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Review Article

**A REVIEW ARTICLE ON C13 NMR SPECTROSCOPY****J.Madhusudhan Reddy, PT. Nagaraju\*,B. Jhansi.**

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**Abstract:**

*Nuclear Magnetic Resonance (NMR) Spectroscopy is not limited to the study of protons. Any element with a nuclear spin ( $^{13}\text{C}$ ,  $^{17}\text{O}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$  and many others) will give rise to an NMR signal. Carbon-  $^{13}\text{C}$  NMR ( $^{13}\text{C}$  NMR or referred to as carbon NMR) is the application of nuclear magnetic resonance (NMR) spectroscopy applicable to carbon. It is similar to proton NMR ( $^1\text{H}$  NMR) and allows the identification of carbon atoms whereas in other identification of H. As such  $^{13}\text{C}$  NMR is an important tool in chemical structure elucidation in organic chemistry.  $^{13}\text{C}$  NMR detects only the  $^{13}\text{C}$  isotope of carbon, whose natural abundance is only 1.1%, because the main carbon isotope,  $^{12}\text{C}$ , is not detectable by NMR since it has zero net spin. The chemical shift of  $\text{sp}^3$  hybridized carbon atoms can be estimated with the help of an additivity rule using the shift value of methane and increments for substituents in  $\alpha, \beta$ , positions.*

**Keywords:** Nuclear magnetic resource, protons, nuclear spin, isotope, additivity rule .

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**INTRODUCTION:**

- Nuclear magnetic resonance concerns the magnetic properties of certain atomic nuclei. It concerns the atoms having spin quantum number.
- $^{12}\text{C}$  nucleus is not magnetically active the spin number I being zero. But C Have  $I = \frac{1}{2}$ .
- $^{13}\text{C}$  account for only 1.1% of naturally occurring carbon  $^{13}\text{C}$ -- $^{13}\text{C}$  coupling is negligible and not observed.
- The gyromagnetic ratio of  $^{13}\text{C}$  is one-fourth of that of  $^1\text{H}$ .
- Each non-equivalent  $^{13}\text{C}$  gives a different signal.

- A  $^{13}\text{C}$  signal is split by the  $^1\text{H}$  bonded to it according to the  $(n + 1)$  rule.
- The most common mode of operation of a  $^{13}\text{C}$ -NMR spectrometer is a hydrogen- decoupled mode.

**DEFINITION:**

NMR is a phenomenon exhibited by when atomic nuclei in a static magnetic field absorbs energy from radiofrequency field absorbs energy from radiofrequency. It results to give a spectrum with frequency on X-axis and intensity of absorption on Y-axis.

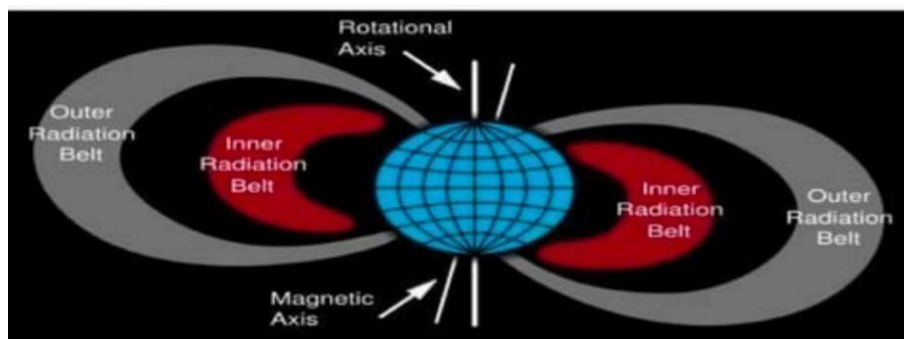


Fig.1: Van Allen Belts

**PRINCIPLE OF NMR:**

When energy in the form of radiofrequency is applied

↓

When applied frequency is equal to precessional frequency.

↓

Absorption energy occurs.

↓

Nucleus in resonance.

↓

NMR signal is recorded.

$E$  absorbed ( $E-1/2$  state  $E+1/2$  state) =  $h\nu$

$$\Delta E = \int (B_0)$$

**Advantages of  $^{13}\text{C}$ -NMR over  $^1\text{H}$ -NMR**

- 1) C-provides information about the backbone of molecules rather than the periphery.
- 2) The chemical shifts range for  $^{13}\text{C}$ -NMR for most organic compounds is 200 ppm compared to 10-15 ppm for H, hence there is less overlap of peaks for C-NMR.
- 3) Homonuclear spin-spin coupling between carbon atoms is not observed because the natural abundance of  $^{13}\text{C}$  is too low for two  $^{13}\text{C}$  to be next to another.

**CHARACTERISTIC FEATURES OF  $^{13}\text{C}$  NMR:**

The chemical shift of the CMR is wider (o is 0-220ppm relative to TMS) in comparison to PMR (8 is 0-12ppm relative to TMS).  $^{13}\text{C}$ - $^{13}\text{C}$  coupling is negligible because of low natural abundance of  $^{13}\text{C}$  in the compound. Thus, in one type of CMR spectrum (proton de coupled) each magnetically non-equivalent carbon gives a single sharp peak that does undergo further splitting.

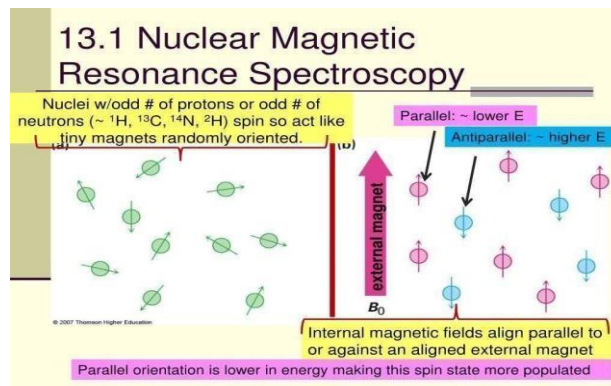
The area under the peak in CMR spectrum is not necessary to be proportional to the number of carbon responsible for the signal. Therefore, not necessary to consider the area under ratio.

Proton coupled spectra the signal for each carbon or a group of magnetically equivalent carbon is split by proton bonded directly to that carbon & the n+1 rule is followed.  $^{13}\text{C}$  nucleus is about one-fourth the frequency required to observe proton resonance.

The chemical shift is greater for  $^{13}\text{C}$  atom than for proton due to direct attachment of the electronegative atom to  $^{13}\text{C}$ .

**Nuclear Magnetic Resonance Spectroscopy:**

- Proton (H) and carbon (C) are the most important nuclear spins to organic chemists.
- Nuclear spins are oriented randomly= absence (a) of an external magnetic field.
- specific orientation= presence (b) of an external field.
- Nuclear Magnetic Resonance Spectroscopy Some nuclear spins are aligned parallel to the external field.
- Lower energy orientation.
- Some nuclear spins are aligned antiparallel to the external field

**Fig. 2: Nuclear Magnetic Resonance Spectroscopy**

- Many nuclei exhibit NMR phenomenon
- All nuclei with odd number of protons.
- All nuclei with odd number of neutrons.
- Nuclei with even numbers of both protons and neutrons do not exhibit NMR phenomenon.

**Why  $^{13}\text{C}$  NMR Required??**

- Proton NMR used for study of number of non-equivalent protons present in unknown compound.
- Carbon NMR can be used to determine the number of non-equivalent carbons and to identify the types of carbon atoms (methyl, methylene, aromatic, carbonyl ... ) which may be present in a compound.
- signals are spread over a much wider range than  $^1\text{H}$  signals making it easier to identify & count individual nuclei.

 **$^{13}\text{C}$  Chemical Shifts:**

**They are measured in ppm ( $\delta$ ) from the carbons of TMS**

- 1) the saturated carbon atom which appears at Up field, nearest to TMS(8-60ppm).
- 2) effect of electronegative atom(40-80ppm)
- 3) Alkenes and aromatic carbon atom (100-170)
- 4) It contains carbonyl carbon bond, which appears at Downfield value (155- 200ppm).
- 5) Transfer of energy is possible from base energy to higher energy levels when an external magnetic field is applied.
- 6) the saturated carbon atom which appears at Up field, nearest to TMS(8-60ppm).
- 7) effect of electronegative atom(40-80ppm)
- 8) Alkenes and aromatic carbon atom (100-170)
- 9) It contains carbonyl carbon bond, which appears at Downfield value (155- 200ppm).

## CORRELATION CHART:


Approximate Values of Chemical Shifts for $^{13}\text{C}$ NMR			
Type of carbon	Approximate chemical shift (ppm)	Type of carbon	Approximate chemical shift (ppm)
$(\text{CH}_3)_4\text{Si}$	0	$\text{C}-\text{I}$	0-40
$\text{R}-\text{CH}_3$	8-35	$\text{C}-\text{Br}$	25-65
$\text{R}-\text{CH}_2-\text{R}$	15-50	$\text{C}-\text{Cl}$	35-80
$\begin{array}{c} \text{R} \\   \\ \text{R}-\text{CH}-\text{R} \end{array}$	20-60	$\text{C}-\text{N}$	40-60
$\begin{array}{c} \text{R} \\   \\ \text{R}-\text{C}-\text{R} \\   \\ \text{R} \end{array}$	30-40	$\text{C}-\text{O}$	50-80
$=\text{C}$	65-85	$\begin{array}{c} \text{R} \\   \\ \text{N}-\text{C}-\text{O} \\   \\ \text{R} \end{array}$	165-175
$-\text{C}$	100-150	$\begin{array}{c} \text{R} \\   \\ \text{RO}-\text{C}-\text{O} \end{array}$	165-175
	110-170	$\begin{array}{c} \text{R} \\   \\ \text{HO}-\text{C}-\text{O} \end{array}$	175-185
		$\begin{array}{c} \text{R} \\   \\ \text{H}-\text{C}-\text{O} \end{array}$	190-200
		$\begin{array}{c} \text{R} \\   \\ \text{R}-\text{C}-\text{O} \\   \\ \text{R} \end{array}$	205-220

Fig: Approximate Values Of Chemical Shift

1

**H and  $^{13}\text{C}$  NMR compared**

$^{13}\text{C}$  signals are spread over a much wider range than  $^1\text{H}$  signals making it easier to identify and count individual nuclei. The term NMR stands for Nuclear Magnetic Resonance. It is a spectroscopic technique used in analytical chemistry for the determination of content, purity and the molecular structures present in a sample. It gives us information about the number and the types of atoms present in a particular molecule. The basis of NMR is the use of magnetic properties of atomic nuclei. NMR is one of the strongest tools that can be used to determine the molecular structure of organic compounds. There are two common types of NMR:  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR. Problems of  $^{13}\text{C}$ :

- **Natural abundance**

$^{13}\text{C}$  natural abundance is very low (1.1%)

- **Gyro magnetic ratio**

$^{13}\text{C}$  nucleus gyro magnetic ratio is much lesser than proton nucleus.

$^{13}\text{C}$ -1.404;  $^1\text{H}$ -5.585.

- **Coupling phenomenon**

$^{13}\text{C}$  &  $^1\text{H}$  have  $I=1/2$  so that coupling between them probably occur.

PROBLEMS IN NMR CAN BE OVERCOME BY

- Fourier Transform Technique-
- Decoupling Technique- 1) Broad Band

Decoupling

- Off Resonance Decoupling
- DEPT (Pulse) Decoupling
- Nuclear Over Hauser Phenomenon-

**Signal Averaging and FT-NMR**

- Low abundance of  $^{13}\text{C}$  is overcome by signal averaging and Fourier- transform NMR (FT-NMR)
- **Signal averaging**
- Numerous individual runs are added together and averaged such that random background noise cancels to zero and NMR signals are enhanced, substantially increasing sensitivity.
- **FT-NMR: (Pulse FT-NMR)**
- Sample is irradiated with entire range of useful frequencies
- $^{13}\text{C}$  nuclei in the sample resonate at once giving complex, composite signal that is mathematically manipulated by Fourier transforms to segregate individual signals & convert them to frequencies.

**Advantages**

- Dramatic increase in the sensitivity of NMR measurements.
- The signals stand out clearly with almost no electronic background noise.

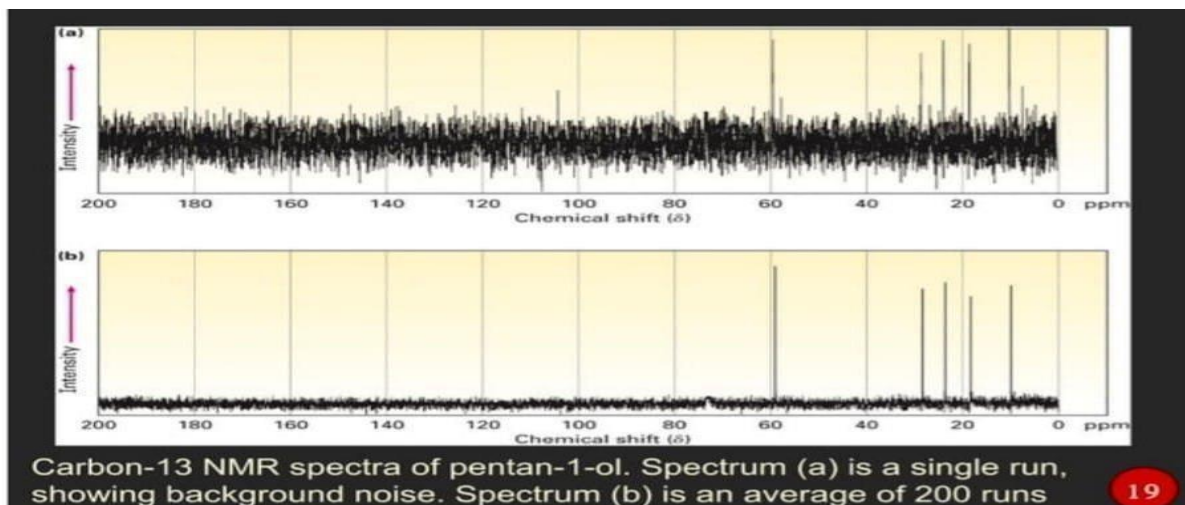


Fig.5: Intensity &amp; Chemical Shift

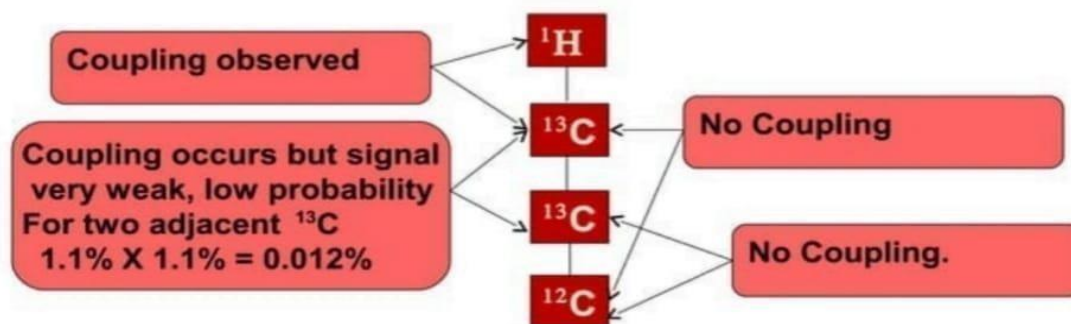
**<sup>13</sup>C-NMR Spin-Spin Coupling****<sup>1</sup>H NMR:** Splitting reveals number of H neighbours.**<sup>13</sup>C NMR:** Limited to nuclei separated by just one sigma bond; no Pi bond.

Fig.6:Flow Chart Decoupling

- **Proton or Noise Decoupling**
  - Irradiation of all <sup>1</sup>H nuclei by using decoupler
  - <sup>1</sup>H nuclei are saturated & C nuclei see zero coupling.
- **Off Resonance Decoupling:**
  - Provides multiple information keeping the spectrum simple.
  - Coupling between C & H attached directly to it
- **DEPT (Distortion less Enhancement by Polarization Transfer):**
  - Involves complex program of pulse & delay times in C & H channel C atoms attached to varying no.

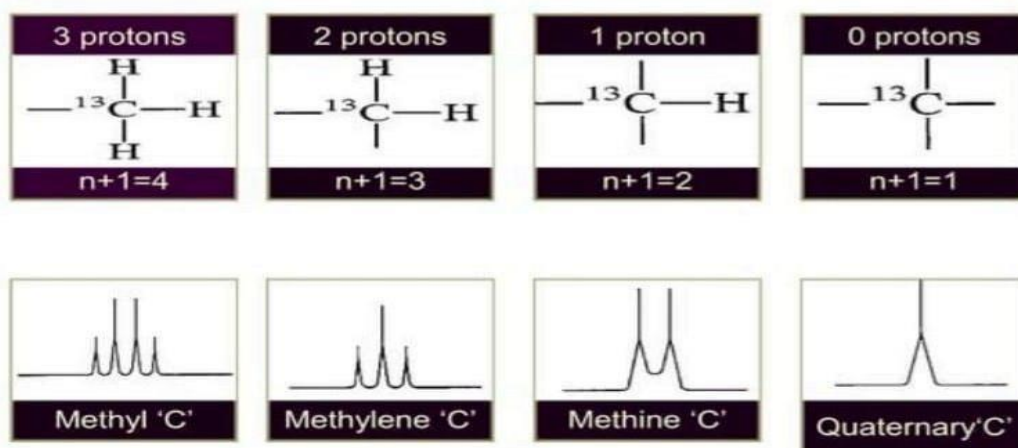
of H exhibit different phases.

**Disadvantage:**

- Information on attached H is lost.

**<sup>13</sup>C off-resonance decoupling**

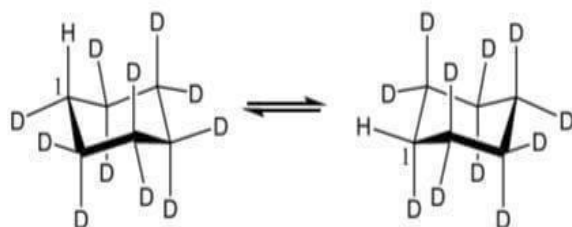
- In this technique the <sup>13</sup>C nuclei are split only by the protons directly bonded to them as a result the multiples become narrow & not removed altogether as in fully decoupled spectra.
- It simplifies the spectrum by allowing some of the splitting formation



### ○ DEPT(Distortionless Enhancement by Polarization Transfer)

#### <sup>13</sup>C NMR Spectroscopy:

The timescale for NMR spectroscopy is longer than for most other spectroscopic methods (UV and FTIR) because relaxation times are of the order of milliseconds to seconds. This makes NMR



spectroscopy a useful technique to study various types of intra- and intermolecular dynamic processes. Intramolecular Processes (Fluxional process):

- 1) Migration of ligand
- 2) Rotation about bond
- 3) Change in conformation
- 4) Spinning of ring

#### Intermolecular Processes (exchange processes): exchange of coordinated and uncoordinated ligands:

If there are two NMR active nuclei present in two different chemical environments, they give two

separate signals with chemical shift difference of  $\Delta\nu$  Hz. However, if a process interchanges their environments with a rate ( $k$ ) much faster than the  $\Delta\nu$ /sec, two nuclei observed as a single peak at an intermediate frequency. This is related to Heisenberg's Uncertainty Principle and also the origin of line broadening by dynamic phenomena. When rate of exchange ( $k$ ) is comparable to the  $\Delta\nu$ , then a broad peak is observed. At coalescence (two signals merge together to give an intermediate signal), the rate of exchange is given by

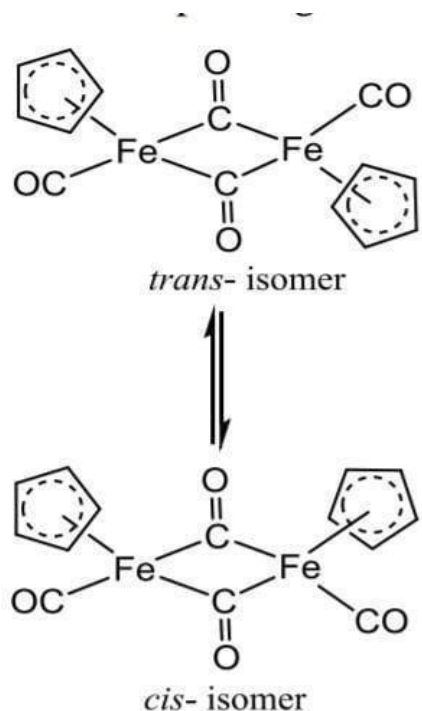
$$k = \frac{\Delta\nu}{\sqrt{2}}$$

The millisecond timescale of NMR spectroscopy makes it particularly useful for monitoring dynamic processes with rates of the order of Hz to kHz, which are virtually impossible to study by any other method.

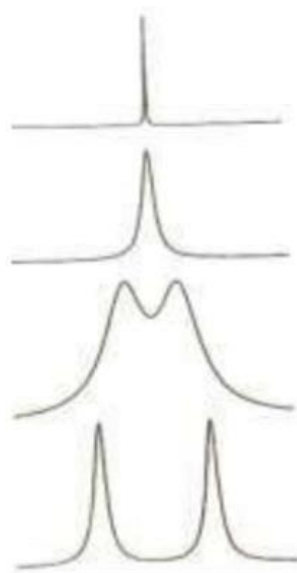
#### Case 1: Cyclohexane interconversion:

The <sup>1</sup>H NMR spectrum of a deuterated cyclohexane molecule (C<sub>6</sub>D<sub>11</sub>H) as a function of temperature.

**Case 2: Cis-Trans Isomerization in  $[(C_5H_5)_2Fe_2(CO)_4]$ :** The  $^1H$  NMR spectrum of complex  $[(C_5H_5)_2Fe_2(CO)_4]$  shows two signals at lower temperature and only one signal at higher temperature. This complex exists as cis and trans isomers, which interconvert rapidly in the solution at



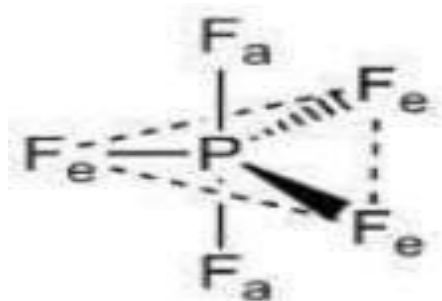
higher temperature and therefore, NMR cannot differentiate cis and trans isomers and single peak is observed. However, at lower temperature, the interconversion is slow and we observe two peaks corresponding to cis and trans isomers.



Variable temperature  $^1H$  NMR spectra of *cis* and *trans*- $[(C_5H_5)_2Fe_2(CO)_4]$

### Case 3:

- Stereochemical non-rigidity in trigonal bipyramidal (TBP) molecules (Berry Pseudo rotation)
- The structure of  $PF_5$  is trigonal bipyramidal with two axial F' and three equatorial 'F'
- NMR spectra compound.



- $PF_5$  is not rigid and hence cannot distinguish axial and equatorial fluorine resulting a doublet with coupling with "p" n  
 $PF_5$  has TBP geometry with two 'F' atoms in axial positions and equatorial positions are occupied by two 'Cl' and one 'F' atoms.  
 At temperatures  $\alpha \geq 22$  deg C and above, all

the fluorine atoms are equivalent and only a doublet ( $J_{FF} = 1048$  Hz) is seen in the  $^{19}F$  NMR spectrum. However, if temperature is lowered to  $-143$  deg \* C the exchange is stopped and two sets of peaks are appeared (i) at  $\delta = 67.4$  ppm for two F, as

'dd' (J<sub>PF</sub>=1048 Hz, J<sub>FF</sub>=124 Hz) and (ii) at  $\delta = -41.5$  ppm for one F<sub>c</sub> as 'dt' (J<sub>PF</sub>=1048 Hz, J<sub>H</sub>=124 Hz).

#### NUCLEAR OVERHAUSER ENHANCEMENT:

- When we obtain a <sup>13</sup>C{<sup>1</sup>H} NMR spectrum, the intensities of many of the carbon resonances increase significantly in comparison to the intensities observed in a proton-coupled experiment.
- Carbon atoms directly attached with hydrogen atoms are enhanced the most, and the enhancement increases (but not always linearly) as more hydrogens are attached to a particular carbon. This effect is known as the Nuclear Overhauser Effect (NOE) and this is applied to the magnitude of the effect as well as to the phenomenon itself.
- Thus, NOE is the change in the intensity of the signal of a nucleus upon irradiation of a nearby nucleus and this operates through space. This effect can be either positive or negative, depending on types of atoms are involved.
- In the case of <sup>13</sup>C interacting with <sup>1</sup>H, the effect is positive; irradiating the hydrogens increases the intensities of the carbon signals. The magnitude of NOE depends on the balance of relaxation mechanisms, and its maximum value for an
  - A{B} experiment in which the B nucleus is saturated is given by
 
$$\text{NOE}_{\text{max}} = \frac{1}{2} \left( \frac{\gamma_{\text{B}}}{\gamma_{\text{A}}} \right) - 1$$
- Where, irr is the magnetogyric ratio of the irradiated nucleus (B), and obs is that of the observed nucleus (A). Remember that NOE<sub>max</sub> is the enhancement of the signal, and it must be added to the original signal strength: Total predicted intensity (maximum) = 1 + NOE<sub>max</sub>
- In a <sup>13</sup>C{<sup>1</sup>H} NMR spectrum, the total NOE for a given carbon increases as the number of nearby hydrogens increases. Thus, we usually find that the intensities of the signals in a <sup>13</sup>C spectrum (assuming a single carbon of each type) assume the order: CH<sub>3</sub> > CH<sub>2</sub> > CH >> C.
- Since, magnitude of NOE depends on relaxation mechanism and dipolar relaxation is particularly important for spin-1/2 nuclei and, as the rate is inversely proportional to the sixth power of the distance between the nuclei, it is most significant when the nucleus being irradiated is directly bonded to the one being observed.
- Therefore, NOE decreases with the 6<sup>th</sup> power of the distance between the interacting nuclei (NOE

is effective typically up to ~4 Å). Signal enhancement due to NOE is an example of cross-polarization, in which a polarization of the spin states in one type of nucleus causes a polarization of the spin states in another nucleus.

- In a <sup>13</sup>C{<sup>1</sup>H} spectrum, when the hydrogens in the molecule are irradiated, they become saturated and attain a distribution of spins very different from their equilibrium (Boltzmann) state. There are more spins than normal in the excited state.
- Due to the interaction of spin dipoles, the spins of the carbon nuclei "sense" the spin imbalance of the hydrogen nuclei and begin to adjust themselves to a new equilibrium state that has more spins in the lower state. This increase of population in the lower spin state of carbon increases intensity of the NMR signal.
- The NOEs experiment is a very useful technique to determine the stereochemistry and structure of the molecule.
- Irradiation of the aldehyde hydrogen leads to a larger NOE for the carbon of the *syn*-methyl group (36.2 ppm) than for that of the anti-methyl group (31.1 ppm), allowing the peaks to be assigned.
- *syn* methyl group is closer to the aldehyde hydrogen.

#### <sup>13</sup>C Interpretation:

- Count how many lines- how many types of carbons
- Symmetry duplicates give same line- if there are more carbons in your spectrum symmetry.
- Check chemical shift window
- Check splitting pattern
- Signal height size

#### <sup>13</sup>C NMR spectroscopy provides information about:

- The number of non-equivalent carbon atoms in a molecule.
- The electronic environment of each carbon.
- How many protons are bonded to each carbon.

#### HETCOR Technique:

- Heteronuclear Chemical Shift Correlation: Carbon Hydrogen:
- <sup>1</sup>H and <sup>13</sup>C spectra plotted separately on two frequency axes.
- Coordinates of cross peak connect signal of



carbon to protons that are bonded to it.

- Other HETCOR technique
- Carbon to Deuterium
- $^{13}\text{C}$  to  $^{19}\text{F}$
- $^{13}\text{C}$  to  $^{31}\text{P}$ .

#### Predicting Chemical Shifts in $^{13}\text{C}$ NMR Spectra:

At what approximate positions would you expect ethyl acrylate,  $\text{H}_2\text{C}=\text{CHCO}_2\text{CH}_2\text{CH}_3$ , to show  $^{13}\text{C}$  NMR absorptions?

- Ethyl acrylate has five distinct carbons: two different  $\text{C}=\text{C}$ , one  $\text{C}=\text{O}$ , one  $\text{C}(\text{O})-\text{C}$ , and one alkyl  $\text{C}$ . From Correlation chart the likely absorptions are,

#### Spin-Spin Splitting in $^{13}\text{C}$ NMR:

- 1) Because of low natural abundance of  $^{13}\text{C}$  nuclei, the probability of finding two  $^{13}\text{C}$  atoms
- 2) adjacent to each other in the same molecule is very low. Hence, no spin-spin splitting between  $^{13}\text{C}-^{13}\text{C}$  nuclei.
- 3) Spin-spin splitting occurs between hydrogen attached to the particular  $^{13}\text{C}$  atom. Such spectrum called proton coupled  $^{13}\text{C}$  NMR spectrum. The splitting of  $^{13}\text{C}$  signal occurs according to  $n+1$  rule.
- 4) The proton coupled  $^{13}\text{C}$  NMR spectrum are very difficult to interpret due to large coupling constant ( $J = 100-320$  Hz).  $^1\text{H}-^{13}\text{C}^1J = \sim 100-320$  Hz;  $^1\text{H}-\text{C}-^{13}\text{C}^2J = 0-60$  Hz;  $^1\text{H}-\text{C}-\text{C}-^{13}\text{C}^3J = 0-60$  Hz

#### NMR OF PARAMAGNETIC COMPOUNDS:

Although, NMR signals of paramagnetic molecules are generally broad, some paramagnetic compounds are useful reagents in high-resolution NMR studies. Also, many times NMR studies of paramagnetic species could be profitable and the consequences of the paramagnetism may be helpful in structure determination of the compounds. The breadth of the resonances and a large expansion of the normal chemical shift scale (e.g. more than 200 ppm for protons) are two most important characteristics of NMR spectra of paramagnetic compounds. The signals from diamagnetic compounds could also show these effects in the presence of paramagnetic species, even in low concentrations. The line broadening of the spectra is caused by fast relaxation (short electronic spinlattice relaxation times) and/or hyperfine electron-nuclear coupling. In general, the chemical shifts of nuclei close to the paramagnetic center are more affected than those of nuclei that are further away. For example, the  $^1\text{H}$  NMR spectra of  $[\text{M}\{\text{dimethyldi}(\text{phenylethynyl})\text{aluminates}\}_3]$

#### HECTOR SPECTRUM:

- 2D-NMR spectra that displays  $^1\text{H}-^{13}\text{C}$ -NMR shift correlations are called HETCOR spectra. It shows coupling between protons and the carbon to which they are attached.
- The methyl doublet of  $^1\text{H}$ -NMR spectrum appears at  $\delta$  1.2 when drawn cross-peak and then dropped down to the  $^{13}\text{C}$  spectrum axis indicates that the  $\text{C}$  peak at 5.20 is produced by methyl carbon of 1-chloro-2-propanol( $\text{C}-3$ )
- down to the  $^{13}\text{C}$  spectrum shows the  $^{13}\text{C}$  NMR signal at 67 arises from the  $\text{C}-2$  carbon is the  $n$  of the compound i.e., the carbon carrying the hydroxyl group.

#### Applications:

- NMR is a non-invasive and non-destructive method, i.e., especially used in repetitive In-vivo analysis of the sample without harming the tissues.
- $^{13}\text{C}$  NMR has elucidated and biochemical structure.
- $^{13}\text{C}$  NMR provides information about the backbone to molecule rather than periphery.
- $^{13}\text{C}$  nuclei are stable isotopes and hence it not danger to radiotracer.
- And also used for quantification of drug purity to determination of the composition of high molecular weight synthetic polymer.
- CMR is a noninvasive and nondestructive method, i.e especially used in repetitive In vivo analysis of the sample without harming the tissues.
- CMR, chemical shift range(0-240 ppm) is wider compared to  $^1\text{H}$ - NMR(0-14 ppm), which permits easy separation and identification of chemically closely related metabolites.
- $^{13}\text{C}$  enrichment, which the signal intensities and helps in tracing the cellular metabolism.
- CMR technique is used for quantification of drugs purity to determination of the composition of high molecular weight synthetic polymers.

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