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Unit-2

NMR spectroscopy: Quantum numbers and their role in NMR,

Principle, Instrumentation, Solvent requirement in NMR,

Relaxation process, NMR signals in various compounds, Chemical shift, Factors influencing chemical shift, Spin-Spin coupling, Coupling constant, Nuclear magnetic double resonance,

Brief outline of principles of FT-NMR and 13C NMR. Applications of NMR spectroscopy.

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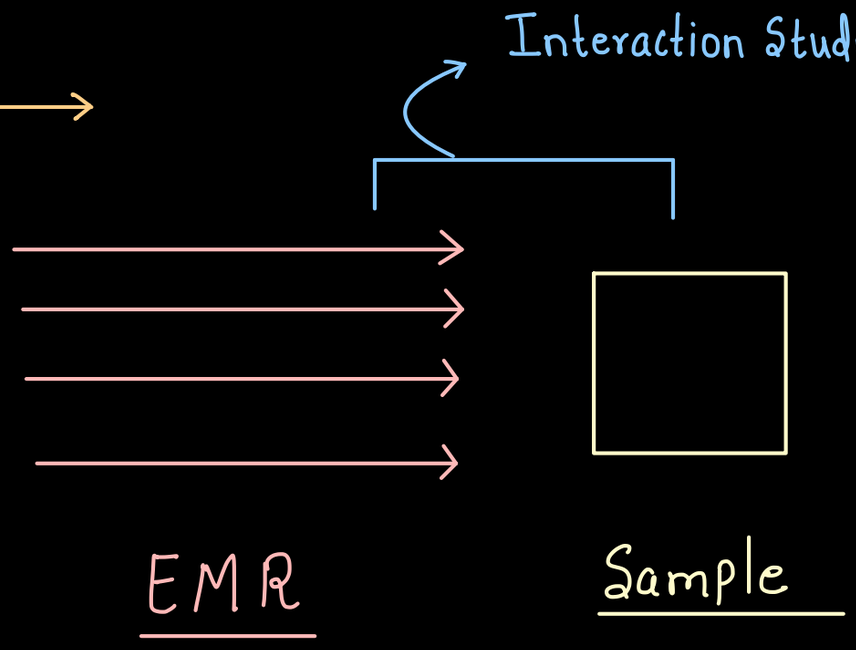
NMR SPECTROSCOPY

Nuclear Magnetic Resonance

Powerful Analytical Technique.

→ Used to Determine the Structure of Organic Compound & it's purity.



-Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical technique used to determine the structure of organic compounds by observing the behavior of nucleus in a magnetic field.

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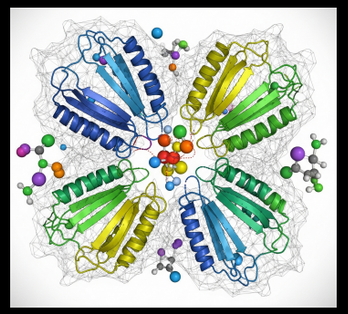
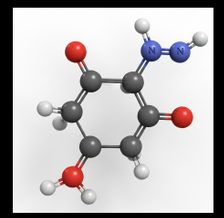
NMR is used in different Industries

1. **In biology lab protein structure is determined by NMR**

It's folding State & Dimerization State.



2. Also Used in Chemical Industry to determine Possible Struct. of Chemical Compound.



3. Also Used in Medical Industry

M.R.I Machine

* Based on NMR Principle.

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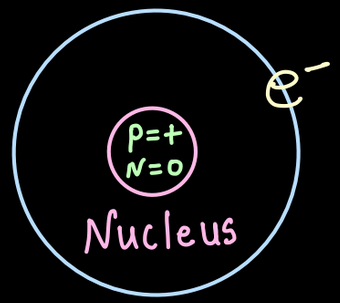
-NMR are of different types but most commonly H-NMR & C-13 NMR is used

- FT-NMR is widely used in organic chemistry, biochemistry, and materials science to characterize the structure of wide range of chemical compounds.



Atom

If proton & Neutron Spining

Nucleus also Spinning.

This Spinning is Seen by NMR.

Quantum Number

The Number which tells about Nucleus Spinning or Not.

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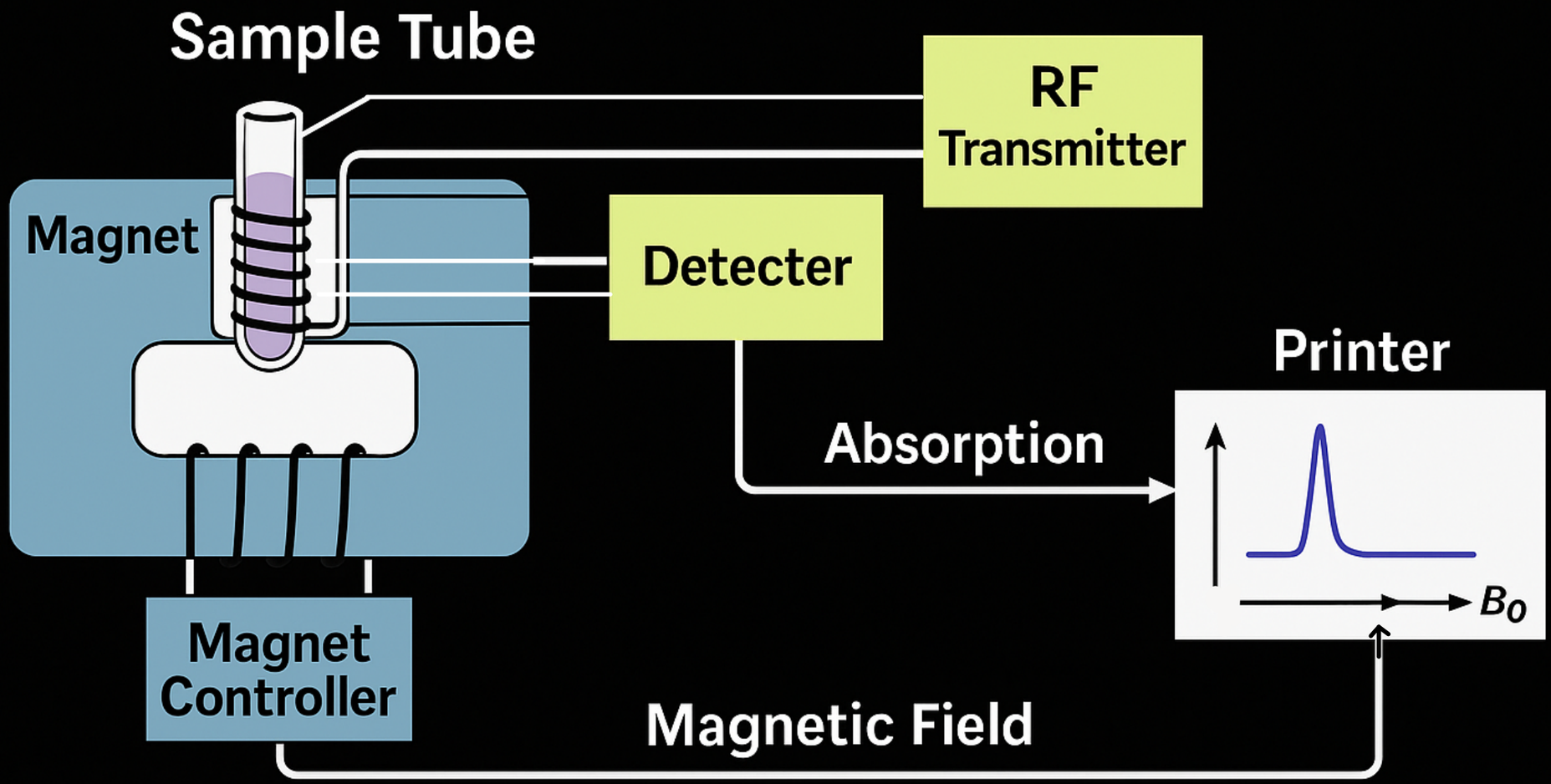
—NMR Machine are of 2 types

→ Large Size → Advance ||| hd NMR research spectrometer

→ Small Size → Benchtop Compact NMR Spectrometer

Nuclear Magnetic Resonance (NMR) Spectroscopy



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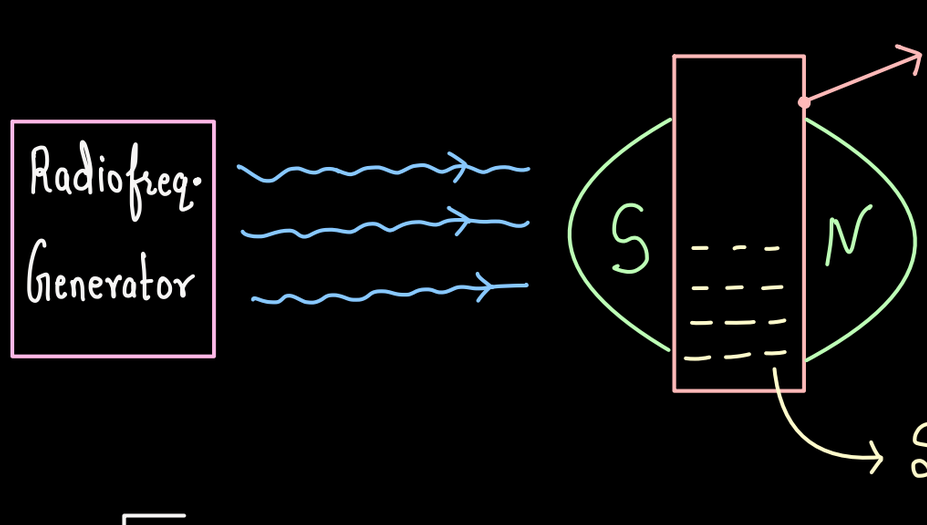
NMR → Contain 2 Magnets. → Produce Strong M.F

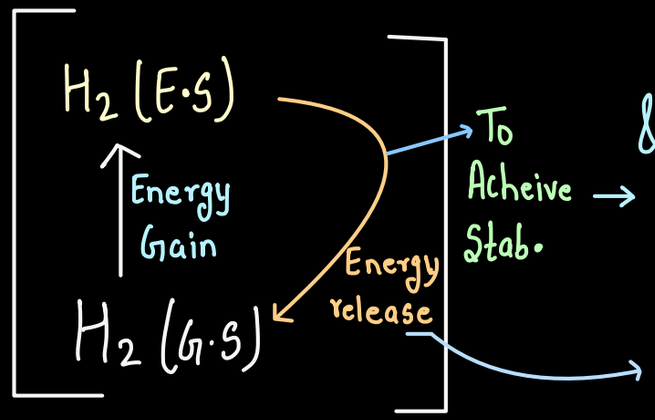


→ If Sample Contain H_2 the Solvent Must be Other then H_2

↓

Deuterium [$^{2}_{1}H$]

To avoid Error.



& this process is known as Relaxation process.

Detect by Detector & Graph forms. [NMR Spectrum].

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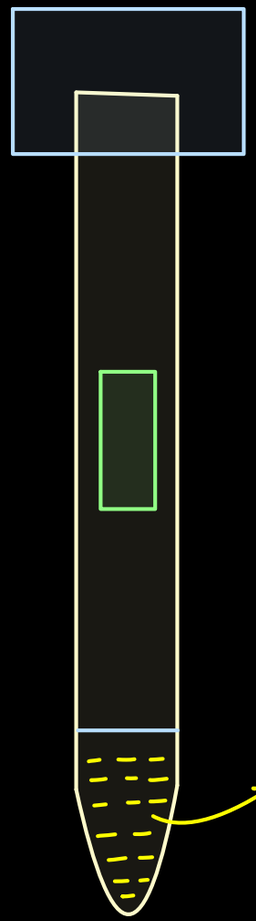
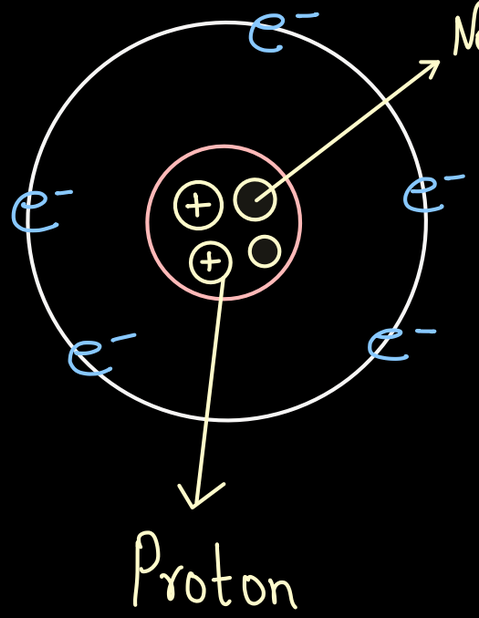


Sample.

Sample Must Contain NMR Active Atom.



In Case of NMR → We talk about Protorn & Neutron [Nucleus].

Proton & Neutron Show Spin around their Axis.

Due to this Spin also Exist on Nucleus.

[Only in Case of [1,3,5,7] odd.]

In Case of Even Number of Proton & Neutron then Spin Paired up & overall No Spin of Nucleus.

→ Even = 2, 4, 6, 8.

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→ 1, 3, 5, 7

-If odd number of proton & Neutron is present then Nucleus show Spin & this will be NMR active Atom.

→ 2, 4, 6, 8.

-If even number of proton & Neutron is present then Nucleus do not show Spin & this will be NMR Inactive Atom.

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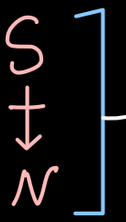
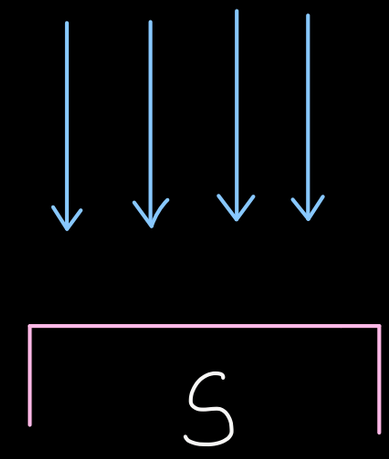
Number of Proton.	Number of Neutron.	Spin	Example
Even	Even	Zero	
Odd	Odd	Non Zero	H^2
Even	Odd	Non Zero	C^{13}
Odd	Even	Non-Zero	N^{15}

→ NMR Active

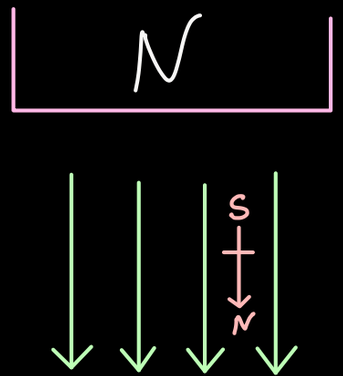
Principle of NMR Spectroscopy



H_2 atom act like small Magnet.



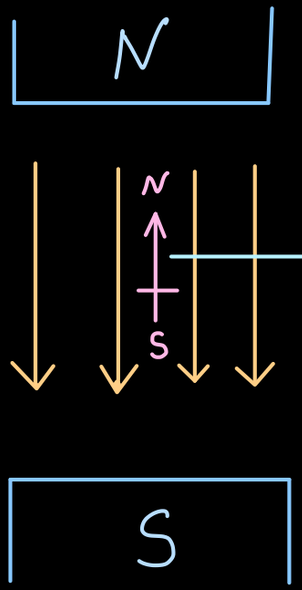
External Magnetic field.



α-Spin State

↓

Low Energy State. (Stab.↑).



β-Spin State

↓

High Energy.

&

Less Stability.

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→ Sample = H_2 ⟶ Contain proton [Charge Particle].

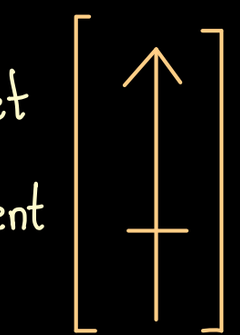
Rotate around axis.

& due to this rotation Charge Particle behave like a Magnet.

∴

Here Magnetic field direction is from North to South.

Magnet represent



Magnetic Vector.

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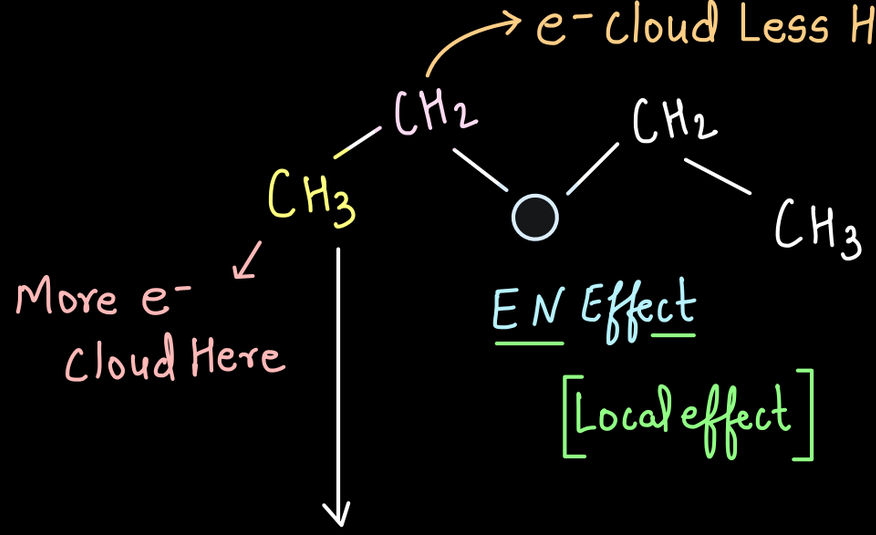
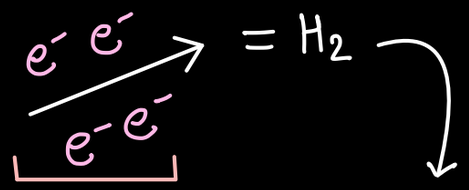
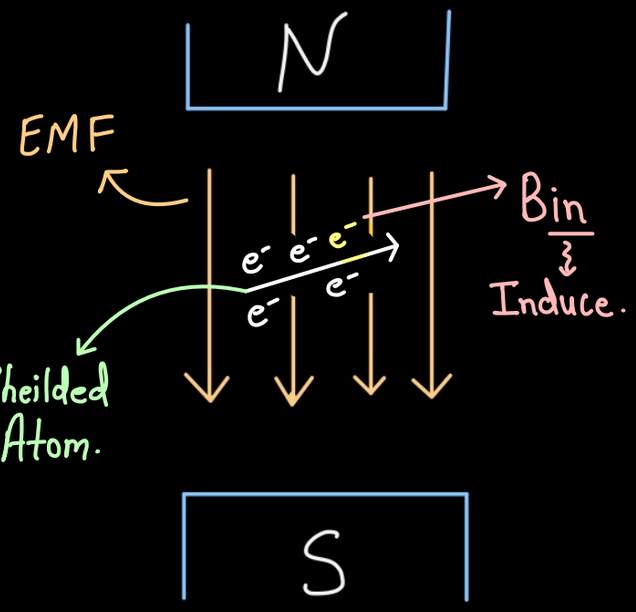
-But if Sample is molecule like ⟶

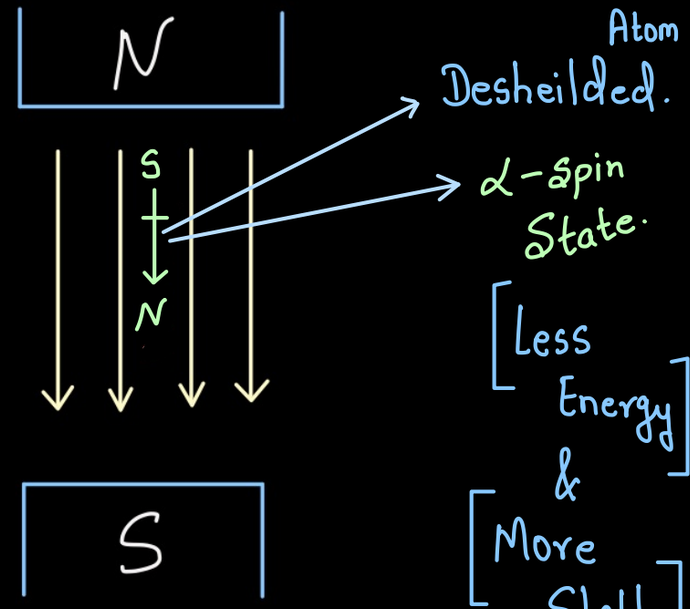




• In the ⊕nsce of Magnet, e⁻ Induce Magn• Field ⟶ Direction opp• to Ext• MF

Here direction ⟶ Orientation. is not too Much CHange.





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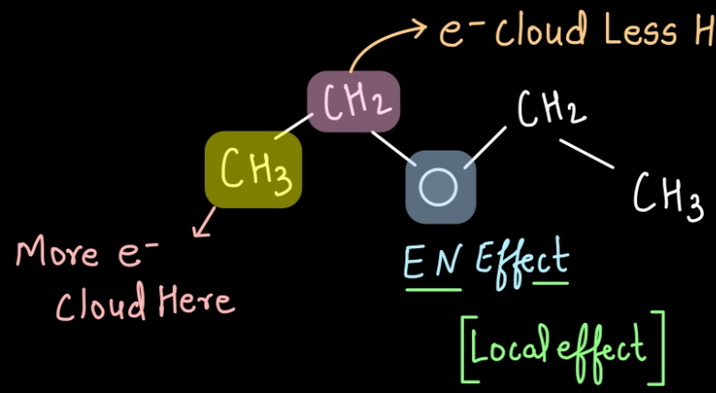
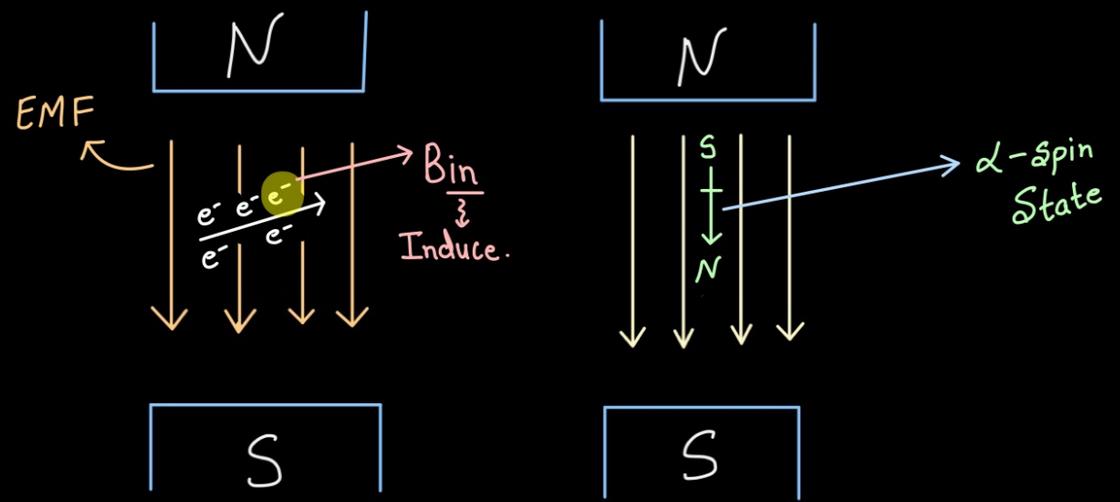
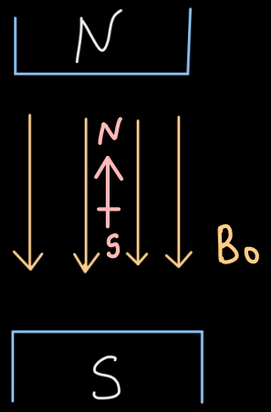




Less Energy required for Converting into β-State.

More Energy req. [α → β].
because Complete Orientation change.

Radiowaves (Energy).



Sample absorb Energy & goes to G.S to E.S

This is Resonance State.

β - State.

[High Energy State].

Less Stability.

1. Chemical Shift ⟶

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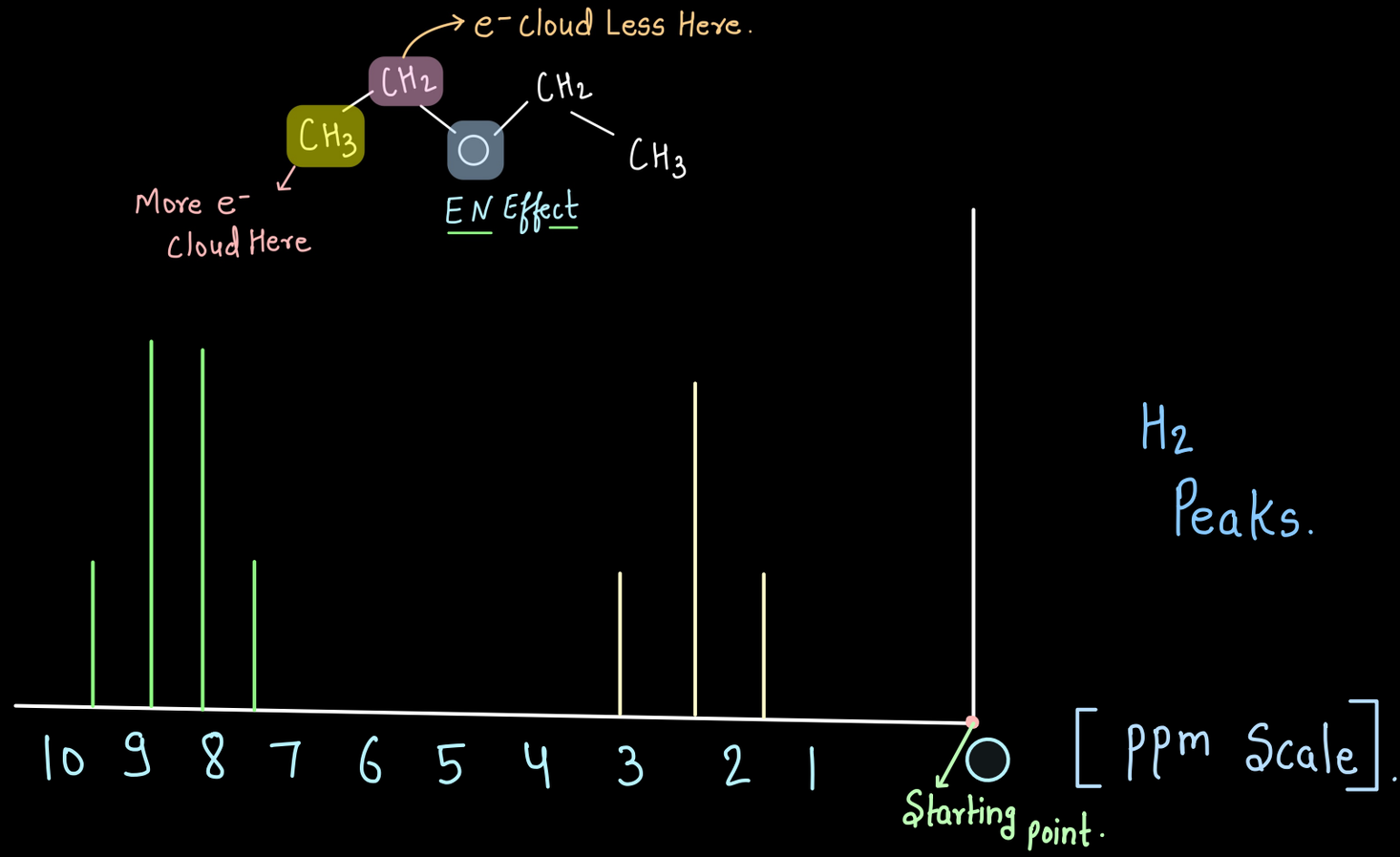
-When we do NMR (Nuclear Magnetic Resonance), we are looking at how the tiny nuclei (like hydrogen) behave in a magnetic field.

- Different hydrogen atoms (or other nuclei) in a molecule are in different environments — some near electronegative atoms, some near double bonds, etc.

➡️ Because of this, they absorb energy at different positions.

This difference in where they absorb energy is called the chemical shift.



[Desheilded H_2 Atom].

Downfield ⟵

↓

More Energy req.

to Come in E-State.

Upfield ⟶

↓

Less Energy req.

to Come in E-State [Resonance State].

[Sheilded H_2 Atom]

2. Peak Splitting-

To know the Spliting we follow $[N+1]$ rule

$[N = \text{Number of Neighb. } H_2]$

Eg → In Case of CH_3

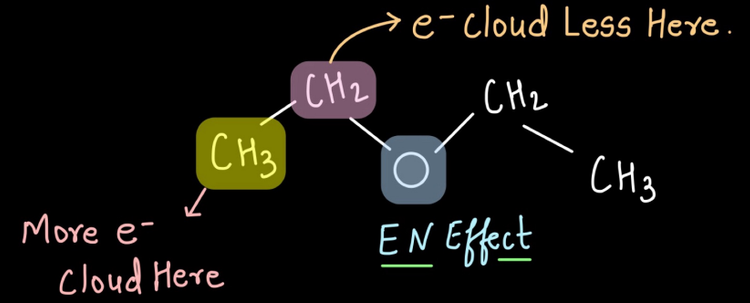
$[N+1]$

$[2+1] = 3$ Peaks.

CH_2

$[N+1]$

$[3+1] = 4$ Peaks.



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Number of Neighbors (n)	Splitting Pattern	Called a...
0	1 peak	Singlet
1	2 peaks	Doublet
2	3 peaks	Triplet
3	4 peaks	Quartet
4	5 peaks	Quintet

Solvent Requirement in NMR (Especially for ^{1}H and ^{13}C NMR)

1. Use of Deuterated Solvents

NMR solvents are usually deuterated solvents, meaning they contain deuterium (^{2}H) instead of normal hydrogen (^{1}H).

💡 *Why deuterated solvents are needed:*

Normal hydrogen (^{1}H) gives a strong signal in ^{1}H NMR and would interfere with your sample's signals.

Deuterium (^{2}H) does not appear in the ^{1}H NMR spectrum.

2. Solvent Must Dissolve the Sample Completely

The solvent should completely dissolve the sample to ensure a homogeneous solution.

It should not react with the sample.

3. Solvent Should Be Chemically Inert

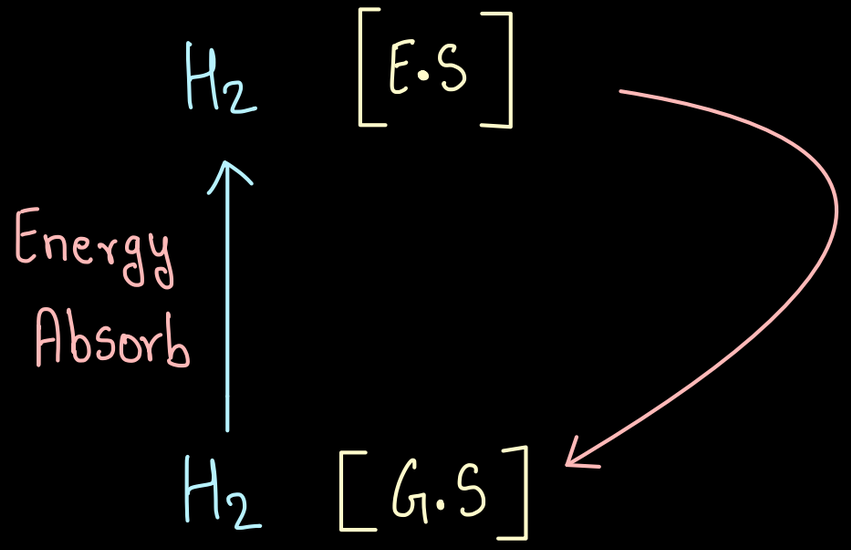
It should not react with your compound.

It should not exchange protons with functional groups (especially –OH, –NH).

🧪 Common Deuterated Solvents Used:

Solvent	Abbreviation	Chemical Formula	Use
Deuterated Chloroform	$CDCl_3$	C^2HCl_3	Most common for nonpolar samples
Deuterated DMSO	DMSO-d_6	$(CD_3)_2SO$	For polar, high-boiling samples
Deuterated Water	D_2O	^2H_2O	For water-soluble compounds
Deuterated Acetone	Acetone-d_6	$(CD_3)_2CO$	Polar samples
Deuterated Methanol	MeOD	CD_3OD	Polar, protic solvents
Deuterated Benzene	C_6D_6	C_6D_6	Aromatic compounds

Relaxation Process in NMR



Relaxation Process.

There are 2 types of Relaxation process.

T_1 relaxation or Spin Lattice relaxation

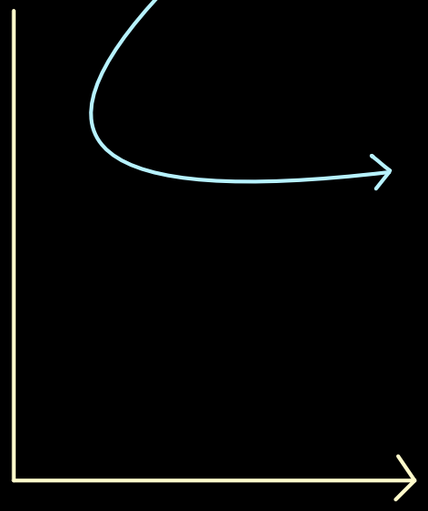
T_2 Relaxation or Spin Spin Relaxation.

DEPTH OF BIOLOGY

There are 2 types of Relaxation process.

- T_1 Relaxation or Spin Lattice Relaxation → Atom give back Energy to Surrounding. [Lattice]
  - → Time taken = T_1
  - → Help in return to G.S (Align with M.F).
  - → Important for getting Clear Signal.
- T_2 Relaxation or Spin Spin Relaxation. → Energy is not given Outside

T_2 time.



→ Atom Share Energy With Each other.

→ Cause Signal to fade & become broader.

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🧪 Example 1: Methanol (CH_3OH)

CH_3 group → 1 signal (around 3.3 ppm)

OH group → 1 signal (around 4–5 ppm)

OH peak is broad (not sharp)

🧪 Example 2: Ethanol ($CH_3–CH_2–OH$)

CH_3 → Triplet (3 small peaks) – around 1.2 ppm

CH_2 → Quartet (4 small peaks) – around 3.6 ppm

OH → Broad singlet – around 4.5 ppm

🧪 Example 3: Benzene (C_6H_6)

All hydrogens are same → only 1 peak

Appears at around 7.3 ppm

🧪 Example 4: Acetone ($(CH_3)_2CO$)

Both CH_3 groups are same → only 1 peak

Appears around 2.1 ppm

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More peaks = different types of H in the molecule

Split peaks = nearby Hs affecting each other

Nearby H_2 Atom (+)nt on Carbon.

Broad peak = OH or NH group

Factors Influencing Chemical Shift in NMR-

Upward Downward.

The chemical shift tells us where a peak appears in the NMR spectrum — it depends on the chemical environment of the hydrogen (or carbon) atoms.
Let's look at the main factors that influence chemical shift:

1. Electronegativity of Nearby Atoms-

Electronegative atoms (like O, N, F, Cl) pull electrons away.
This deshields the hydrogen (less electron density).
Result → Peak shifts downfield (toward higher ppm).

Example:
CH_4 (methane) = ~0.2 ppm → Position of Peak in NMR Graph.
CH_3Cl = ~3.1 ppm (Cl is electronegative)

2. Hybridization of the Carbon

Hydrogen attached to sp^2 carbon = more deshielded → higher ppm

Hydrogen on sp^3 carbon = more shielded → lower ppm

Example:

CH_3- (sp^3) = ~1 ppm

=CH− (sp^2, alkene) = ~5–6 ppm

−C≡CH (sp, alkyne) = ~2.5 ppm

3. Aromaticity (Ring Currents)-

Aromatic rings (like benzene) create ring currents that deshield nearby Hs.

Result → Peaks shift downfield.

Benzene protons: ~7.3 ppm

flow of e⁻ in
Aromatic ring
in the Influence of M.F

4. Magnetic Anisotropy

Caused by pi-electrons in alkenes, alkynes, aromatics.

They create local magnetic fields that affect nearby hydrogens.

Example:

Alkyne (C≡C–H) = ~2.5 ppm

Alkene (=CH) = ~5–6 ppm

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5. Hydrogen Bonding

OH or NH groups can form hydrogen bonds → causes broadening and chemical shift variation.

Can move downfield depending on strength of H-bond.

Example:

Alcohol (OH) H = ~1–5 ppm (broad, variable)

6. Solvent Effects

The solvent used (especially if polar or capable of H-bonding) can slightly shift peaks.

Deuterated solvents are preferred to reduce interference.

→ Alternate Single or = bond.

that allow e⁻ to Move freely.

Conjugation and Resonance

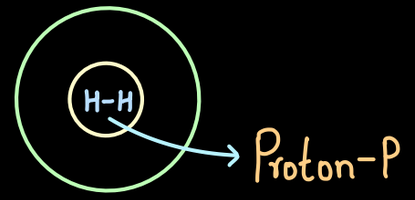
Conjugated double bonds or resonance systems delocalize electrons, reducing shielding.

Result → Higher ppm

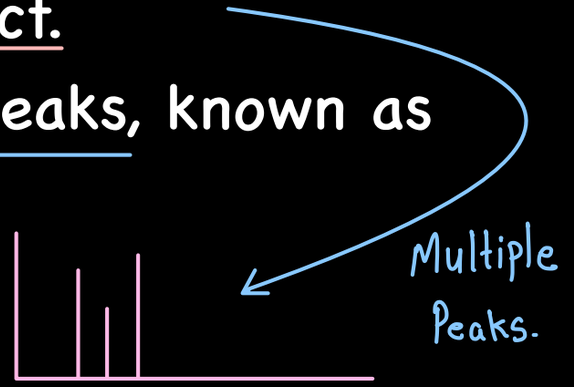
Example:

$CH_3-C=O$ (acetone) methyl = ~2.1 ppm

Coupling Constant (J)



When a proton is near another proton, their magnetic fields interact.
This interaction splits the NMR signal of one proton into multiple peaks, known as multiplets.
The spacing between these peaks = coupling constant (J).



It's measured in hertz (Hz), and it tells us the distance between split peaks in a multiplet (like doublet, triplet, etc.).

It tells us how many hydrogens are nearby. [More H_2, More Splitting].

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Why is it important?

It helps confirm how many neighboring hydrogens (H) are interacting.

Also tells us what kind of coupling is happening (cis, trans, geminal, etc.).

Example:

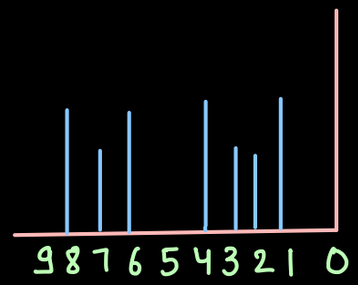
CH_3-CH

-The CH hydrogen is split by 3 hydrogens from CH_3 → it becomes a quartet

-The distance between each line in the quartet is the coupling constant (J)

Let's say:

The lines are 7 Hz apart → J = 7 Hz



Nuclear Magnetic Double Resonance (NMDR or Double Irradiation)

NMDR is an advanced NMR technique where two radiofrequency (RF) fields are applied simultaneously to the sample:

One to observe the spectrum
Another to irradiate a specific nucleus (usually a proton) continuously
It helps identify spin-spin coupling relationships between nuclei.

Why NMDR ?

Sometimes, NMR peaks are complex because of overlapping coupling.
By irradiating one peak, we can see if another peak is split by it or not — this confirms if they are coupled

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🔍 How it works-

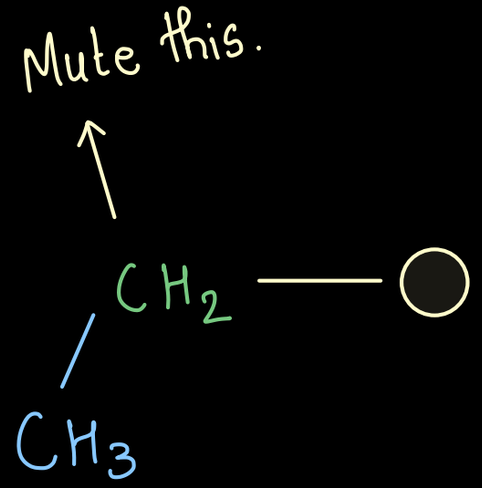
Suppose you have two peaks:

Peak A is being split because of Peak B.

You irradiate Peak B continuously.

This removes the effect of B.

Now Peak A collapses to a singlet if it was split by B.



Use of Double Resonance:

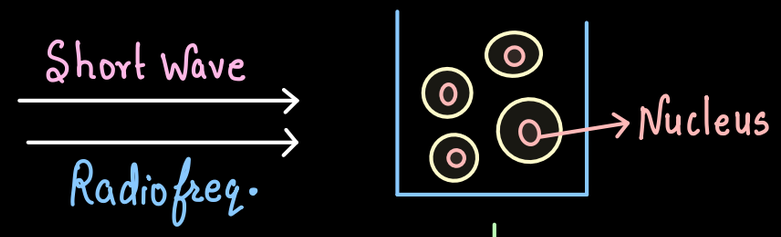
To find out which hydrogens are coupled
To simplify complex spectra
Helps in structure determination

Term	Meaning in Simple Words
Coupling Constant (J)	Distance between split peaks; tells strength of interaction between nearby Hs
Double Resonance	Special NMR method where one signal is "turned off" to see how it affects other signals

FT-NMR = Fourier Transform Nuclear Magnetic Resonance

Instead of scanning one frequency at a time (like old continuous-wave NMR), FT-NMR uses a short, powerful radiofrequency (RF) pulse to excite all nuclei at once, and then measures how they relax.

Step 1: Wake up all atoms with a quick signal-



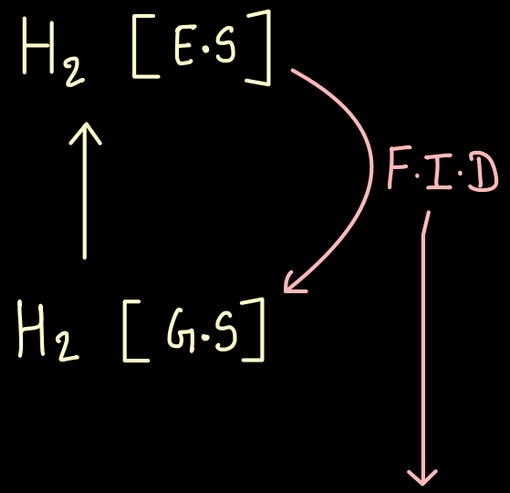
To, Excite all Nucleus at Once. & then Measure how they relax.

The NMR machine sends a short, strong energy pulse (radio wave).

This excites all hydrogen atoms at once.
They "wake up" and start spinning differently.
This takes just a tiny fraction of a second.

Step 2: Atoms calm down (relax) and send signals-

After the pulse, the atoms slowly return to normal.
As they relax, they release signals (like a sound wave).
These signals are called FID = Free Induction Decay
Think of it like a bell ringing after being hit.



Step 3: Computer records the signal-

The FID signal is saved.
But it's in a form that we can't understand directly (like noise or wavy lines).

We Can't Understand Directly

So, we Use Computer

It CHanges the Wavy Signal into Peaks.

This peaks form the NMR Spectrum.

We Can See Graph peak at diff. ppm.

1. Faster
2. More Sensitive
3. Better Quality.

Step 4: Computer changes it using "Fourier Transform"

The computer uses math called Fourier Transform (FT).
It changes the wavy signal (FID) into peaks.
These peaks form the NMR spectrum.

Step 5: You see the NMR spectrum

Now we see a graph with peaks at different ppm.
Each peak tells you what type of hydrogen or carbon is in your compound.

DEPTH OF BIOLOGY

Why do we use FT-NMR-

Because it is faster, more sensitive, and gives better-quality spectra compared to old continuous-wave (CW) NMR.

1. Excites all atoms at once-

Old method (CW NMR) scanned one frequency at a time

FT-NMR gives a short pulse → excites all nuclei at once

Result: Faster data collection

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2. Saves time

Instead of spending minutes scanning each peak,

FT-NMR gets the whole spectrum in 1 second (or a few seconds)

3. Better sensitivity

FT-NMR allows signal averaging: the machine repeats scans many times and adds them together to remove noise

You can detect even very small amounts of sample

4. More accurate and sharper signals

With FT-NMR, peaks are clearer and better resolved

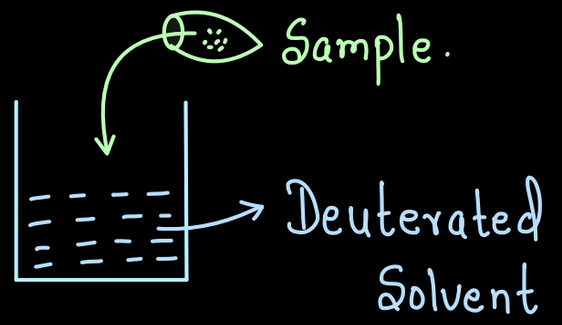
Helps in analyzing complex molecules

5. Works for low-abundance nuclei

Like ^{13}C or ^{31}P → FT-NMR makes it easier to see them because of signal enhancement

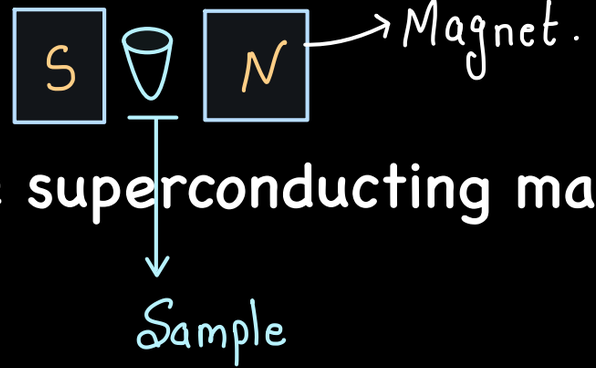
Advantage	Why it's Important
Fast scanning	Saves time in analysis
High sensitivity	Detects weak signals (small samples)
Better resolution	Gives sharp, clear peaks
Allows averaging	Reduces noise in spectra
Multi-nuclei capable	Works with ^{1}H, ^{13}C, ^{31}P, etc.

How it works in detail



1. Sample Preparation-

The chemical sample (liquid or solid dissolved in a deuterated solvent, like $CDCl_3$ or D_2O) is placed in an NMR tube.



This tube is inserted into the center of the superconducting magnet in the NMR machine.

2. Magnetic Field (B_0) Application

A very strong magnetic field (B_0) is created by the superconducting magnet.

Nuclei with spin (like ^{1}H, ^{13}C, ^{31}P) behave like tiny magnets and align with or against B_0.

A few more nuclei align with the field, creating a small net magnetization.

3. Radiofrequency (RF) Pulse Excitation

A short burst of RF energy is sent to the sample via RF coils.

This energy temporarily tips the aligned nuclear spins away from the magnetic field direction.

Think of it like nudging spinning tops off balance.

4. Signal Detection – Free Induction Decay (FID)

After the RF pulse is turned off, the excited spins relax back to their original position.

As they relax, they emit tiny radio signals. → Signal picked up by R.F Coils. (Signal Called F.I.D)

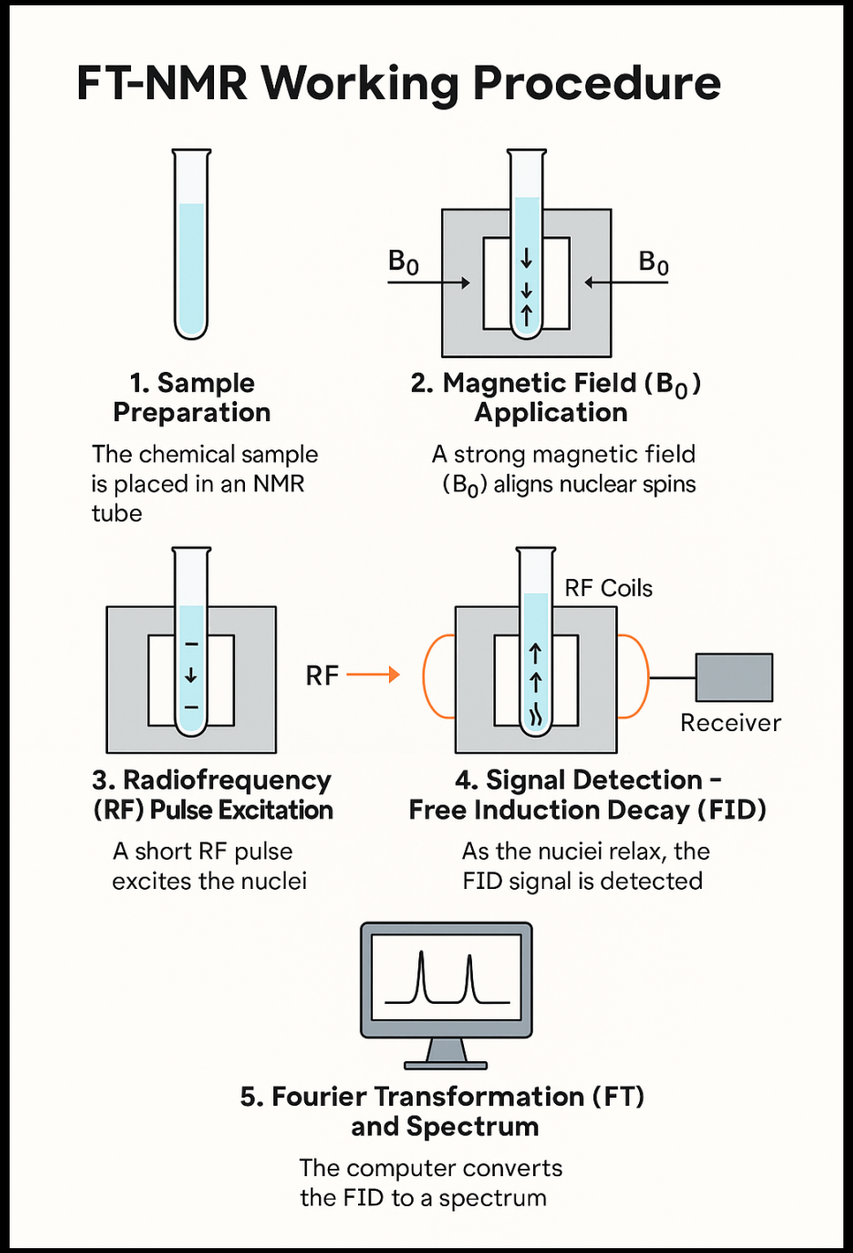
These signals are picked up by the RF coils (now working as receivers).

The raw signal is called Free Induction Decay (FID) – a time-based signal with all the frequency information.

5. Fourier Transformation (FT) and Spectrum-

The FID signal is sent to a computer, where a mathematical process called Fourier Transform is used.

FT changes the FID (time domain) into an NMR spectrum (frequency domain).
The resulting spectrum shows peaks – each peak tells us about the type and environment of nuclei in the molecule.



13C-NMR Spectroscopy (Carbon-13 Nuclear Magnetic Resonance)

13C-NMR is based on the absorption of radiofrequency radiation by the carbon-13 isotope (^{13}C), which has a nuclear spin (I = ½) and is magnetically active.

When placed in a strong magnetic field, ^{13}C nuclei align either with or against the field.
Irradiating them with radiofrequency causes transition between these energy levels.

The energy absorbed (measured in ppm) depends on the chemical environment of the carbon atom.
Each chemically distinct carbon atom in a molecule gives a separate signal in the ^{13}C-NMR spectrum.

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Basic Outline / Steps:

1. Sample Preparation

Dissolve in deuterated solvent (e.g., $CDCl_3$).

2. Placement in Magnetic Field

Sample is placed in a strong magnetic field (usually superconducting magnet).

3. Radiofrequency Pulse

A short RF pulse excites ^{13}C nuclei.

4. Relaxation & Signal Detection

Excited nuclei relax to ground state, emitting energy (Free Induction Decay - FID).

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5. Fourier Transform (FT)

Converts FID signal into an interpretable spectrum (frequency vs. intensity).

6. Spectrum Interpretation

Peaks represent different types of carbon atoms.

Chemical shifts (in ppm) tell us about carbon environments (e.g., alkyl, aromatic, carbonyl).

🧭 Common Chemical Shifts (approximate):

Carbon Type	δ (ppm) Range
Methyl / Alkyl (CH_3, CH_2)	0 – 50
Carbon next to electronegative atom	50 – 90
Alkene / Aromatic	100 – 160
Carbonyl (C=O)	160 – 220

Instrumentation of 13C-NMR Spectrometer

1. Superconducting Magnet

Generates a strong, stable magnetic field (typically 200–900 MHz).

Maintains low temperature using liquid helium and nitrogen.

2. Radiofrequency (RF) Transmitter

Produces radiofrequency pulses to excite the ^{13}C nuclei.

Tuned to the resonance frequency of ^{13}C (~25 MHz for 100 MHz ^{1}H NMR).

3. Sample Probe

Holds the sample tube inside the magnet.

Contains RF coils to transmit pulses and detect the NMR signal.

4. RF Receiver / Detector

Detects the weak signal (FID) emitted by nuclei as they relax.

Converts analog signal to digital.

5. Analog-to-Digital Converter (ADC)

Converts Free Induction Decay (FID) into a digital signal.

Sends it to the computer for processing.

6. Computer & Fourier Transform Unit

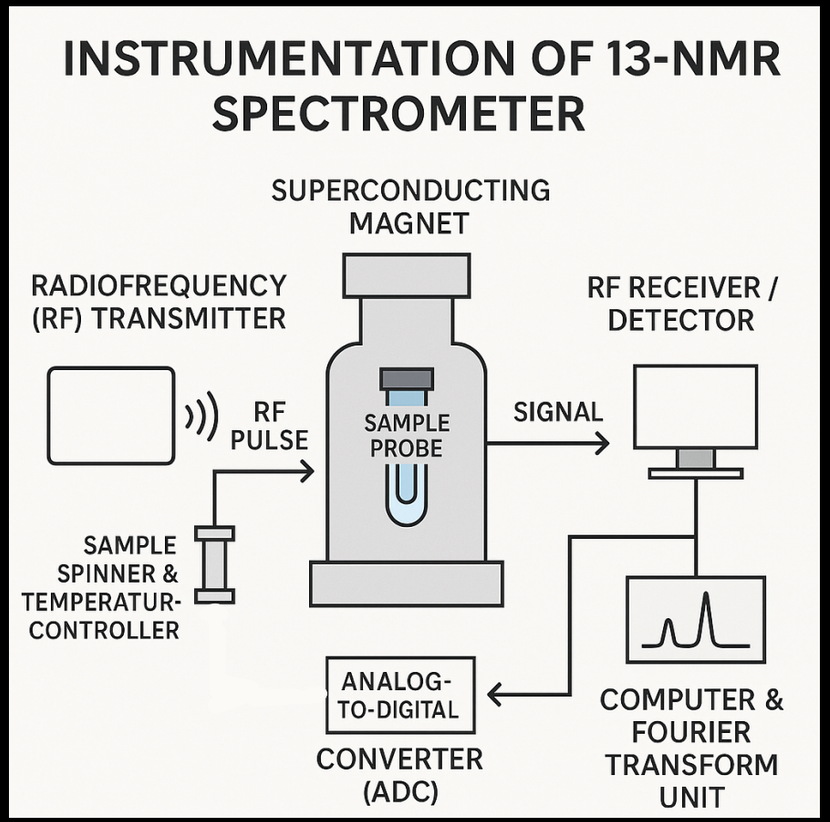
Performs Fourier Transform to convert time domain FID → frequency domain spectrum.

Displays the 13C-NMR spectrum for analysis.

7. Sample Spinner & Temperature Controller -

Spins the sample to average out magnetic field in homogeneities.

Maintains constant temperature to ensure stability of the signal.



DEPTH OF BIOLOGY

Applications of NMR Spectroscopy

1. Structure Determination of Organic Compounds-

Identifies the number and types of hydrogen (^{1}H NMR) and carbon atoms (^{13}C NMR) in molecules.

Determines the molecular framework of unknown compounds.

Helps in stereochemistry and conformational analysis.

2. Identification of Unknown Substances-

NMR provides a fingerprint-like spectrum to recognize unknown samples.

Used in forensic chemistry and quality control labs.

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3. Quality Control in Pharmaceuticals

Confirms the purity and identity of drug compounds.

Detects impurities

Ensures batch-to-batch consistency in pharmaceutical production.

4. Reaction Monitoring

Follows the progress of a chemical reaction in real time.

Monitors formation of intermediates and final products.

5. Drug Discovery & Metabolomics

Used in drug design to study binding of ligands to receptors.

Helps in studying metabolite profiles in biological samples (metabolomics).

Applied in pharmacokinetics to study how drugs behave in the body.

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6. Biological and Biochemical Applications-

Determines the 3D structure of proteins, peptides, and nucleic acids in solution (using 2D and 3D NMR).
Used in studying enzyme-substrate interactions.
Analyzes biological fluids (e.g., blood, urine) in medical diagnostics.

7. Solid-State NMR

Studies solids like polymers, catalysts, membranes, and minerals.
Useful for materials science, nanotechnology, and battery research.

8. Environmental and Food Analysis

Identifies contaminants in water, soil, and food products.
Determines adulteration and composition of oils, wines, juices, etc.

9. Magnetic Resonance Imaging (MRI)-

Based on the principle of NMR.

A powerful medical imaging technique to visualize internal organs and tissues.