR spectroscopy of human brain.
- Many studies are concerned with altered levels of metabolites in various brain diseases.
- To determine the spatial distribution of any given metabolite detected spectroscopically IS (image selected *in vivo spectroscopy).*

Application of NMR in medicine

MRI is specialist application of multi dimensional Fourier transformation NMR





- ✓ Anatomical imaging.
- ✓ Measuring physiological functions
- ✓ Flow measurements and angiography.
- ✓ Tissue perfusion studies.
- ✓ Tumors

References:-

- Organic spectroscopy by William Kemp
- Instrumental methods of chemical analysis by Chatwal
- Instrumental methods of analysis by Willard
- Wikipedia.org

Thank You