NMR Spectroscopy

A spectroscopy tool

Applications

- In medicines (MRI)
- Food technology
- Major application in chemistry is to determine the structure of molecules
- In biology, for determining the structure of biomolecules

Why is it so special ?

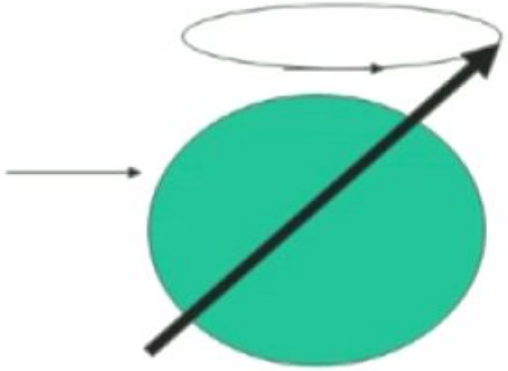
- Non invasive
- Each atom in a molecule can be probed
- Material can be studied in different pH,pressure ,temperature
- All states of matter can be studied
- Dynamics at an atomic scale,for a range of time can be studied

How does it work?

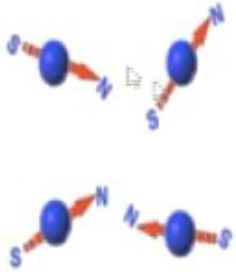
- Only active NMR nuclei show the spectrum

  Eg : H , 13C ,19F ,15N

Proton/Neutron Inside the Nucleus (spin= ½)

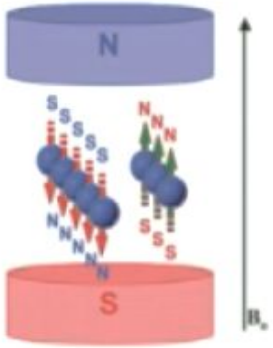


A spinning charge creates a magnetic moment, so these nuclei can be thought of as tiny magnets.



In absence of any external Magnetic field

Applying External Magnetic field



The quantum mechanical picture

For a nuclei which has spin and charge will have a dipole moment

$\mu = \gamma * I$, γ = gyromagnetic ratio. , I=spin angular momentum

Energy of interaction when a magnetic field is applied is

$E = -\mu.B.$

If magnetic field is only in Z direction , we get the energy of interaction to be

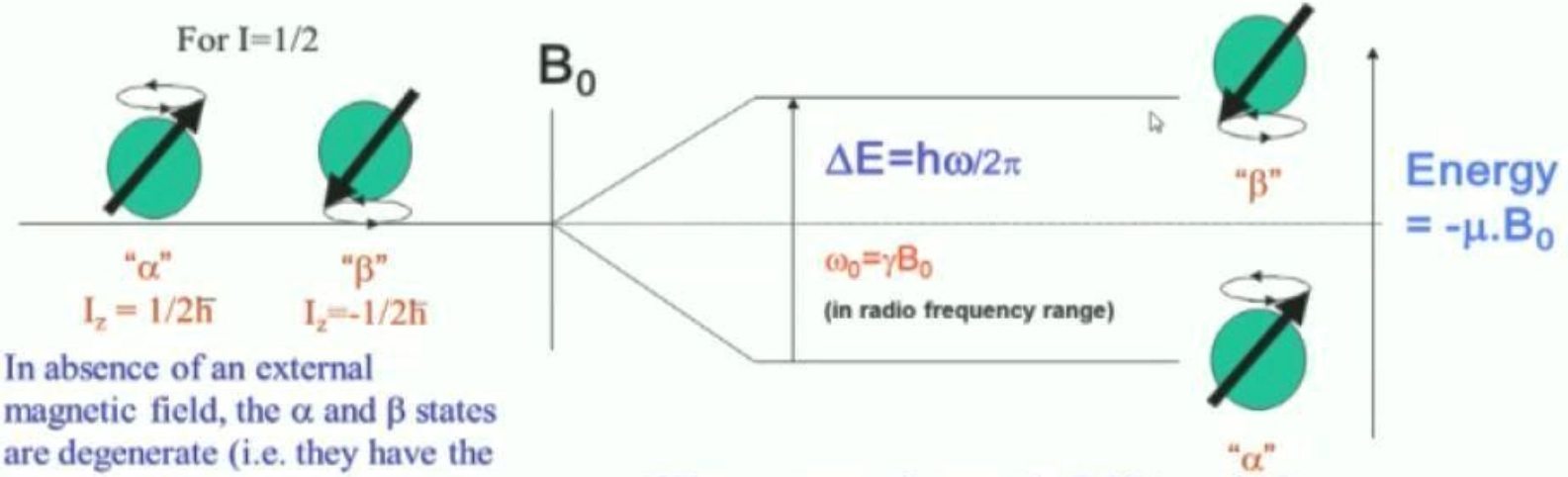
$E = -\mu z.Bz$

Let's consider a spin=½ nuclei

Quantum Mechanical Picture

- The system is thus split into two states with different energies:

$E_{1/2} = -1/2\ h\ B_0$ and $E_{-1/2} = 1/2\ h\ B_0$ => $\Delta E = E_{-1/2} - E_{1/2} = hB_0$



In absence of an external magnetic field, the α and β states are degenerate (i.e. they have the same energy)

When an external magnetic field is applied these two states split into two distinct levels

Boltzmann distribution law

N(higher)/N(lower) =exp(-ΔE/kT) ;

It says that at equilibrium always higher level will be less populated than ground level

ΔE=B.*γ*(h/2)

The population difference can be increased by

1)Increasing B

2)Decreasing T

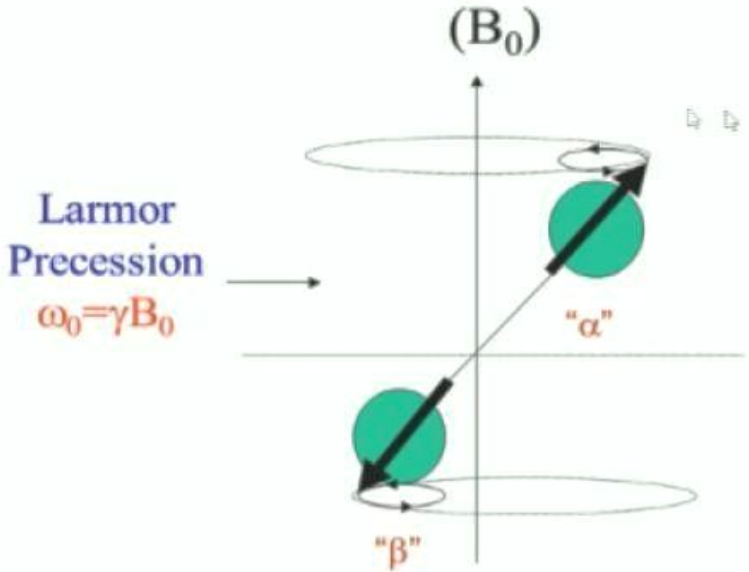
Sensitivity of NMR instruments

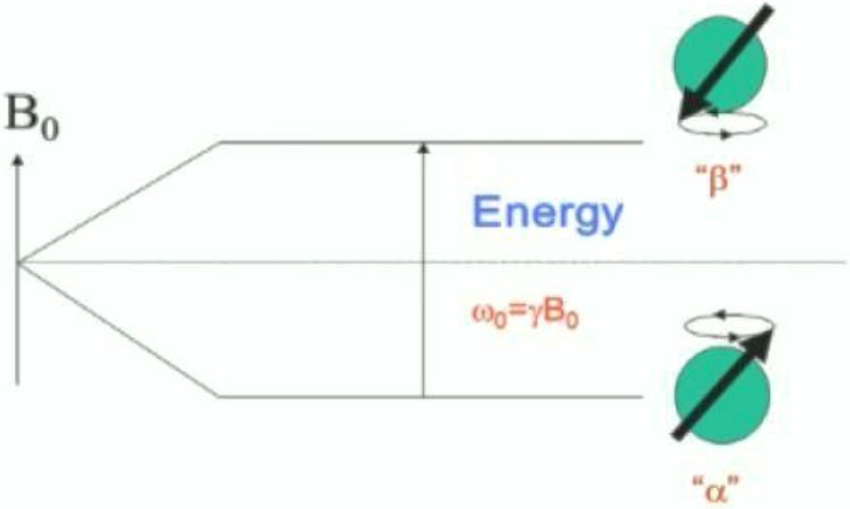
1) Depends on population difference in different states

2) γ of different nuclei. Eg: γ of ‘H’ is four times γ of ‘C’

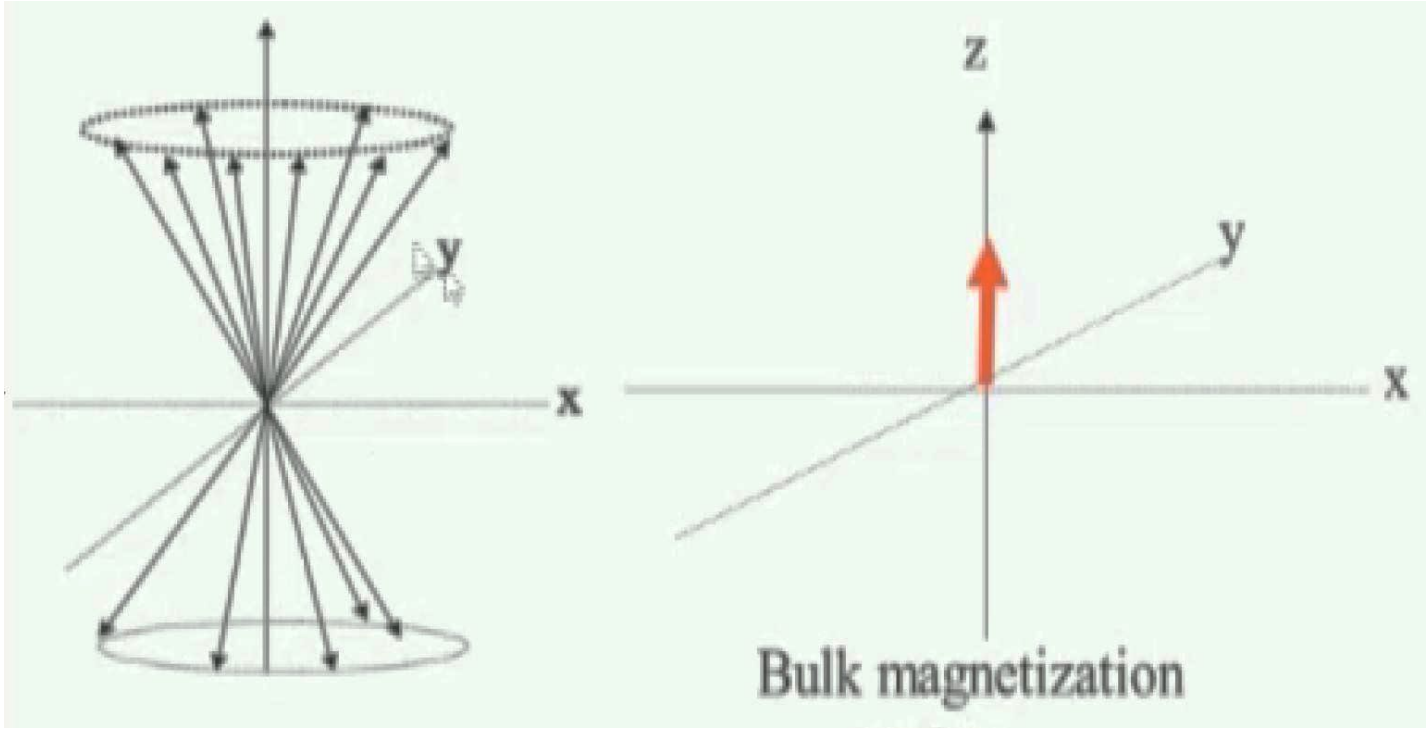
Classical mechanical picture

- The external magnetic field exerts a torque on the spinning nucleus. This causes the nuclear spin to precess around the magnetic field
- The precessional frequency is same as the energy gap between the two spin states

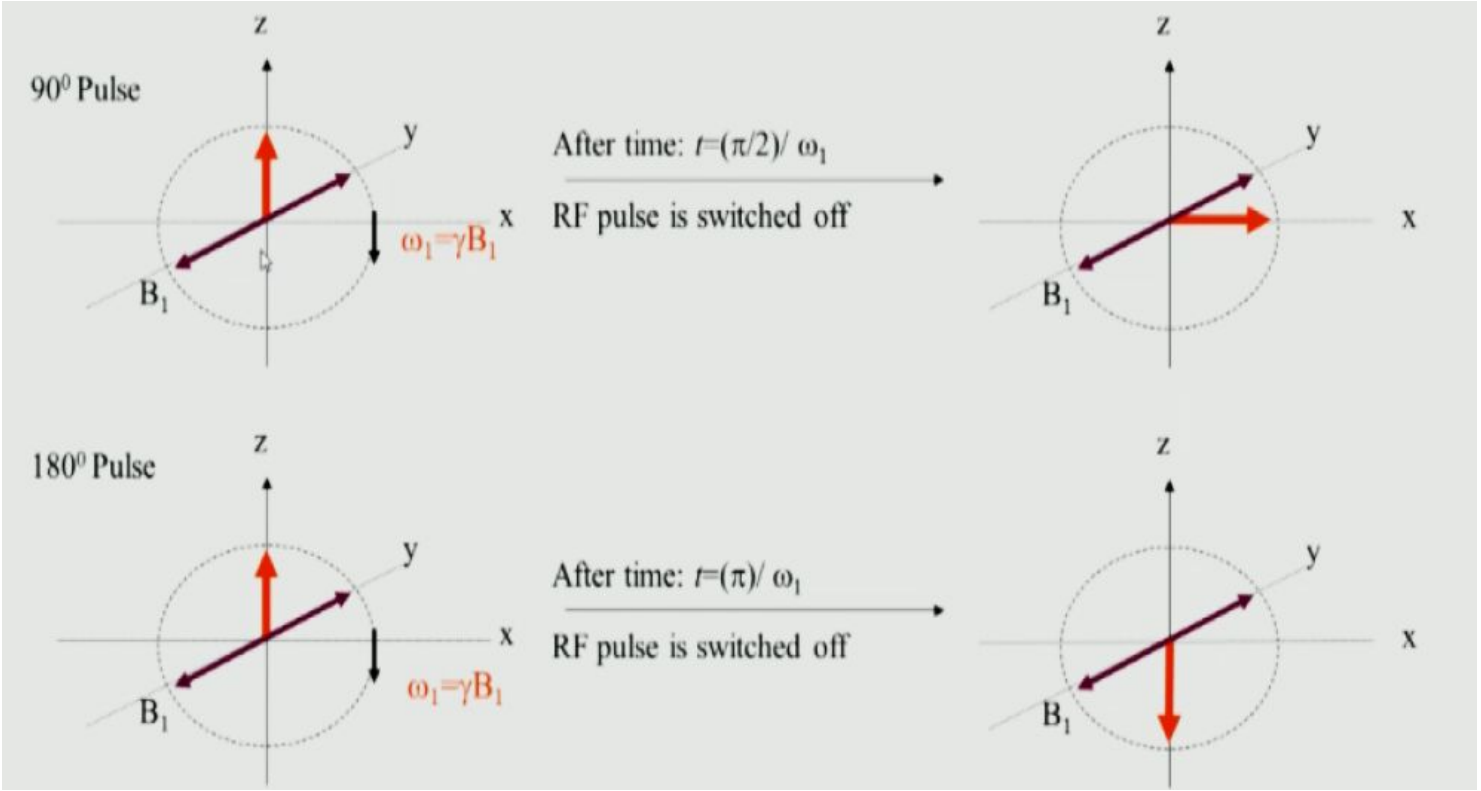




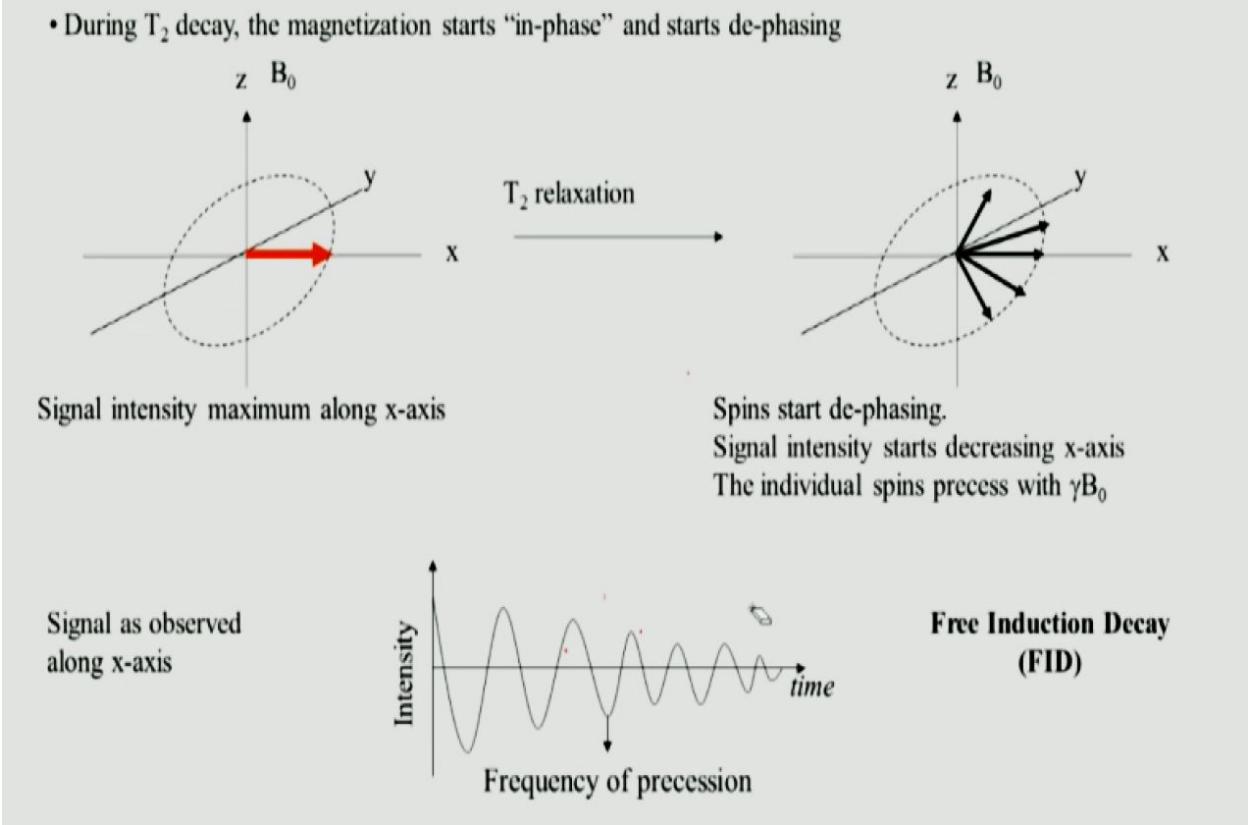
Bulk magnetisation



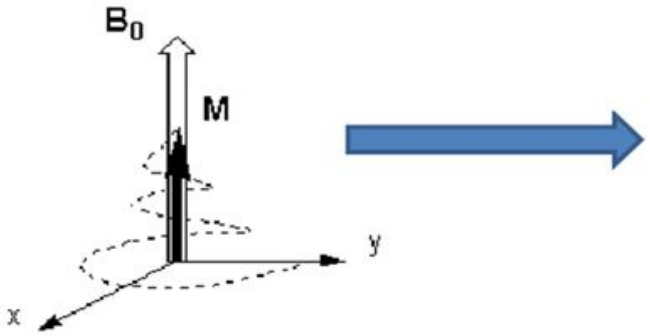
Different RF pulses

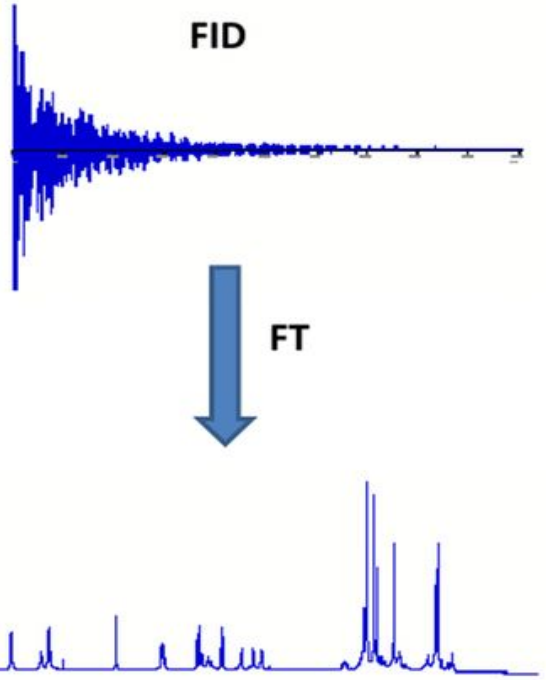


How do we get a NMR signal ? . FID?



How do we get the spectra ?





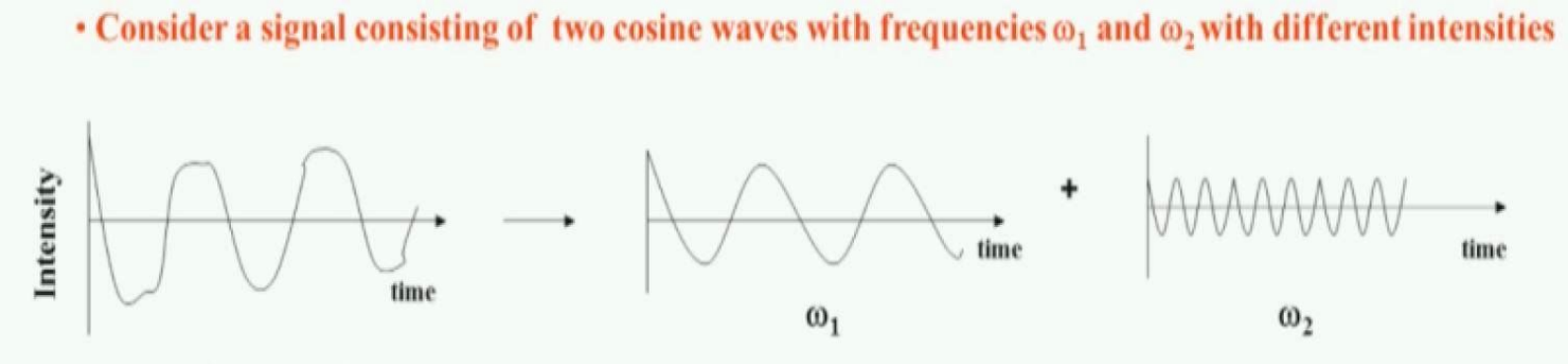
FID is thus nothing but a projection of the net magnetisation along the X plane, along the X axis.

Basically an oscillation, oscillation + decrease, damped

oscillation.

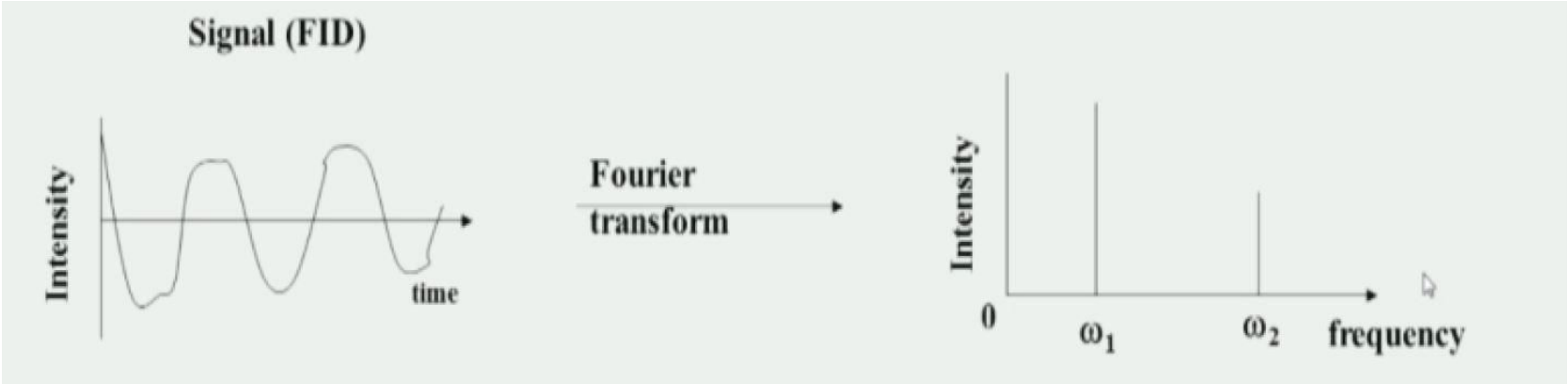
Not all the nuclei will dephase around and precess around B_o with the same frequency ω_o , they will differ from ω_o a little reason being they are all not in the same chemical environment.

So, the FID produced by this molecule will be a superposition of all this “chemical environmentally “ different nucelis.

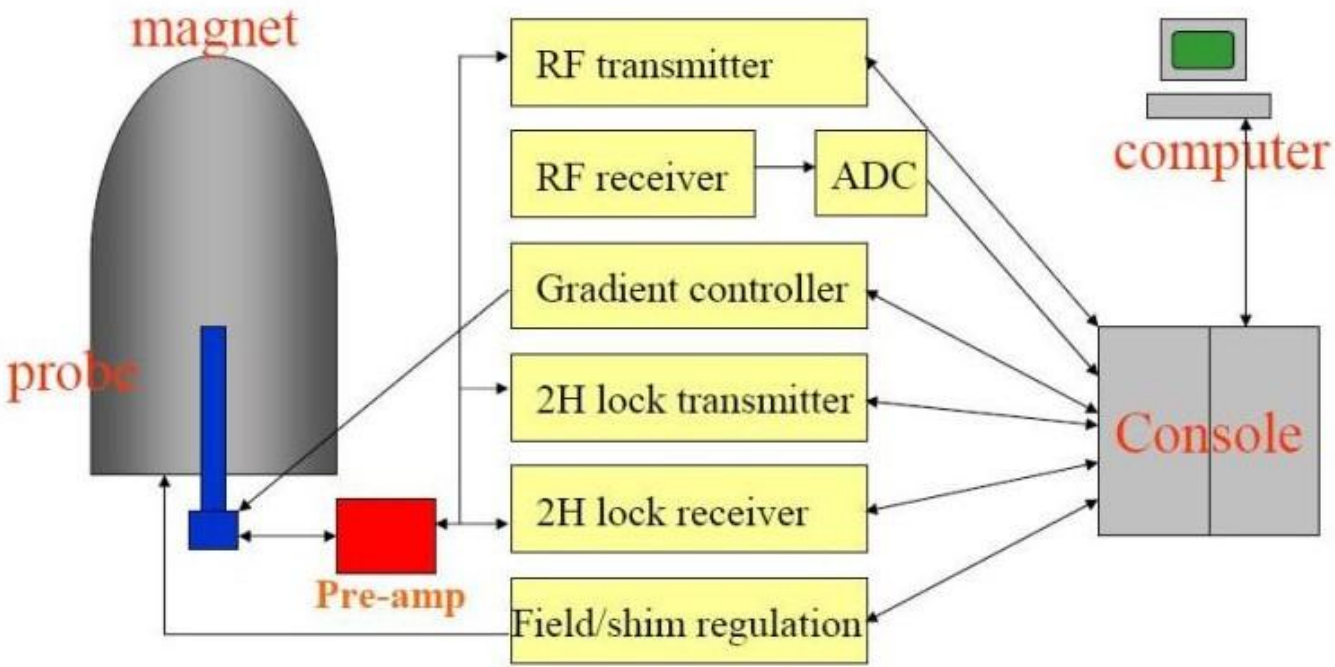


Fourier transform

- will tell us how many different frequencies are there in the molecules
- how many nuclei different in their chemical environment are present.
- It takes intensity/time ---->intensity/frequency
- Fourier transformation gives different peaks where in the area of the peak is proportional to the number of hydrogen



NMR Hardware



Magnet

Superconducting magnets,shim coil,liq He and N2 container

Probe

Rf coil to pulse and receive signal,gradients,temperature probes,lock coil

Console

Electronics for generating RF pulses,power and gradient amplifiers,lock system,temperature control

Computer

Data storage ,processing and analysis

Magnet

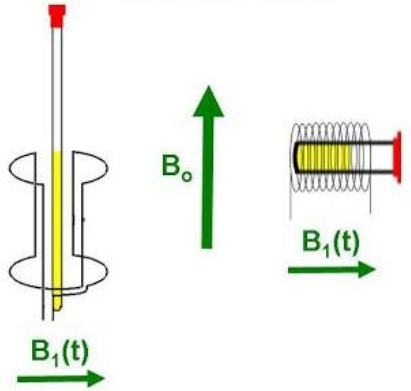
- Magnetic field are produced my neobium,titanium or neobium tin alloy,
  These are placed in chamber containing liq He at around 4K so these wires are now in superconducting states.
  These are further surrounded by alternating vacuum and liq N2 chambers so as to decrease evaporation of He in the core chamber
- Sensitivity of the instrument is directly proportional to magnetic field.
  $S \propto B.^{(3/2)}$
- Another problem faced to keep a steady magnetic field is vibrations from ground.
  This is tackled by placing the NMR instruments on vibration free stands.

Probe

- Probe is basically where our sample goes and sits.
  It has RF coils which supply energy (magnetic radiation)
  Which helps in the resonance of sample .

- The same RF coil receive the radiation emitted by the sample which is used for
- getting the spectrum

- The Probe has to be tuned accordingly for different nuclei
  To get good signal to noise ratio.



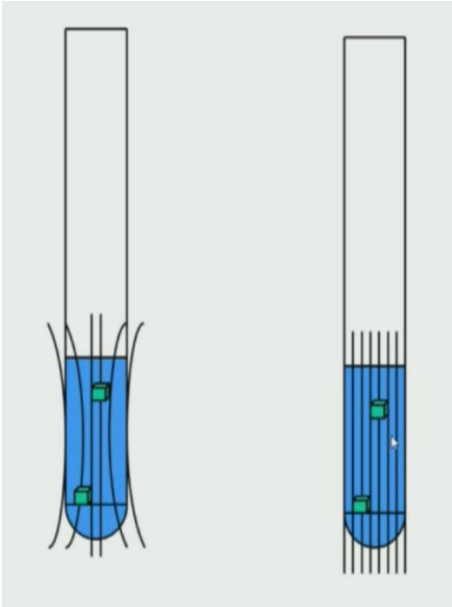
A very widely used probe nowadays is cryogenic probe
Whose main property is that it works under low temperat
So it can decrease the noise which arises due to thermal attributes.

Shimming

When the magnetic field is not homogeneous throughout our sample nuclei in different regions experience different magnetic field ,So a particular nuclei will show resonance for a range of magnetic field.

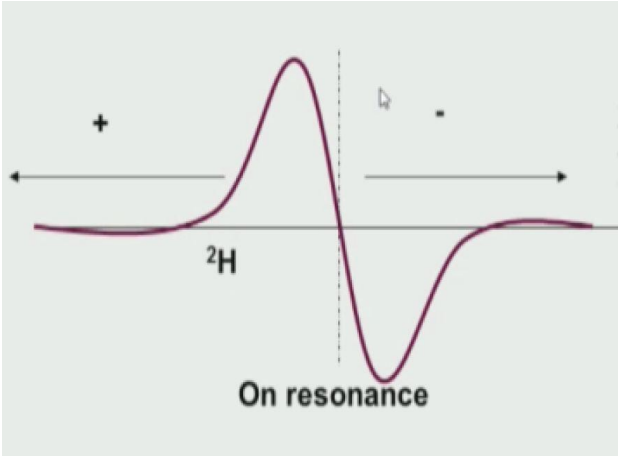
Hence, the peak will broaden for it ,
which will also decrease resolution.

The process which makes the magnetic field homogeneous is called shimming



Locking

- The magnetic field keeps shifting as we saw earlier,
  To solve that we have this locking system.
- We add some amount of deuterated solvent whose resonance is known to us beforehand.
  Current in the magnetic field generating coil is adjusted such that this deuterated solvent's resonance peak
  is kept constant.



1 D NMR

How do we analyse a 1D spectra ?

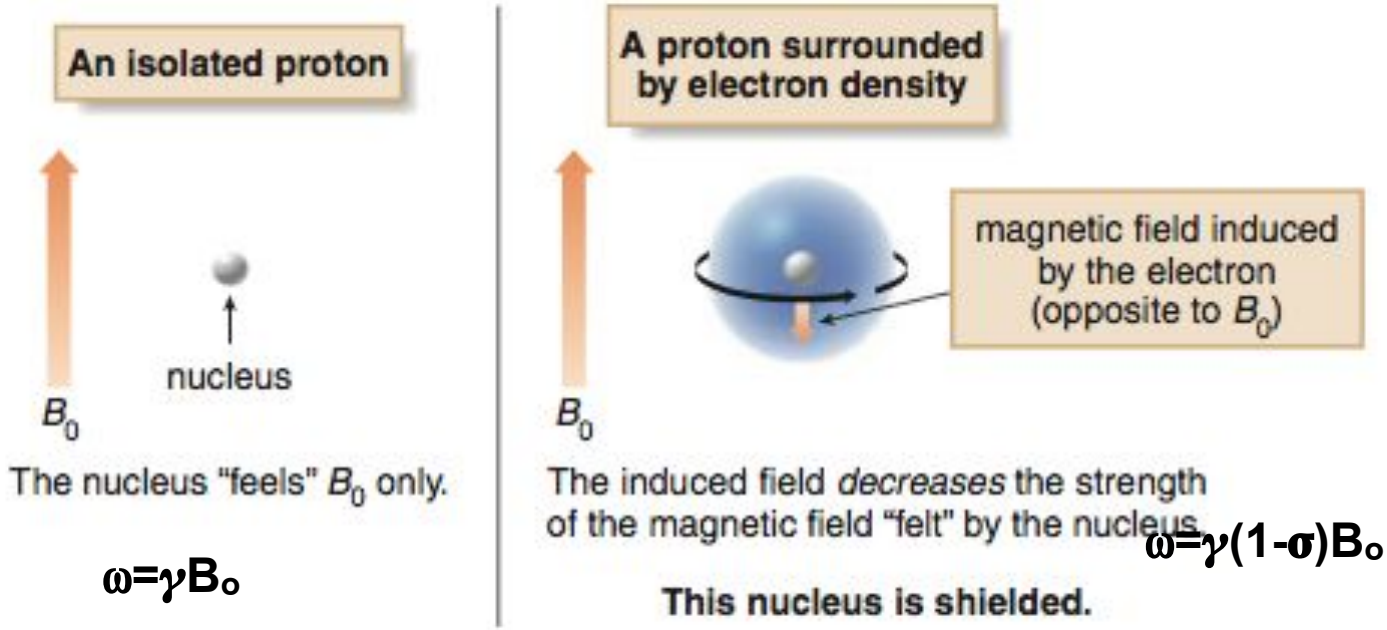
1)Chemical shift : will tell us how many different protons are there

2) Integral : will tell us about the relative no. of protons giving rise to one signal

3) Spin-Spin splitting : will tell us about the neighbouring protons of a particular proton.

4) J coupling : will give us extra information about the arrangement of atoms around a particular proton

Chemical Shift



Chemical Shift's value doesn't change with the magnetic field applied .It's based on a reference

Let $\omega_{ref}=\gamma B_o$ be the precessional frequency of the reference nucleus

$\omega=\gamma(1-\sigma)B_o$ be the precessional frequency of the nucles of interest

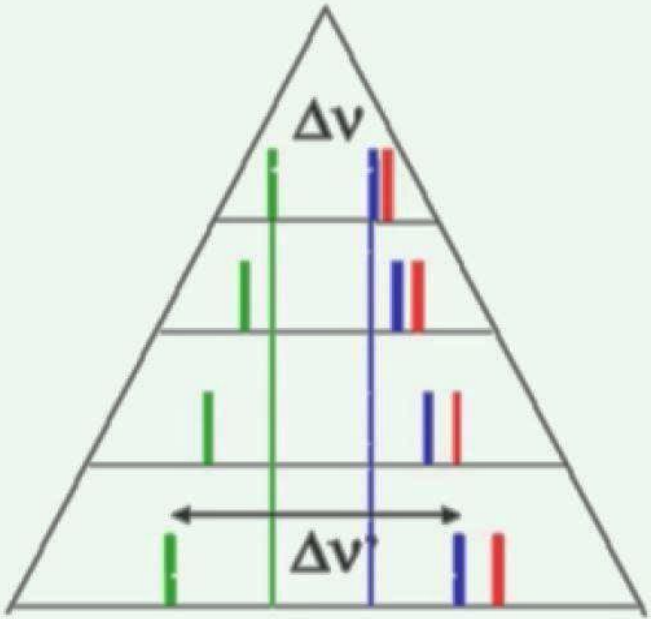
$\sigma=(\omega-\omega_{ref})/\omega_{ref}$

$=(\nu-\nu_{ref})/\nu_{ref} \cong 10^{-6}$

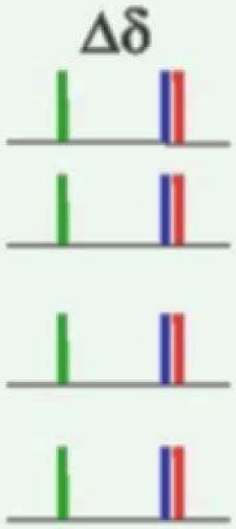
Hence chemical shift is given in **ppm**

$\nu_1-\nu_2=(\sigma_1-\sigma_2)*\nu_{ref}$;

Δν depends on B_0 o



Δδ is independant of B_0

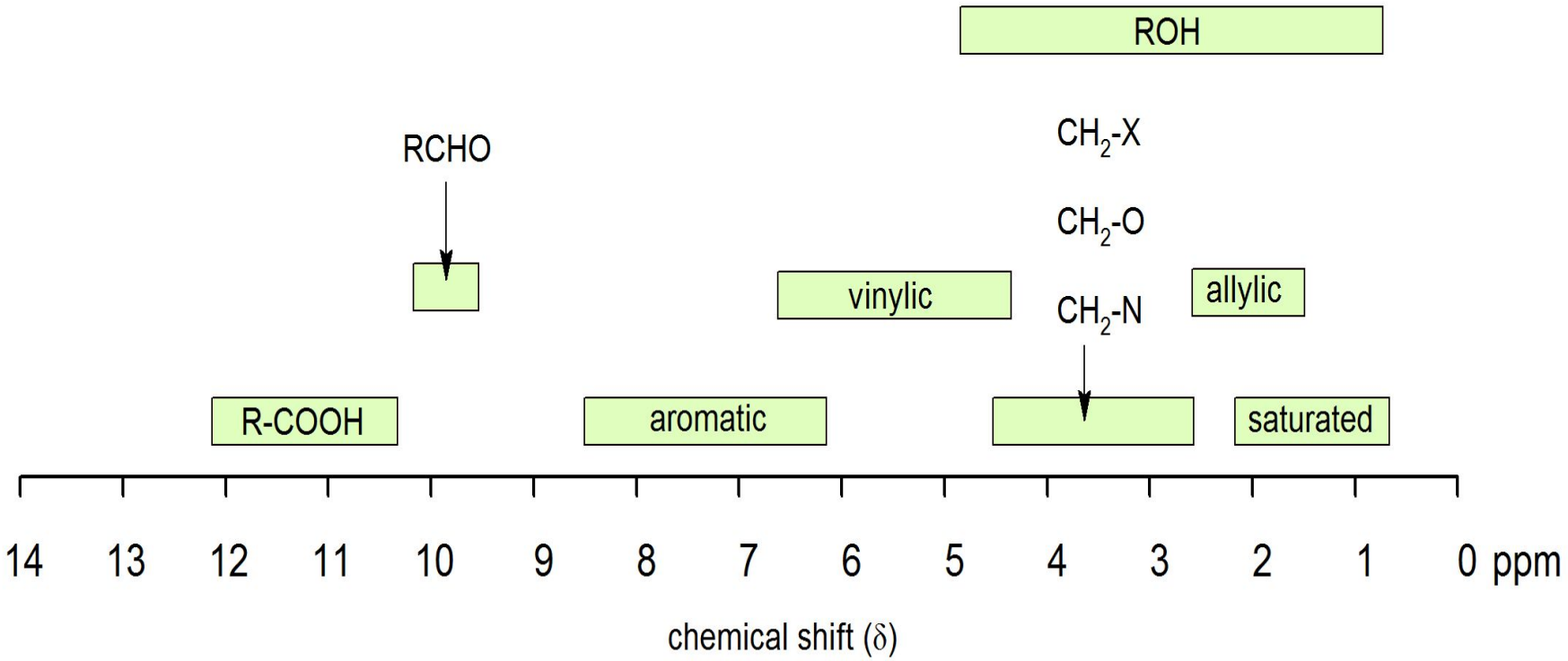


$$\delta = \left(\frac{\nu_A - \nu_{ref}}{\nu_0}\right).10^6$$

$\nu 1 - \nu 2 = (\sigma 1 - \sigma 2) * \nu ref$

Frequency scale

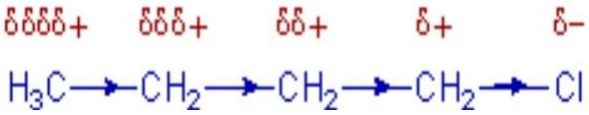
Delta scale


ROH
RCHO
CH_2-X
CH_2-O
vinylic
allylic
CH_2-N
R-COOH
aromatic
saturated
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
chemical shift (δ)

Factors affecting chemical shift

- Inductive effect
- Hybridisation
- Magnetic Anisotropy
- Mesomeric Resonance effect
- Aromatic current effect
- Steric Effect
- Hydrogen Bonding

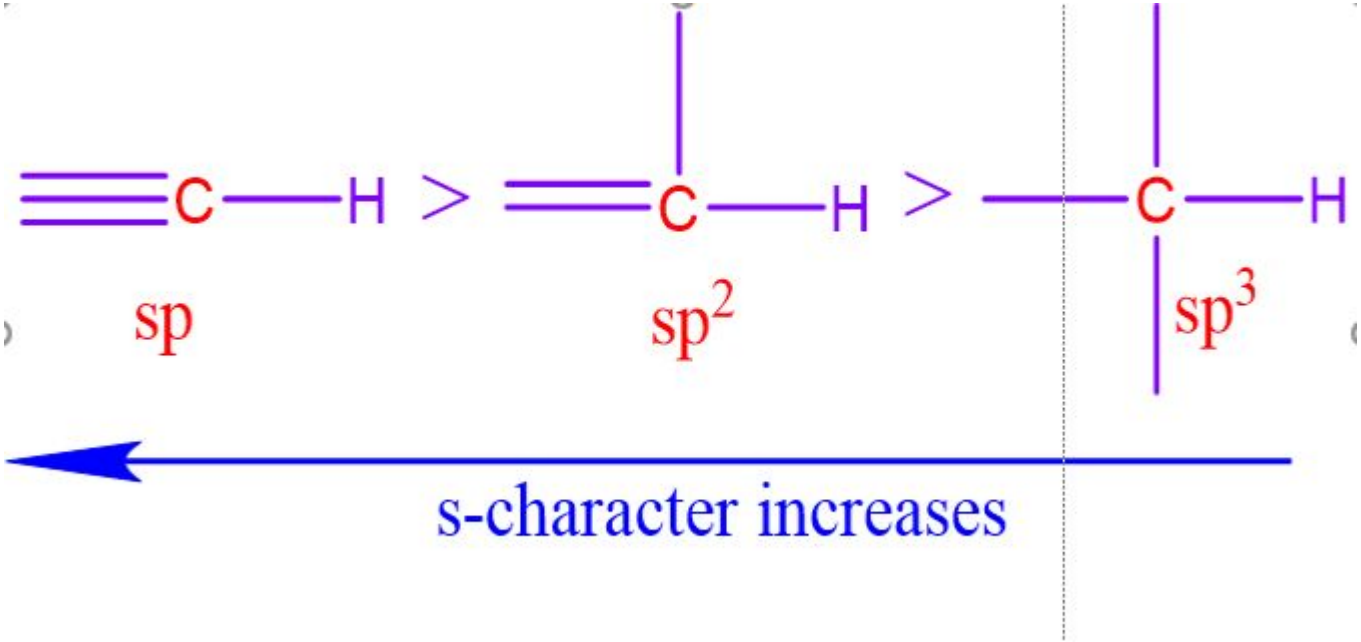
Inductive Effect



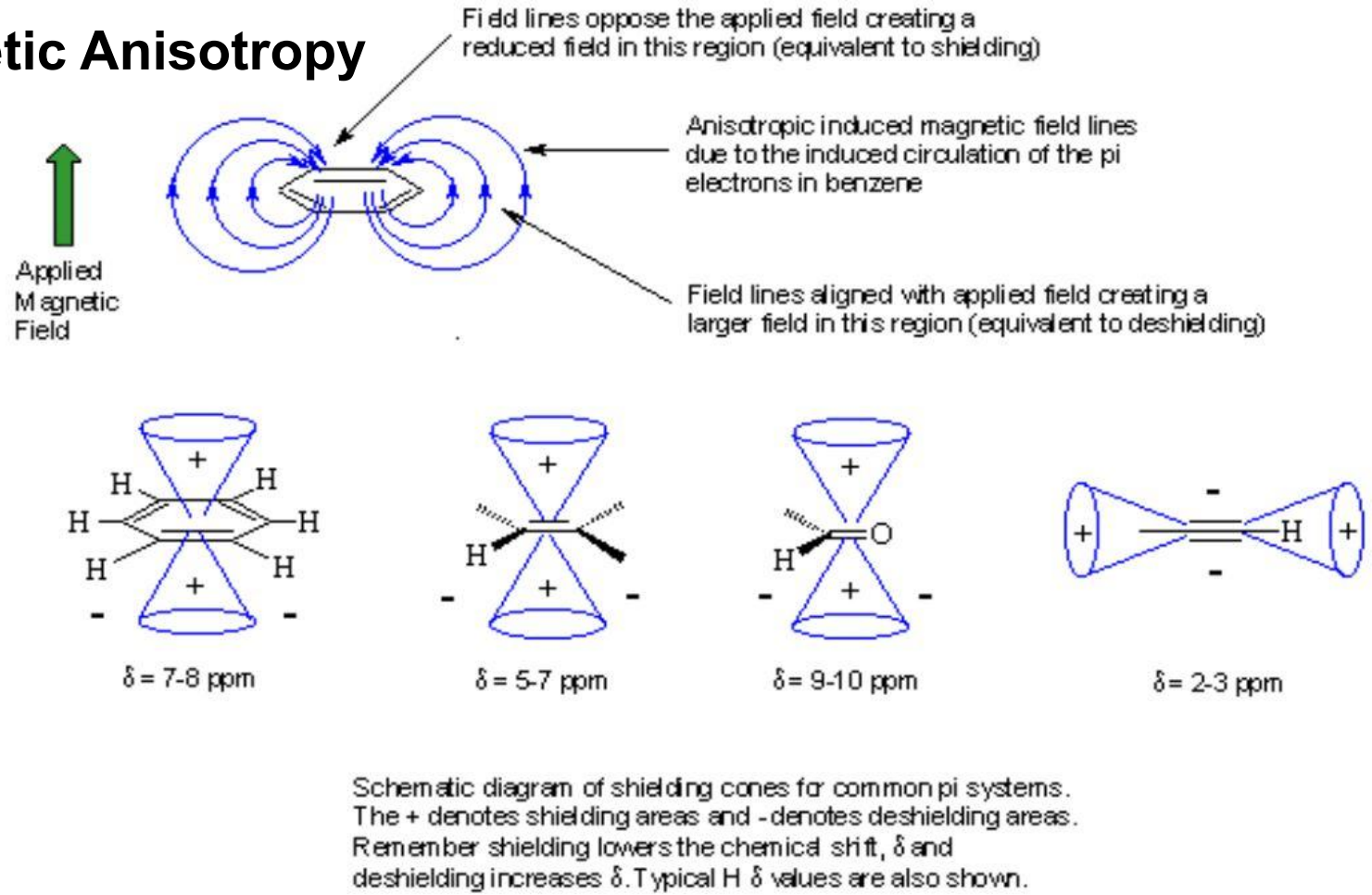
Hybridisation

Type of Hydrogen (R = alkyl)	Name of Hydrogen	Chemical Shift (δ)
RCH_3, R_2CH_2, R_3CH	Alkyl	0.8 - 1.7
$R_2C{=}C(R)CHR_2$	Allylic	1.6 - 2.6
$RC{\equiv}CH$	Acetylenic	2.0 - 3.0
$R_2C{=}CHR$, $R_2C{=}CH_2$	Vinylic	4.6 - 5.7
RCHO	Aldehydic	9.5-10.1

Hybridisation

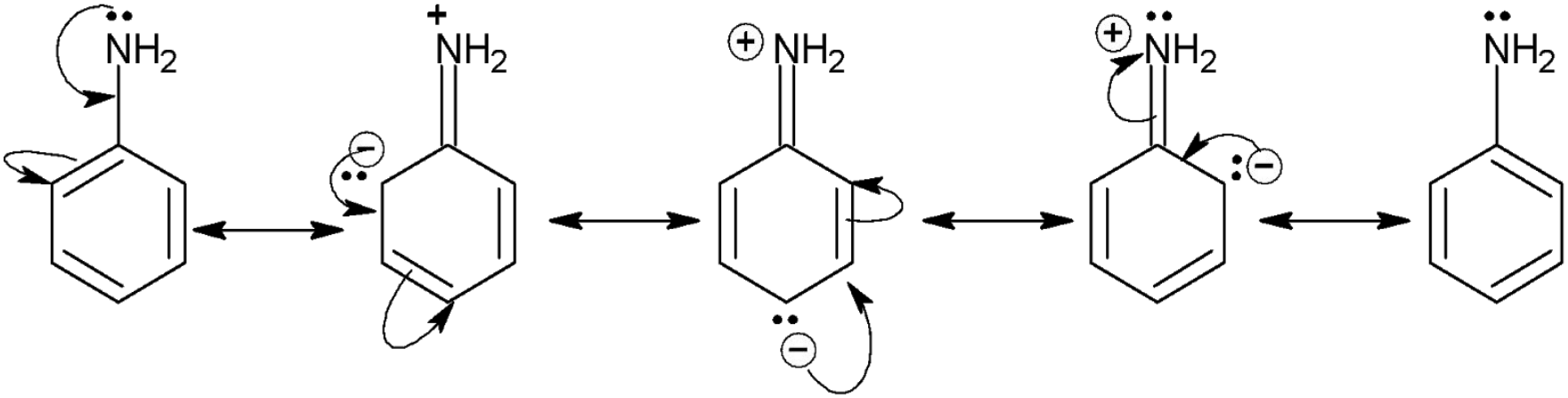


Magnetic Anisotropy

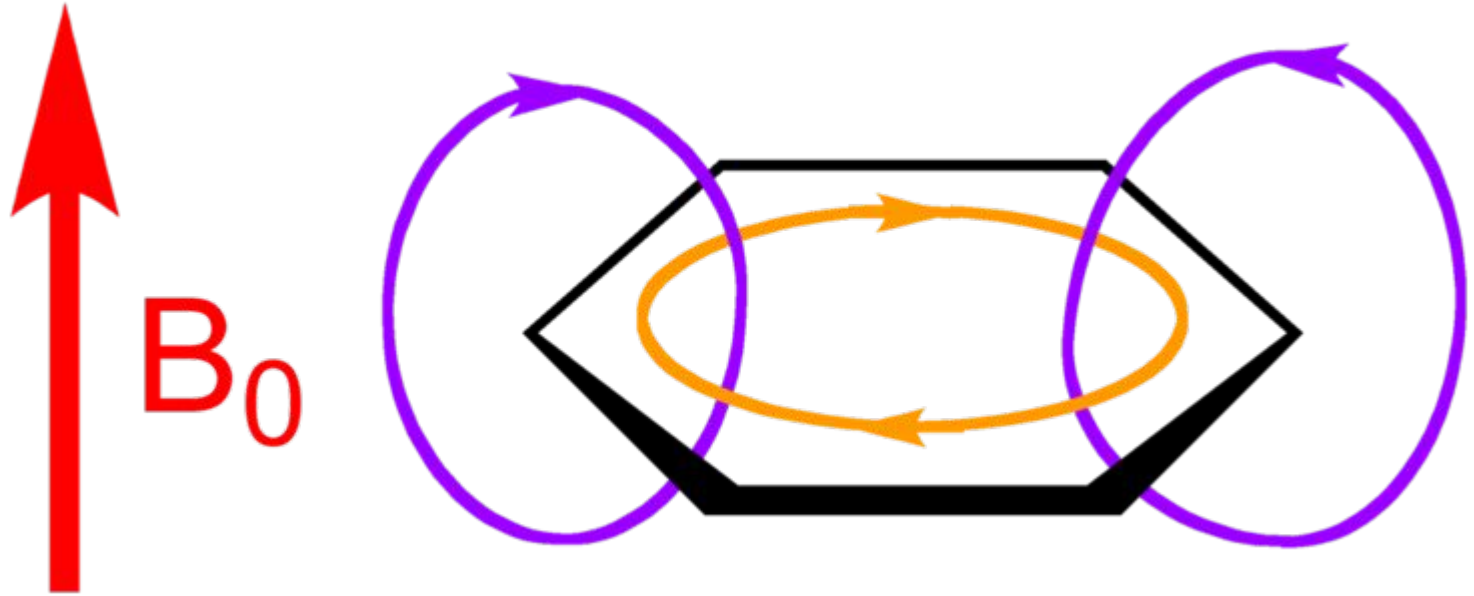


Schematic diagram of shielding cones for common pi systems. The + denotes shielding areas and - denotes deshielding areas. Remember shielding lowers the chemical shift, δ and deshielding increases δ. Typical H δ values are also shown.

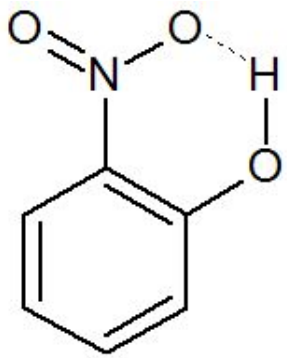
Mesomeric effect



Aromatic Ring current

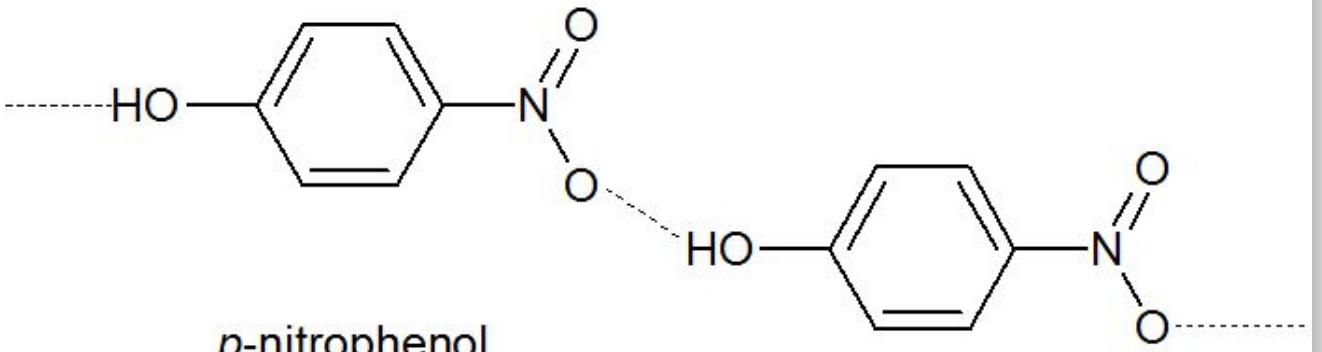


Hydrogen bonding



o-nitrophenol

(intramolecular hydrogen bonding)



p-nitrophenol

(intermolecular hydrogen bonding)

Chemically equivalent means ?

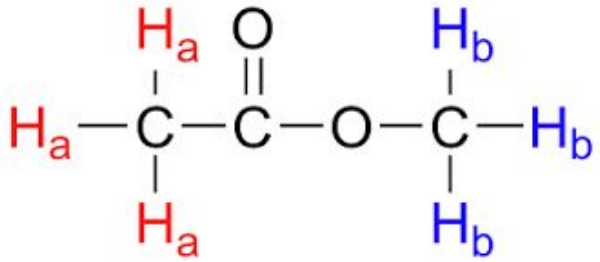
They have identical chemical environment.

The two nuclei are interconvertible in their positions by symmetry operations or rapid time dependent mechanisms.

Two chemically equivalent nuclei have same chemical shift.

BUT

Two nuclei with same chemical shift doesn't mean they are chemically equivalent.



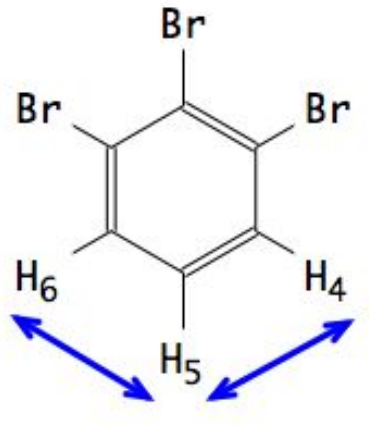
Magnetically equivalent

To nuclei are magnetically equivalent if:

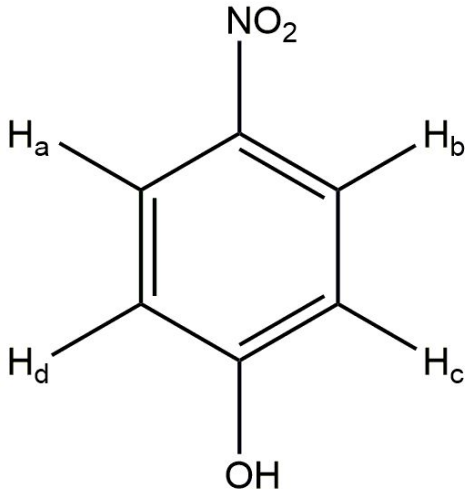
1) They are chemically equivalent

2)They should couple equally with any other nuclei in the molecule

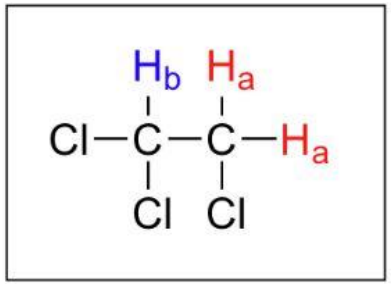
Magnetically equivalent

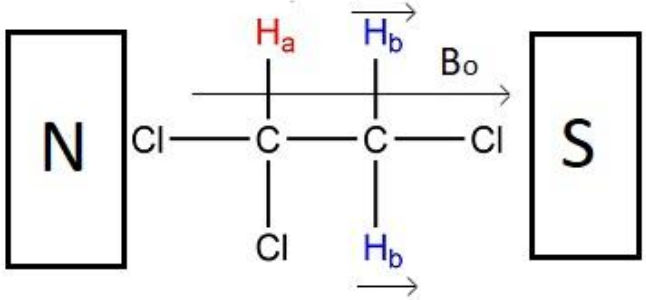


NOT magnetically equivalent



Observation




N
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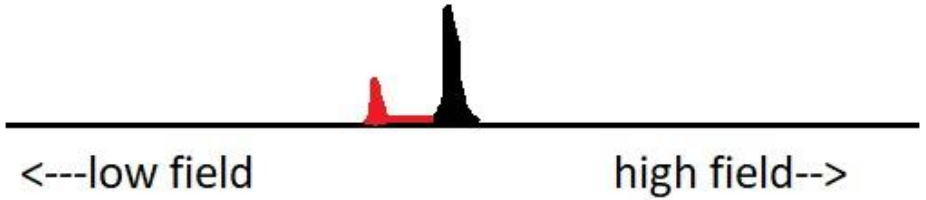
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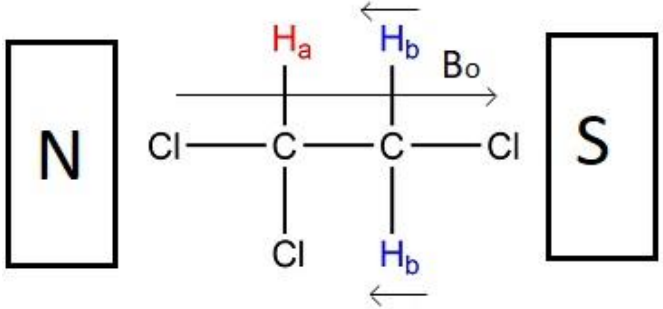
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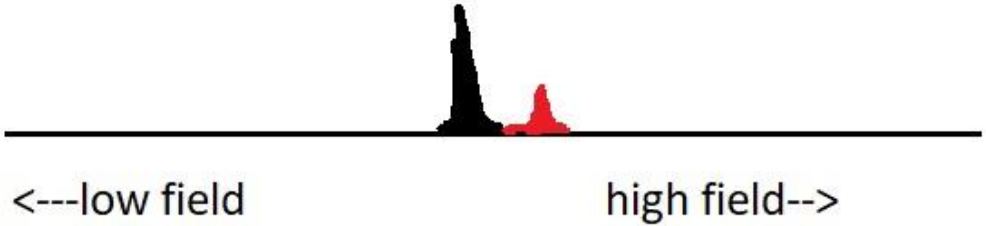
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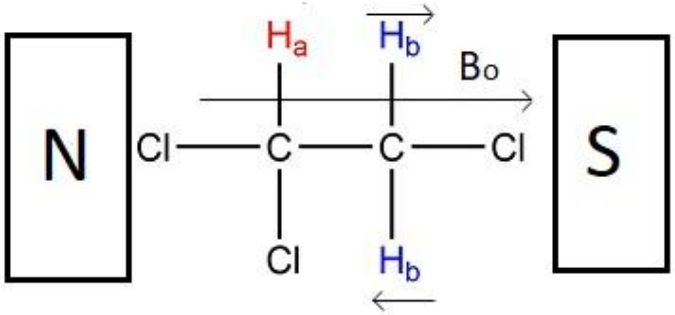
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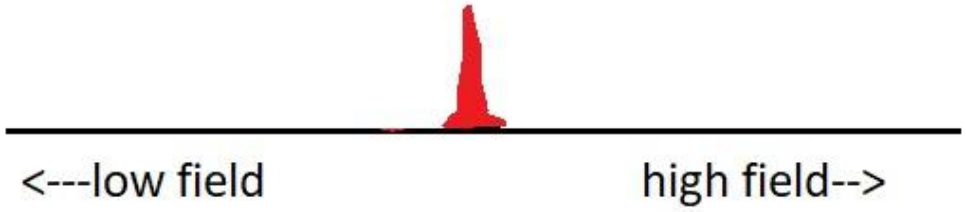
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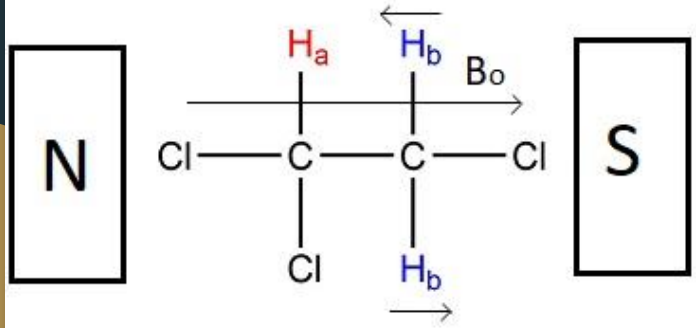
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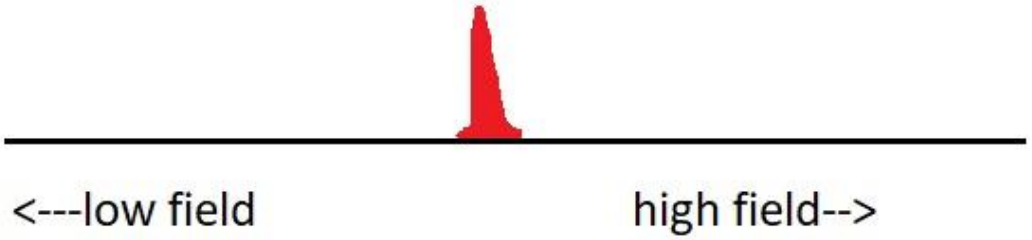
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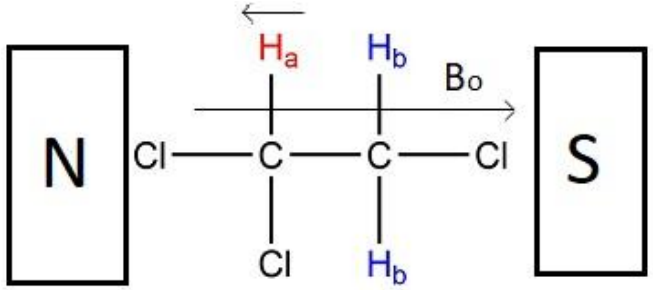
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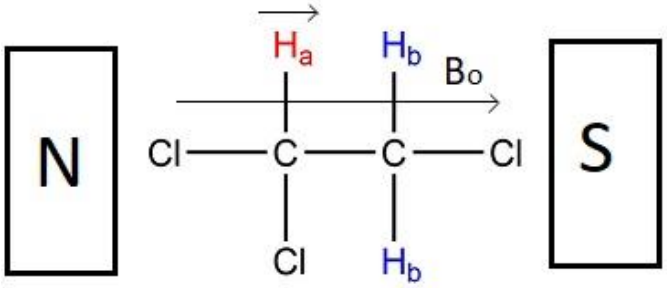
high field-->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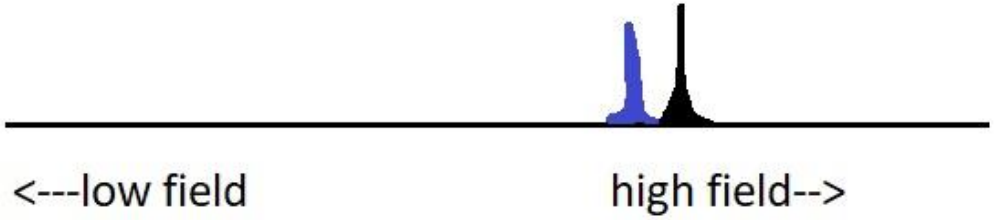

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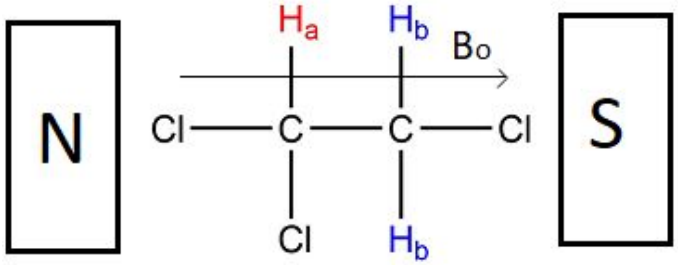
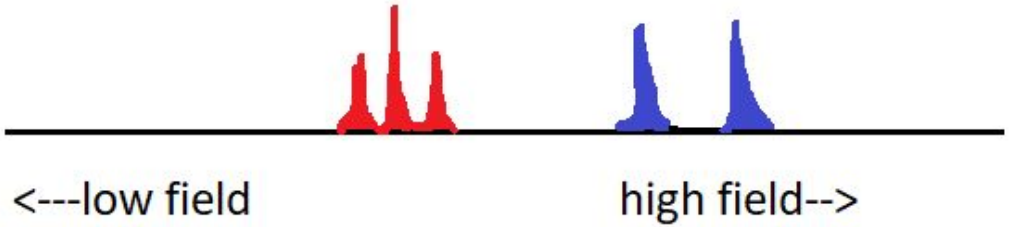
high field-->

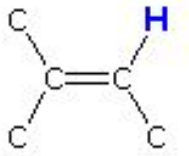
Final spectrum



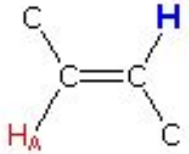


J coupling

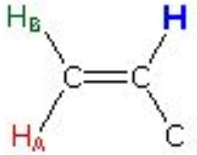
No Coupled Hydrogens



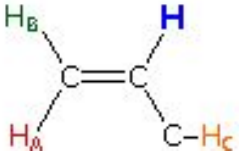
One Coupled Hydrogen

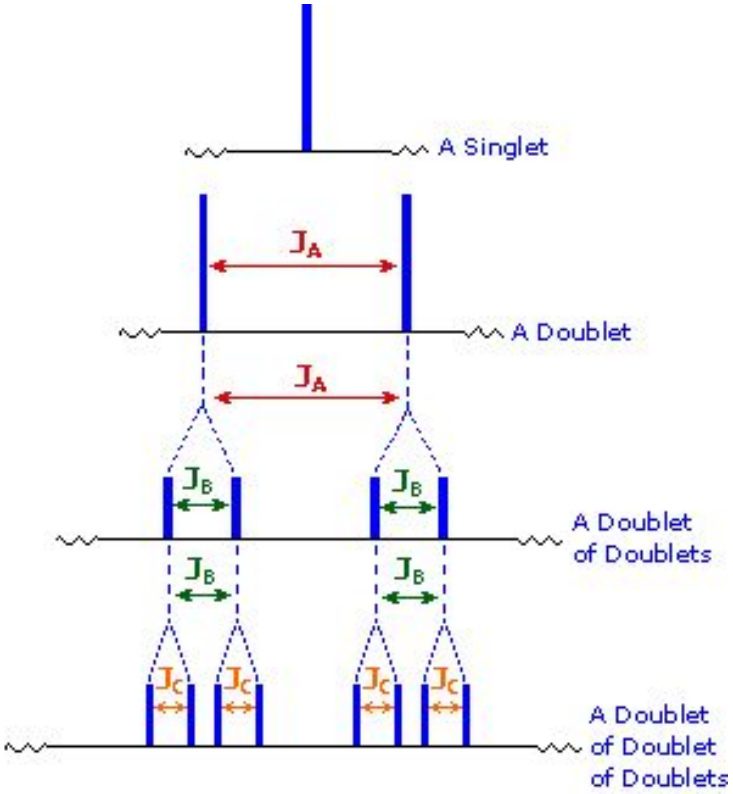


Two Coupled Hydrogens



Three Coupled Hydrogens





Strength of J coupling depends on

- Gyromagnetic ratio
- No. of bonds separating them
- The conformation of a molecule
- The hybridisation state of the bond
- The substituents attached to C13

Sensitivity

Sample concentration. S/N ∝ concentration

Temperature. S/N can increase or decrease with temperature

Magnetic field . S/N ∝ B0^3/2

Type of nucleus being observed S/N ∝ γ of nucleus

Type of probe being used. Cryogenic probes have higher sensitivity.

Measurement time to record the data. S/N ∝ T^3/2

1-D H NMR

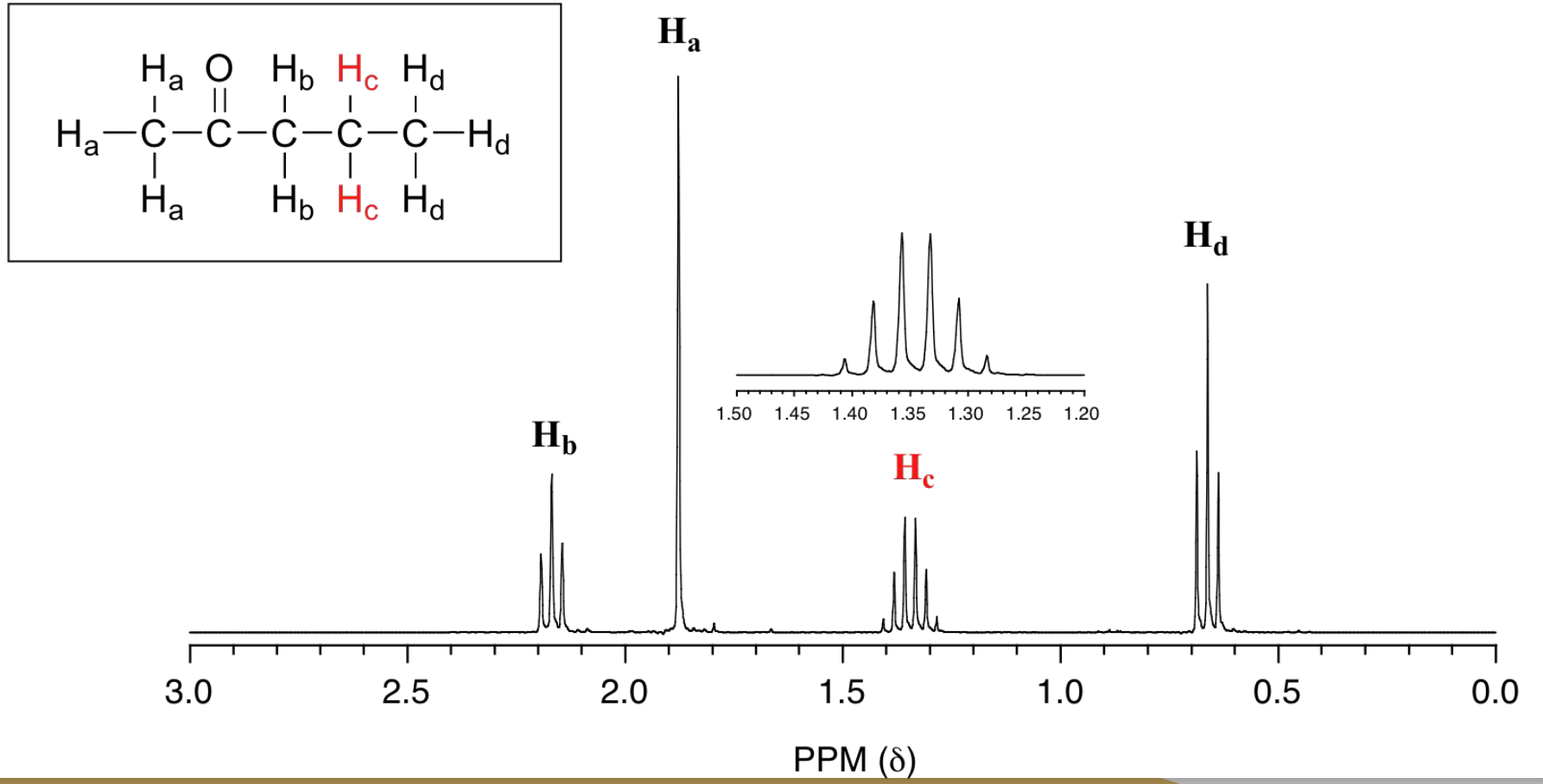
To analyze a 1D spectrum we should know the molecular formula of the compound

Once we know the molecular formula of the compound we scan the compound and see the no. of peaks in the compound which will tell us how many “types” of protons are there.

Once the type of protons are known we analyze the J coupling .

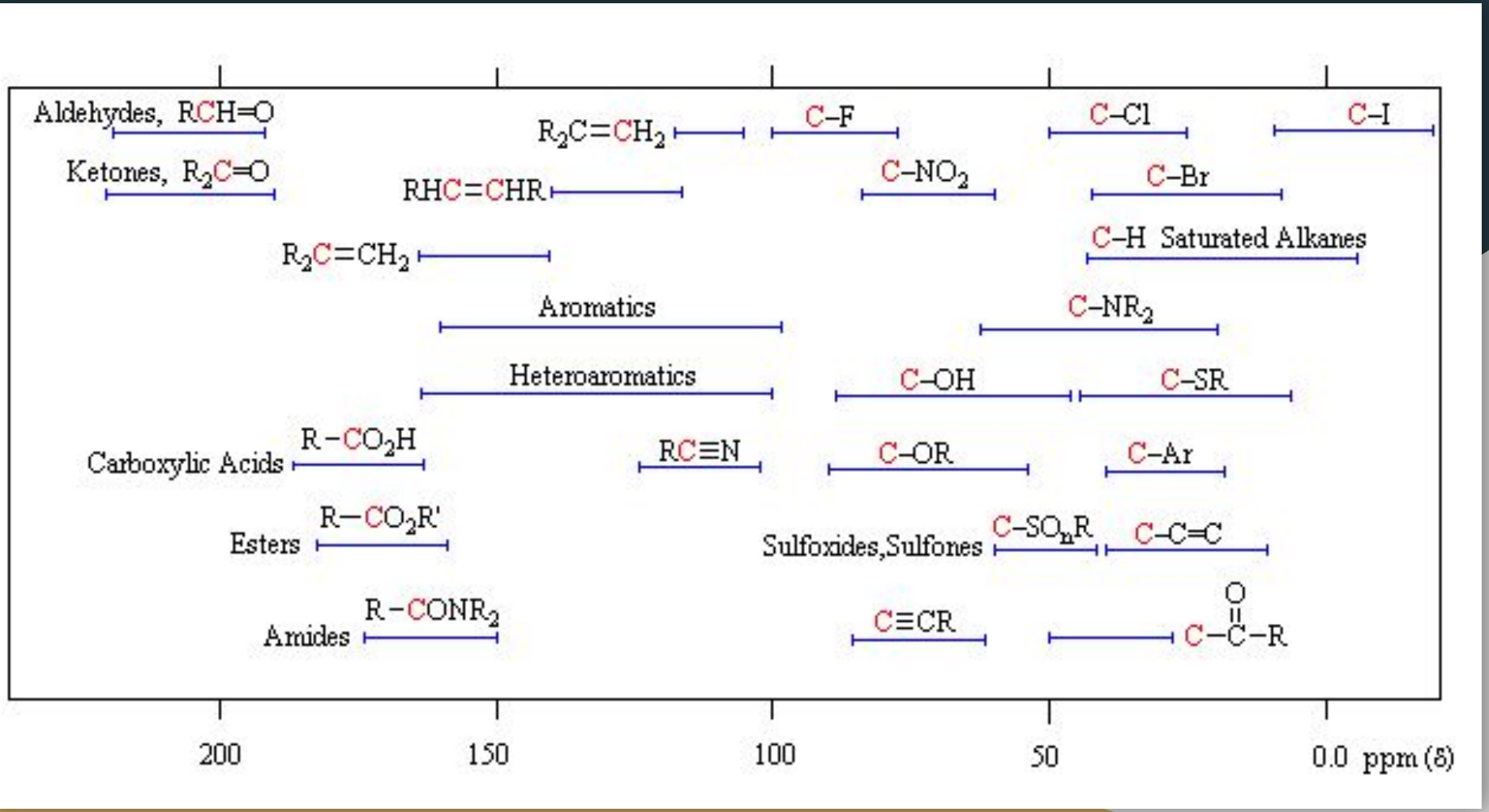
Last step is to analyze the relative peak integral.

Methods like Shoolery’s rule and Pascual Meier Simon’s rule are used to predict chemical shift of compound based on molecular structure


Ha O Hb Hc Hd
Ha C C C C C Hd
Ha Hb Hc Hd
Ha
Hb
Hc
Hd
1.50 1.45 1.40 1.35 1.30 1.25 1.20
3.0 2.5 2.0 1.5 1.0 0.5 0.0
PPM (δ)

CARBON -13 NMR

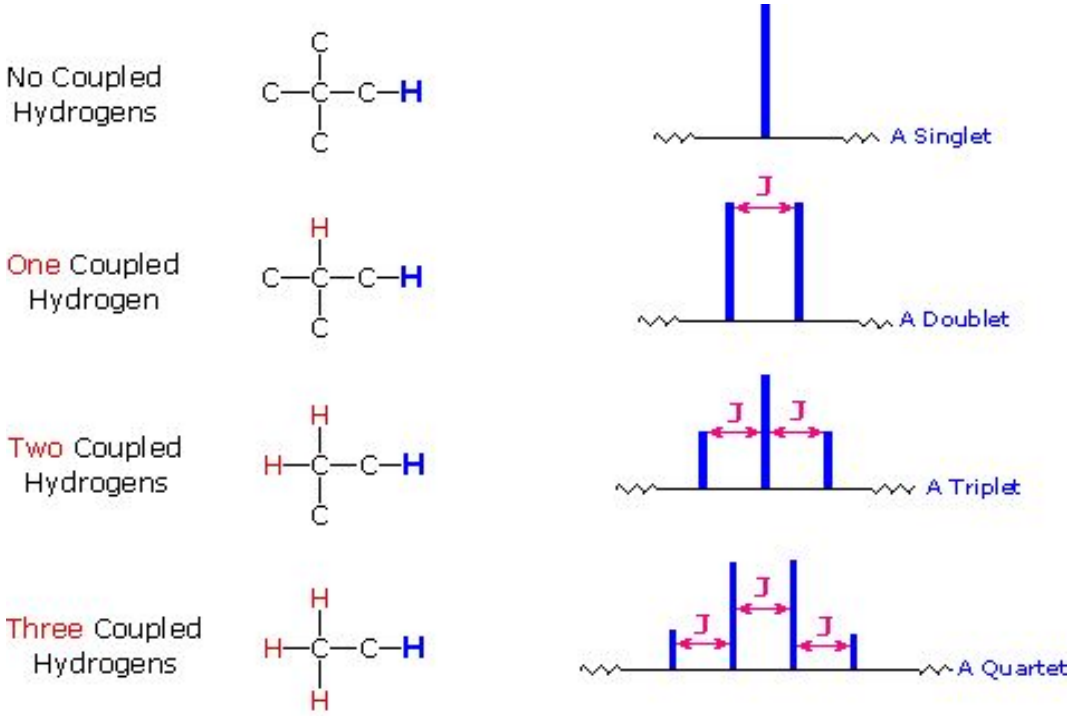
- Carbon 13 is an active nuclei with spin =½
- It's signal are weak because natural abundance of C13 is very low
- γ value of C 13 is four times less than γ value of H
- The chemical shift range for C 13 is 0-200 ppm


Aldehydes, RCH=O
Ketones, R2C=O
R2C=CH2
RHC=CHR
R2C=CH2
Aromatics
Heteroaromatics
Carboxylic Acids
R−CO2H
Esters
R−CO2R'
Amides
R−CONR2
R2C=CH2
C–F
C–NO2
RC≡N
C–OH
C–OR
C≡CR
C–Cl
C–Br
C–H Saturated Alkanes
C–NR2
C–SR
C–Ar
C–SOnR
Sulfoxides,Sulfones
C–C=C
C–C–R
200
150
100
50
0.0 ppm (δ)

Carbon nmr splitting

C13-C13 splitting (VERY LESS PROBABLE)

C13-1H splitting (VERY COMMON)

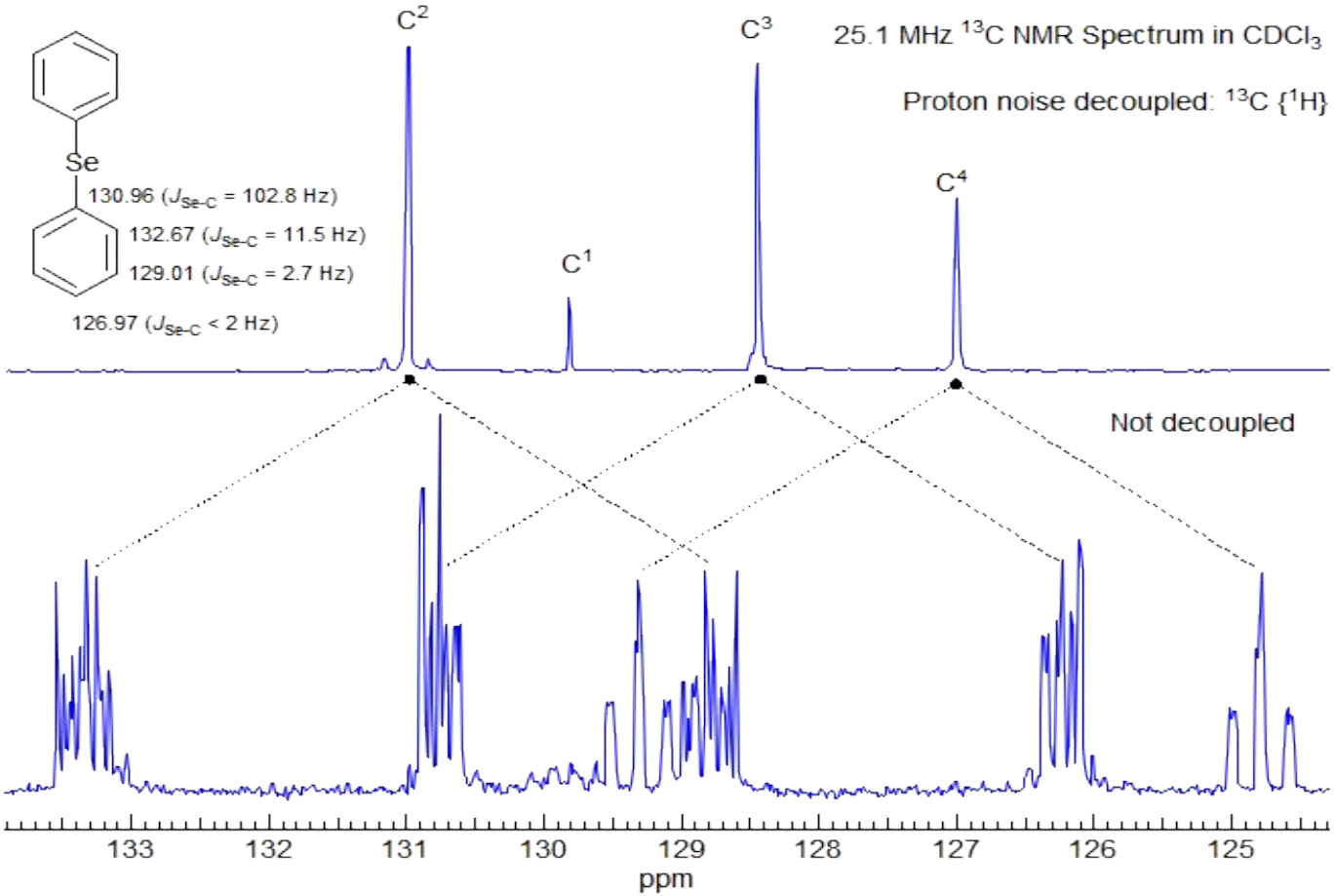


C 13 decoupling

C13 splits because its coupled to 1H and it has two states α and β

Similar is the case for **1H** splitting, but proton is 99% of the time attached to **12C**, so this splitting due to **13C** is not observable or that intense.

In case of **13C** spectra it's always attached to a **1H** so splitting is observed .
To decouple this a continuous RF radiation is applied to **1H** to make the α and β state equally populated.So, **13C** doesn't split.


C2
C3
25.1 MHz 13C NMR Spectrum in CDCl3
Proton noise decoupled: 13C {1H}
C4
C1
Se
130.96 (JSe-C = 102.8 Hz)
132.67 (JSe-C = 11.5 Hz)
129.01 (JSe-C = 2.7 Hz)
126.97 (JSe-C < 2 Hz)
Not decoupled
133
132
131
130
129
128
127
126
125
ppm

Practical aspects of C13

TMS is used as reference here too, as in **1H** NMR

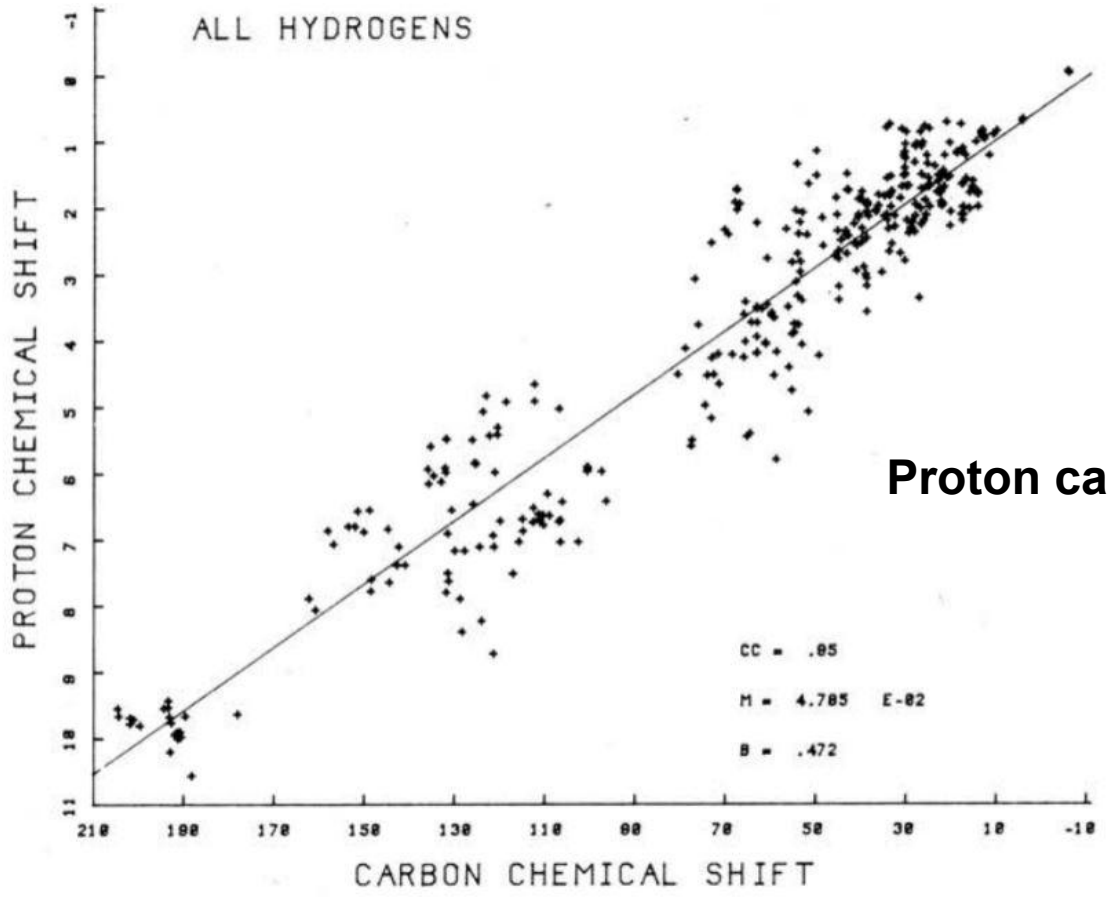
The T1 and T2 relaxation times of 13C are longer than 1H

Due to long T1 relaxation time,longer relaxation delays are required. (several 10's of seconds usually)

T1 cannot be estimated everytime ,this leads to distortion of intensities due to incomplete relaxation

Distortion also is due to proton decoupling.

This is reason why area under C13 graph dosen't give us relative no. of carbons



Plot of δ_C versus δ_H for the compounds listed in the Appendix.

Proton carbon correlation

Proton–Carbon Chemical Shift Correlations[a]

$$\delta_H = m\delta_C + b \text{ or } \delta_C = \frac{\delta_H - b}{m}$$

Type CH	number data pts	*m*, ppm H/ppm C	*b*, ppm H	CC
methyl	55	0.0545	0.280	0.819
methylene	117	0.0531	0.327	0.849
methine	62	0.0609	−0.158	0.816
acetylenic	9	0.124	−6.55	0.942
aromatic	27	0.0413	2.30	0.675
vinyl	42	0.0392	1.02	0.637
formyl	23	0.0364	2.67	0.697
all	335	0.0479	0.472	0.955

[a] Slope *m* and intercept *b* are derived from linear least-squares regression analysis. CC = correlation coefficient.

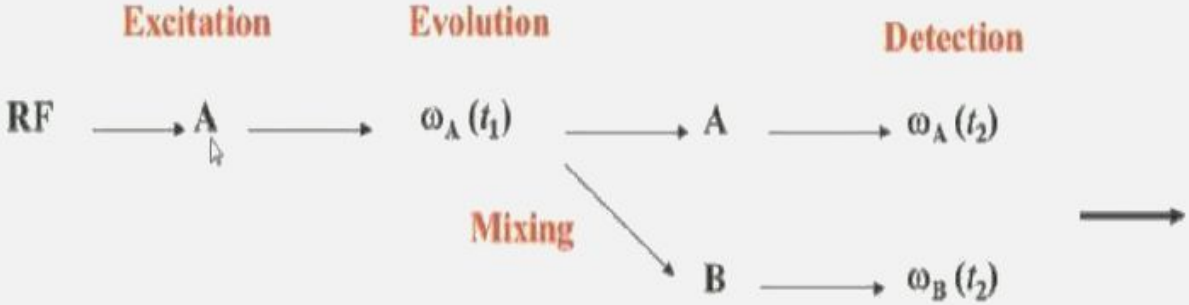
Limitations of 1D NMR

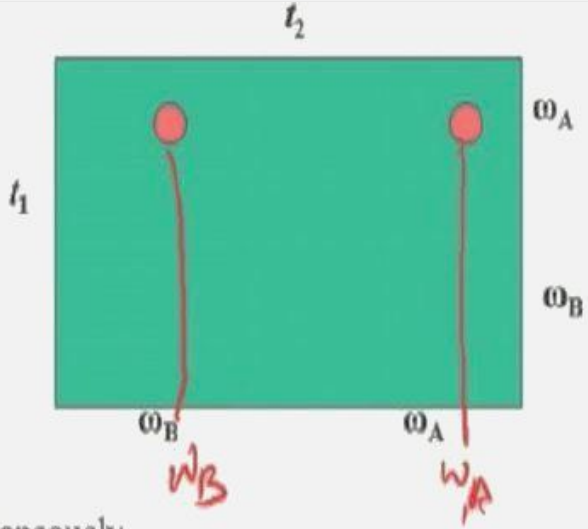
It is not possible to correlate two peaks of **1D NMR**

It is difficult to analyze **1D** NMR spectrum due to overlapping of peaks

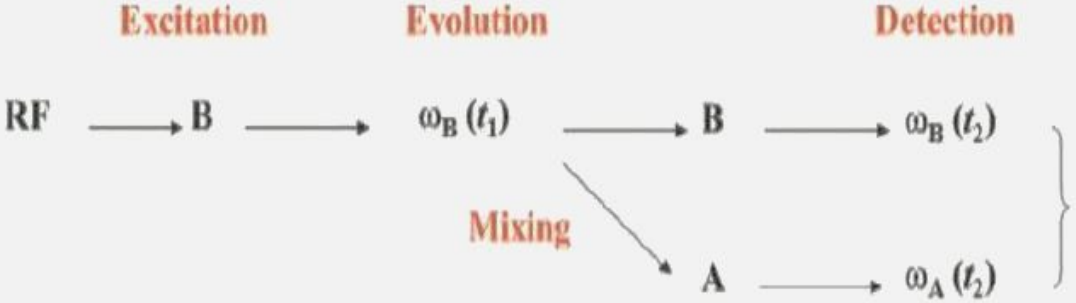
We can’t correlate **1D 1H** and **13C** peaks to, as in which peak in the proton spectrum corresponds to which peak in the **13C** spectrum.

2 D NMR Spectroscopy





- Generally the RF pulses are non-selective: they excite both spins A and B simultaneously. Thus, the B spin will undergo the following:



2D NMR experiments

HOMO-NUCLEAR EXPERIMENTS

COSY (**C**Orrelation **S**pectroscop**Y**)

TOCSY(**TO**tal **C**orrelation **S**pectroscop**Y**)

INADEQUATE(**I**ncredible Natural **A**bundance **D**ouble **QU**Antum **T**ransfer **E**xpt)

NOESY (**N**uclear **O**verhauser **E**ffect **S**pectroscop**Y**)

ROESY(**R**otating frame **O**verhauser **E**ffect **S**pectroscop**Y**)

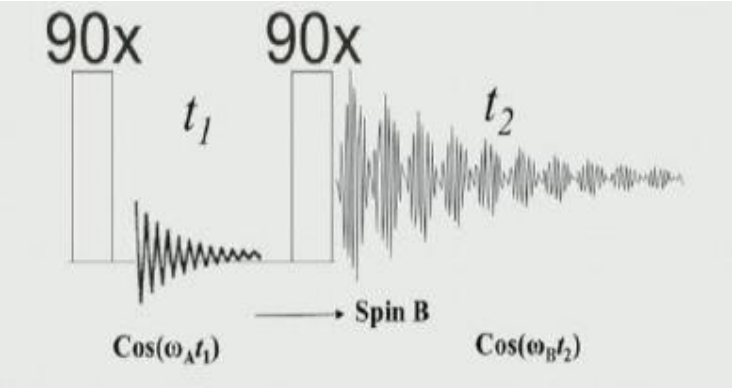
HETERO-NUCLEAR EXPERIMENTS

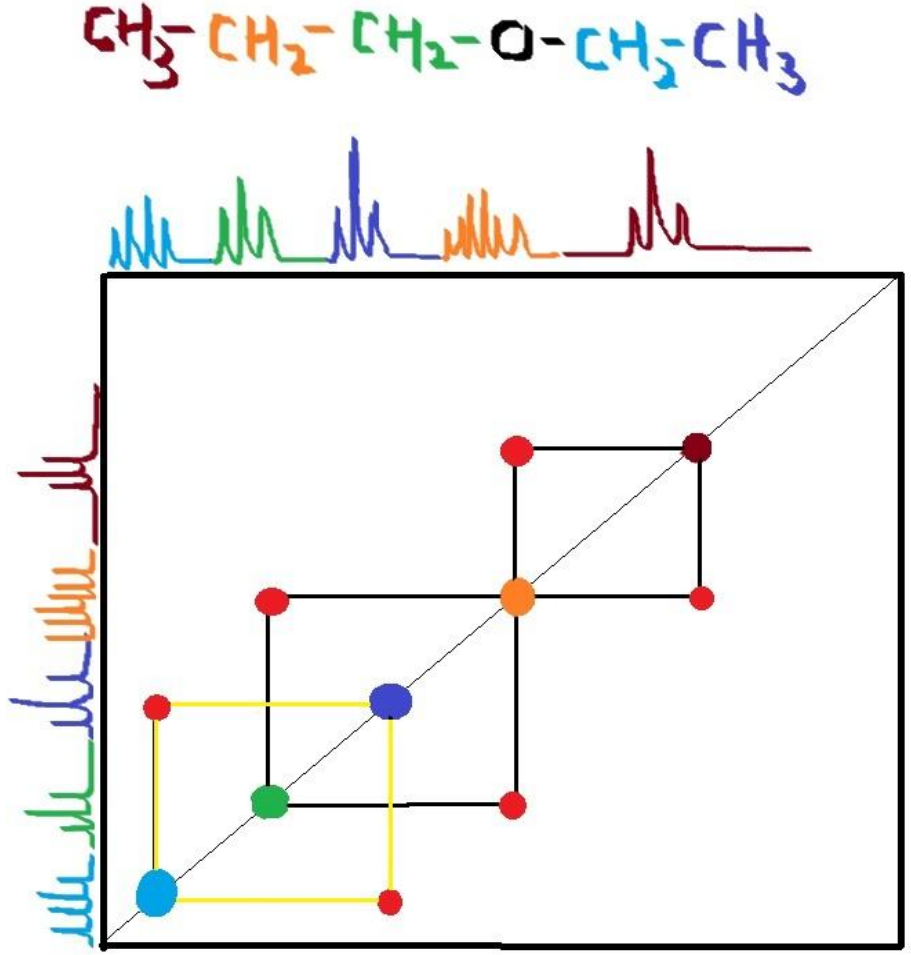
HSQC (**H**eteronuclear **S**ingle **Q**uantum **C**orrelation)

HMQC (**H**eteronuclear **M**ultiple **Q**uantum **C**orrelation)

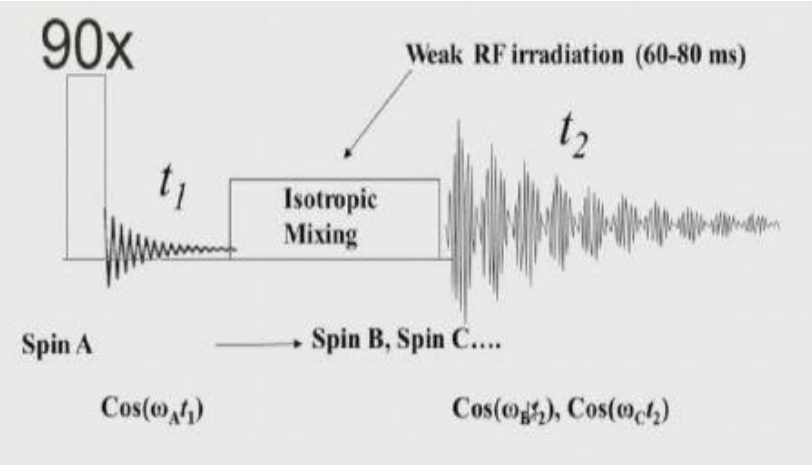
HMBC(**H**eteronuclear **M**ultiple **B**ond **C**orrelation)

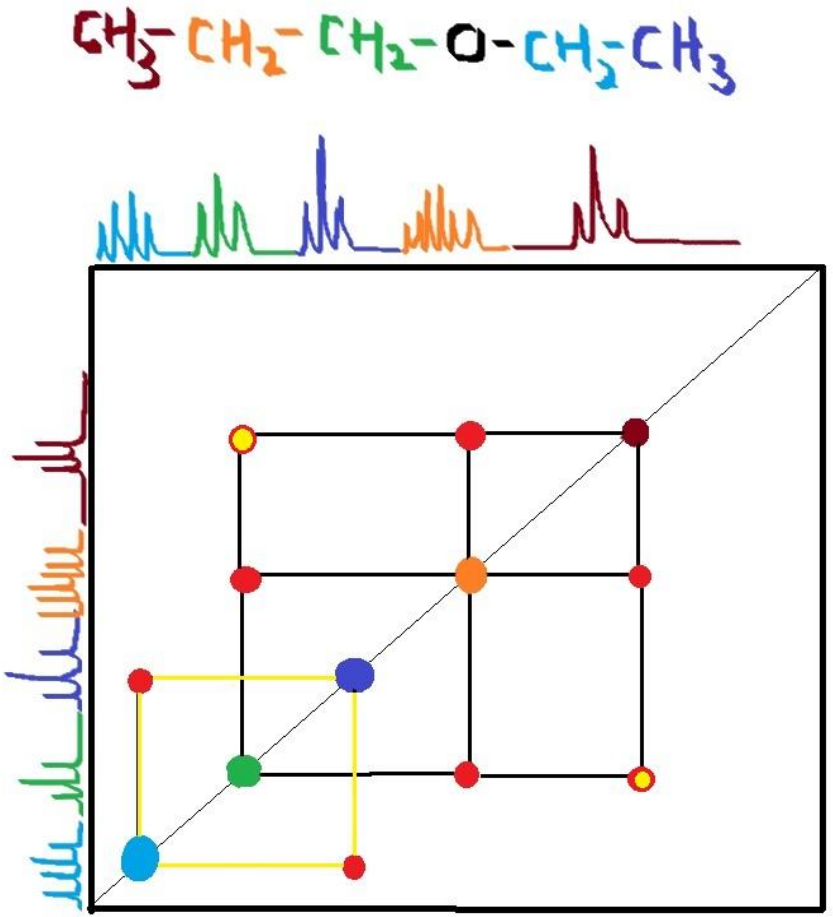
COSY


90x
90x
t_1
t_2
Spin B
$\mathrm{Cos}(\omega_A t_1)$
$\mathrm{Cos}(\omega_B t_2)$


CH3-CH2-CH2-O-CH2-CH3

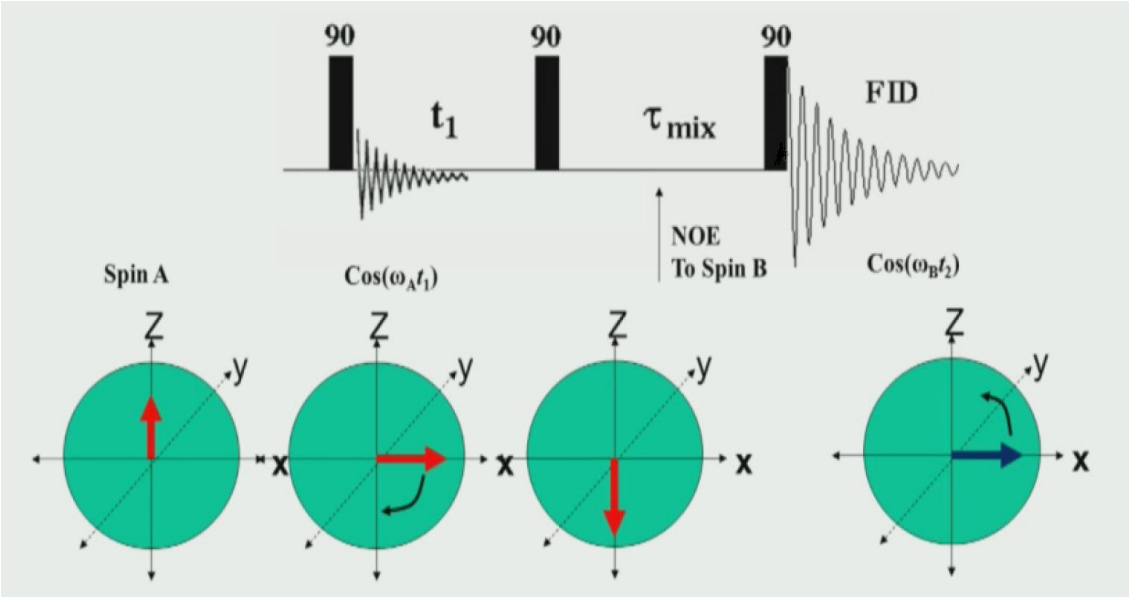
TOCSY


90x
Weak RF irradiation (60-80 ms)
t_1
t_2
Isotropic
Mixing
Spin A
Spin B, Spin C….
$\cos(\omega_A t_1)$
$\cos(\omega_B t_2)$, $\cos(\omega_C t_2)$


CH_3-CH_2-CH_2-O-CH_2-CH_3

NOESY

Nuclear overhauser effect is a phenomena in which signal of a 1H is affected if another 1H close in space is eradicated or inverted .



NOESY can result in '**+**' '**-**' or '**0**' peak enhancement depending on the size of the molecule,

Which is correlated to a parameter called rotational correlation time τ_C

NOESY is '**0**' if [size of molecule (τ_C) $\times$ spectrometer frequency(ω) = 1.]

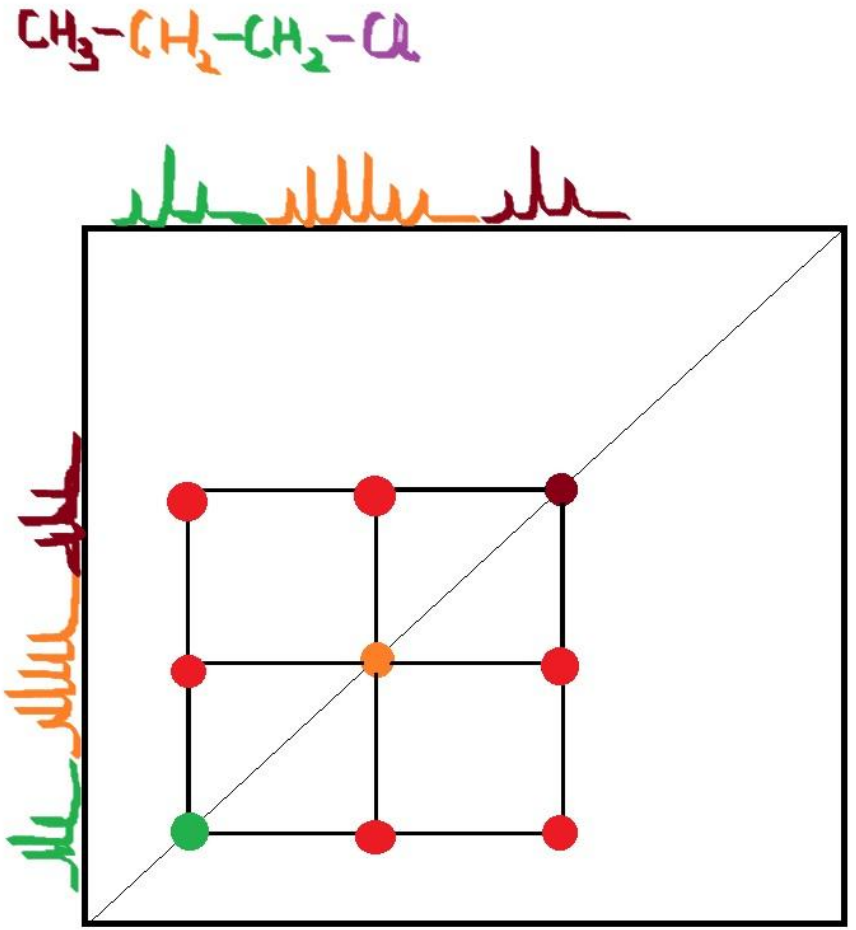
So molecules which satisfy this condition won't give a peak even if there are nuclei which are space correlated.

But, τ_C is temperature dependent . So, we can just go to another temperature and do our measurements.

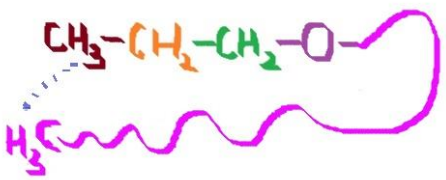
BUT

If we can't change temperature we go for ROESY.

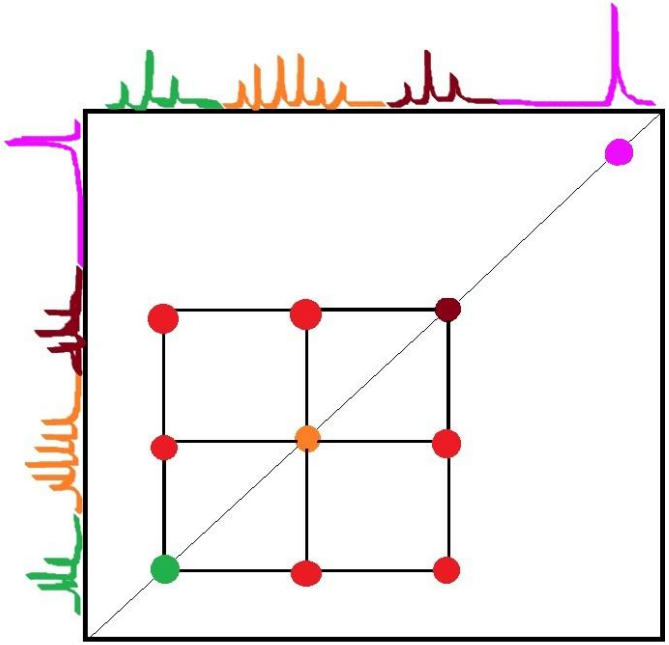
NOESY spectra

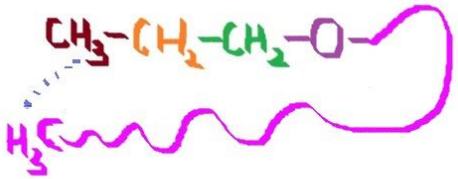


What is the difference between NOESY and TOCSY then ?

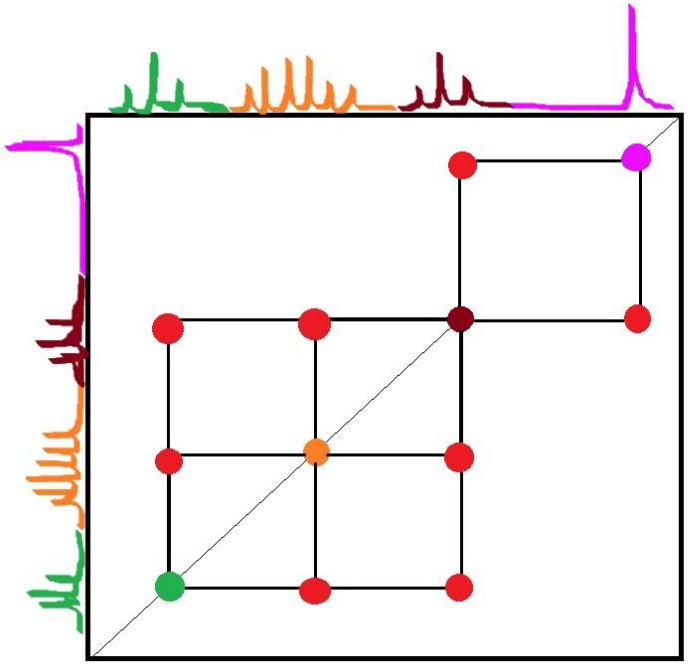


TOCSY





NOESY

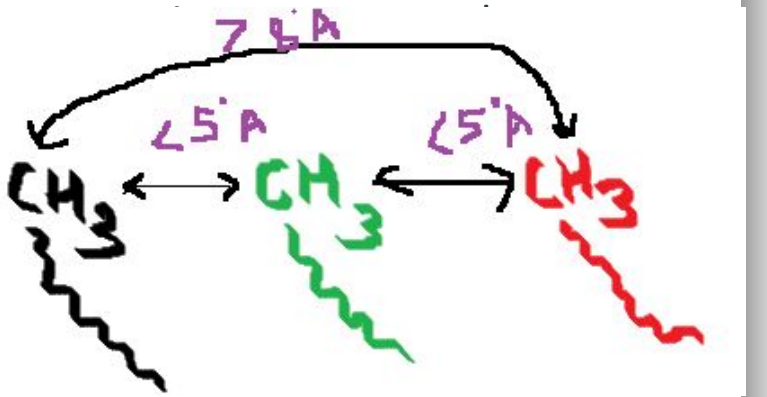


Disadvantages of NOESY

1) It doesn't work for all size of molecules.
2) Some cross peaks in **NOESY** might me due to spin diffusion and not space interaction. But from spe

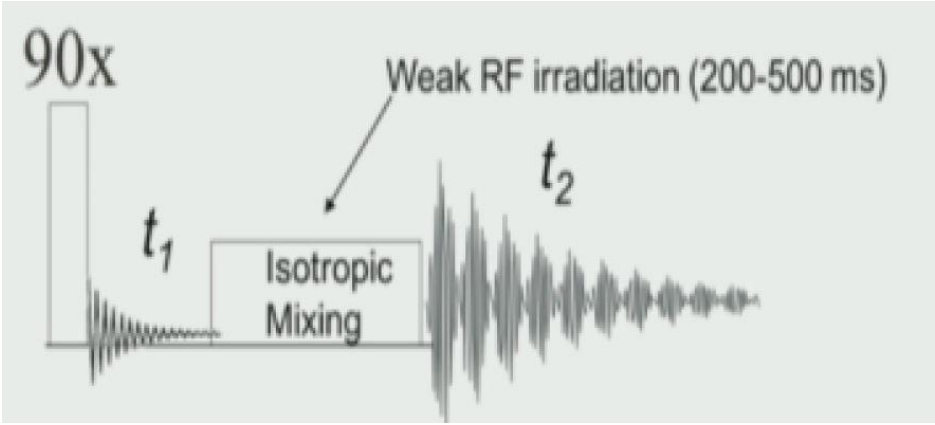
FOR ALL SUCH CASES

ROESY



ROESY

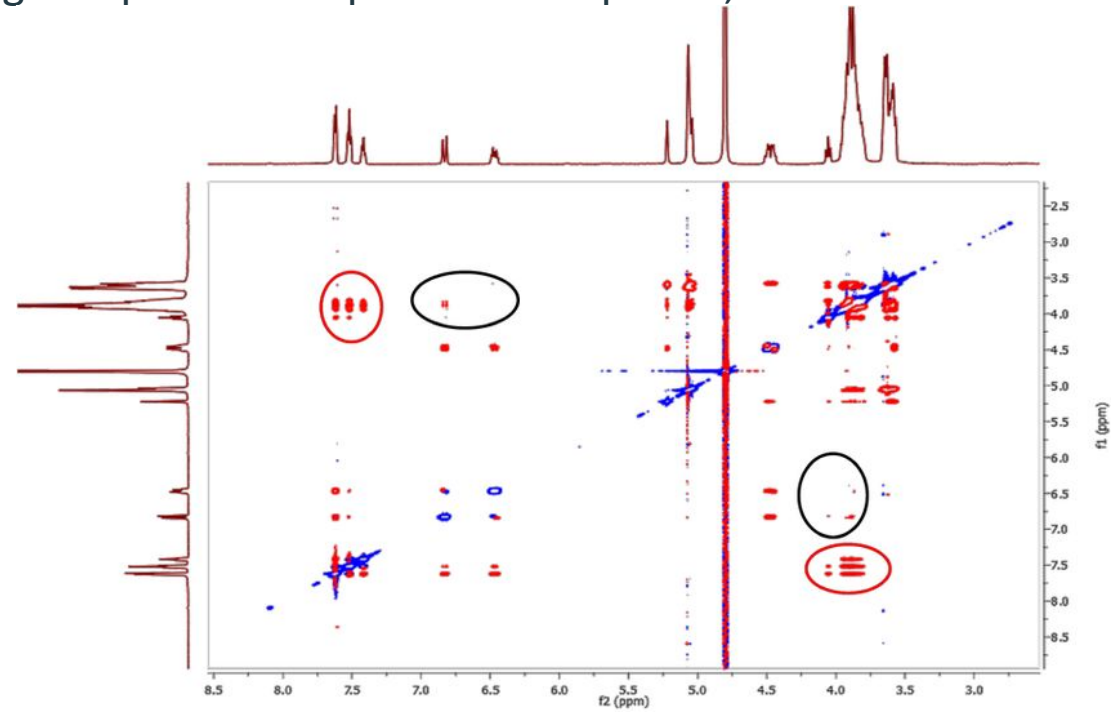
Pulse diagram of ROESY AND TOCSY are very similar.
But,here the weak RF irradiation is applied for much longer time
and the pulse program is optimised in such a way that TOCSY peaks don’t come in the spectrum.



Advantages of ROESY

It works for all molecules of different sizes

It gives a colour say blue for diagonal peaks and spin diffusion peaks , while it gives red colour for NOE peaks

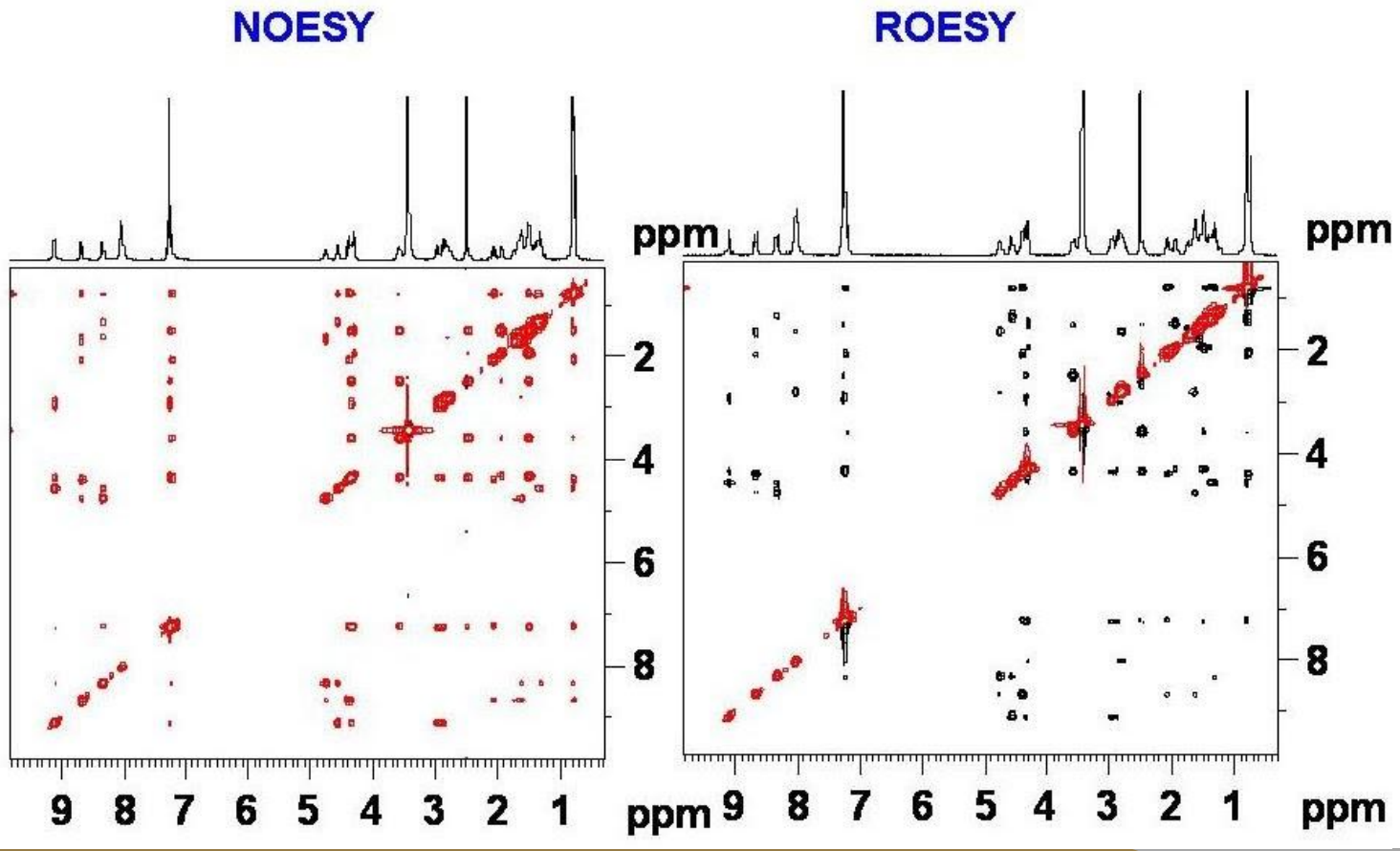


Disadvantages of ROESY

It’s less sensitive compared to NOESY

So, we go for ROESY only if necessary

NOESY vs ROESY for Gramicidin at 300 MHz



NMR Spectra of Hetero Nuclei

Will tell us about the correlation between 1H and different nuclei . eg 19F, 31P, 13C etc.

Different NMR spectra are

2D HECTOR

2D HSQC

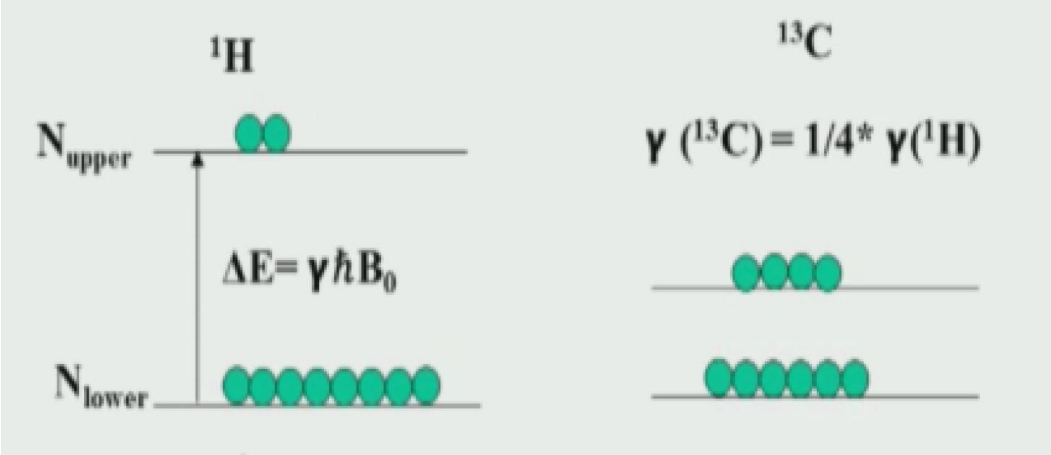
2D HMQC

2D HMBC

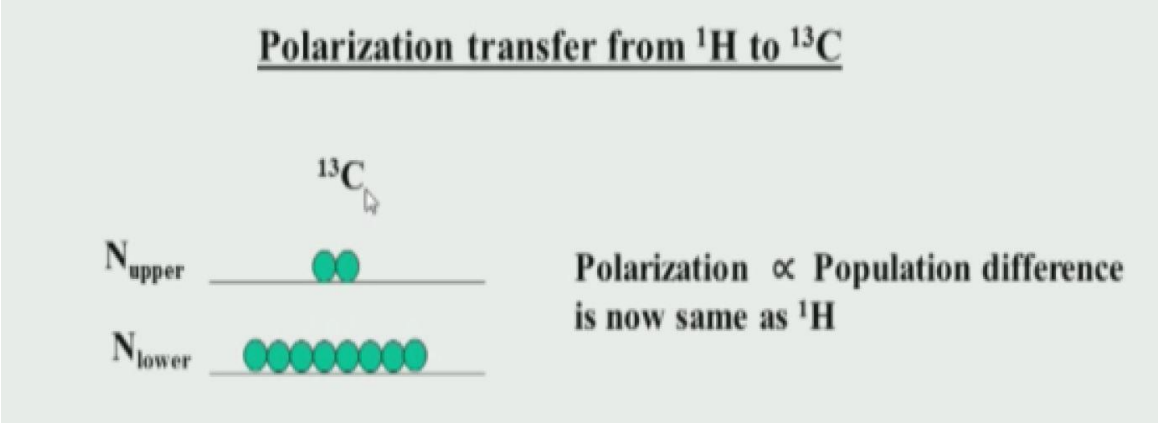
Sensitivity issues

1H and **13C** have different sensitivities so we can't get them to be equally intense.

Sensitivity depends on population difference.



Sensitivity can be attained by polarization transfer

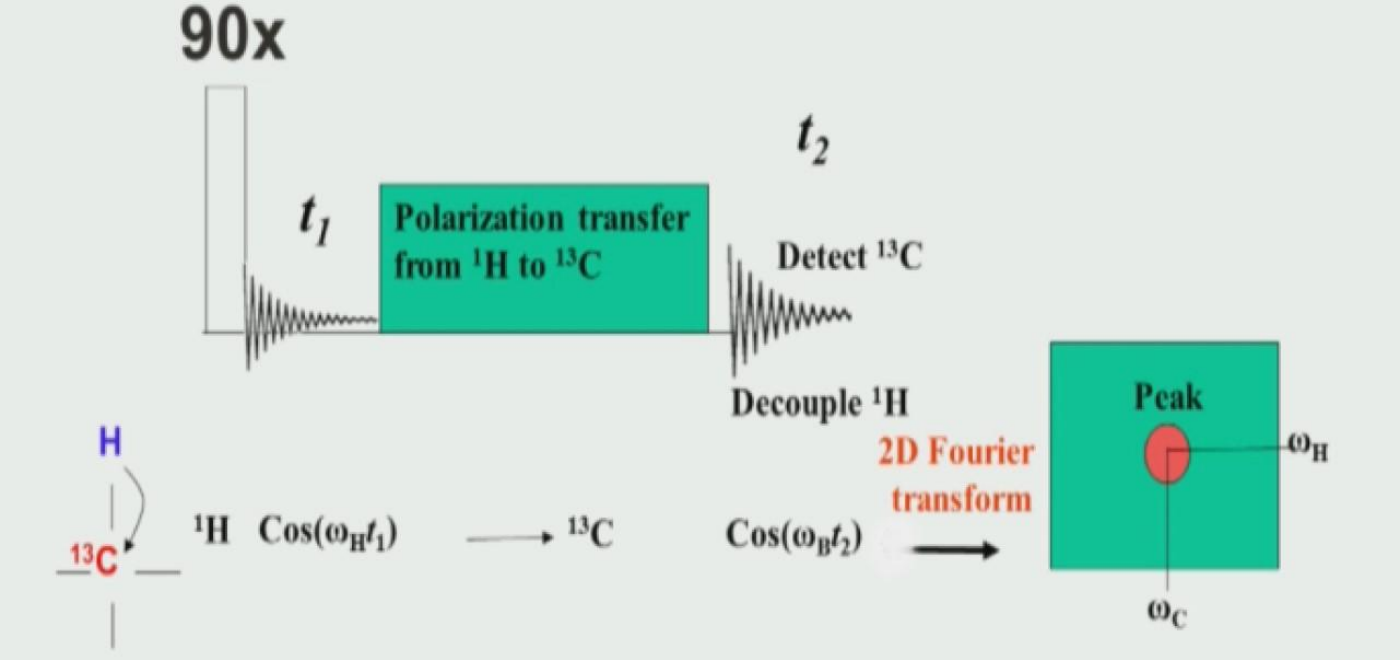


There are two ways to do this

1) Polarization transfer through space(NOE effect)

2) Polarization transfer through bond (J coupling)

2D HECTOR


90x
t_1
t_2
Polarization transfer from ^{1}H to ^{13}C
Detect ^{13}C
Decouple ^{1}H
H
^{13}C
^{1}H Cos($\omega_H t_1$)
^{13}C
Cos($\omega_B t_2$)
2D Fourier transform
Peak
ω_H
ω_C

2D HECTOR not a good technique because,

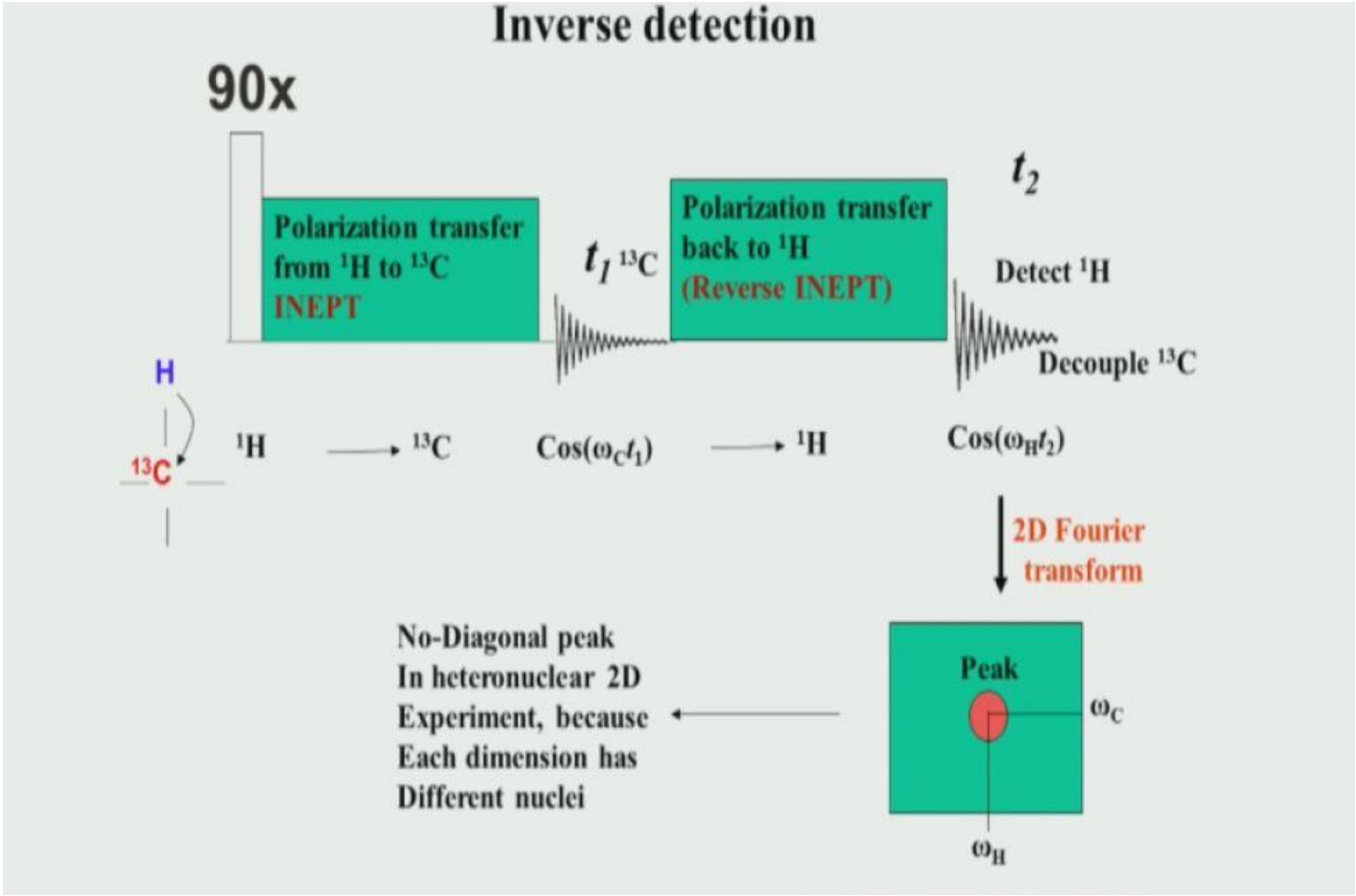
SENSITIVITY $\propto \gamma$(excited)*γ(detected)^3/2

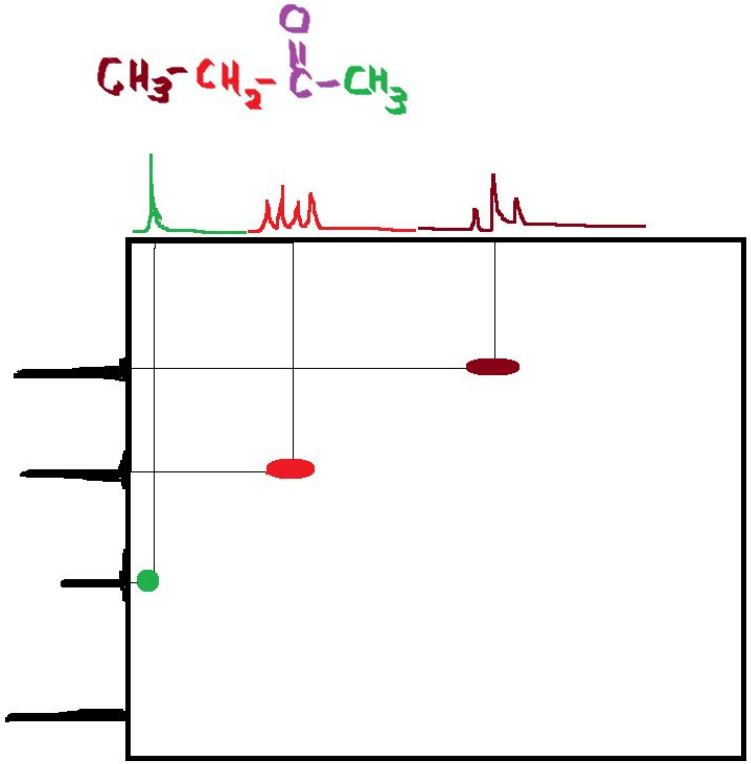
And we are using 1H for excitation and 13C for detection thereby decreasing the sensitivity.

So, we go the next good method.

2D HSQC

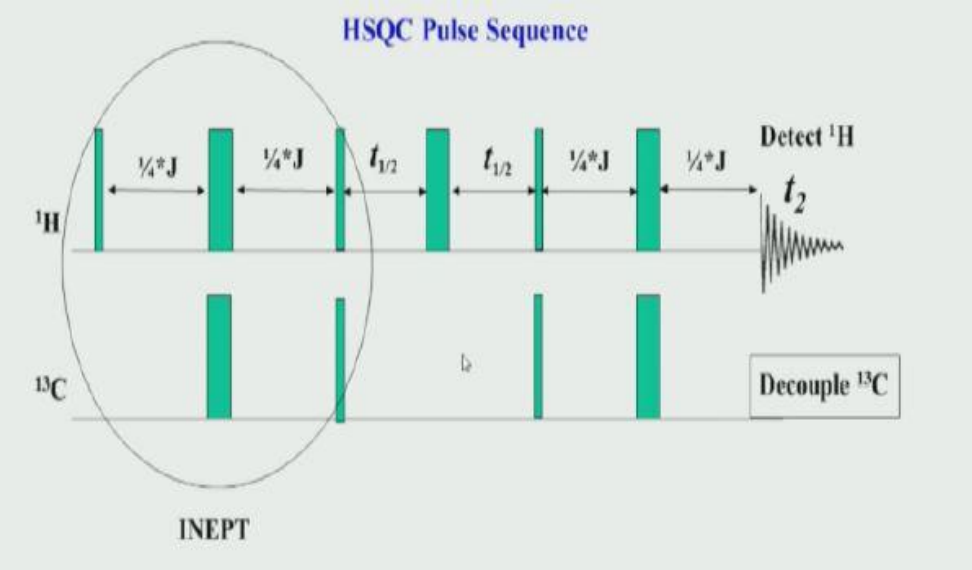
Inverse detection

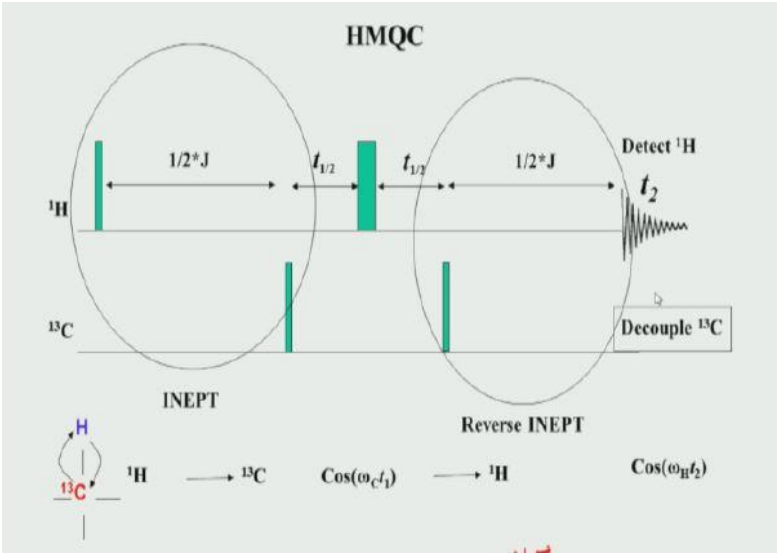



O
CH3-CH2-C-CH3

HSQC v/s HMQC

It gives all the same info as HSQC but the pulse program makes it different





Thank you...

-Rohit B Raj