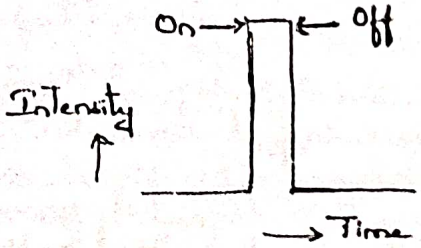
2D-NMR SPECTROSCOPY

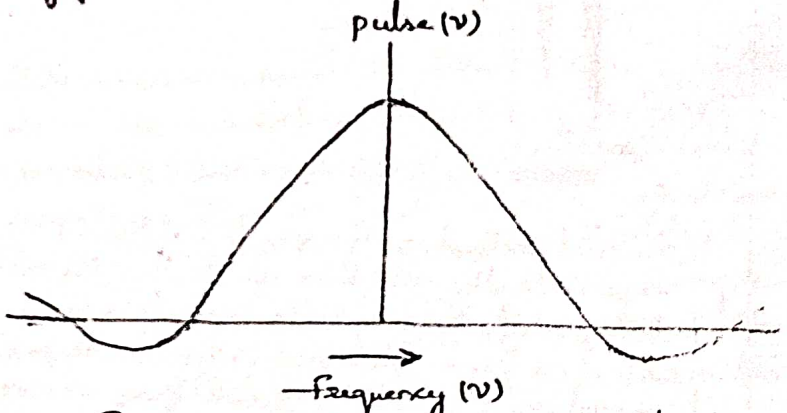
Spectral techniques are very much useful for the structural elucidation of molecules. Among various spectral techniques, NMR, CMR & FMR etc are very useful in the structural elucidation of molecules. The most commonly encountered difficulties in nuclear magnetic resonance (NMR) spectral studies, are strong couplings, unresolved signals etc. These difficulties can be eliminated by using new techniques. 2D NMR is Two Dimensional NMR spectroscopy such modern technique used to eliminate difficulties of the classical NMR spectroscopies like ^{1}H-NMR & ^{13}C-NMR.

The CW type of NMR spectrometer operates by exciting the nuclei of the istope under observation one type at a time. In case of ^{1}H nuclei, each distinct type of proton is excited individually, and its resonance peak is observed and recorded independently of all the others. In this method scanning is done until all of the types have come into resonance.

In the modern sophisticated instruments, a powerful but short burst energy, called a pulse, is used to excite all of the magnetic nuclei in the molecule simultaneously. The pulse actually contains a range of frequencies centered on the fundamental as shown in the figure. When the pulse is discontinued, the ex

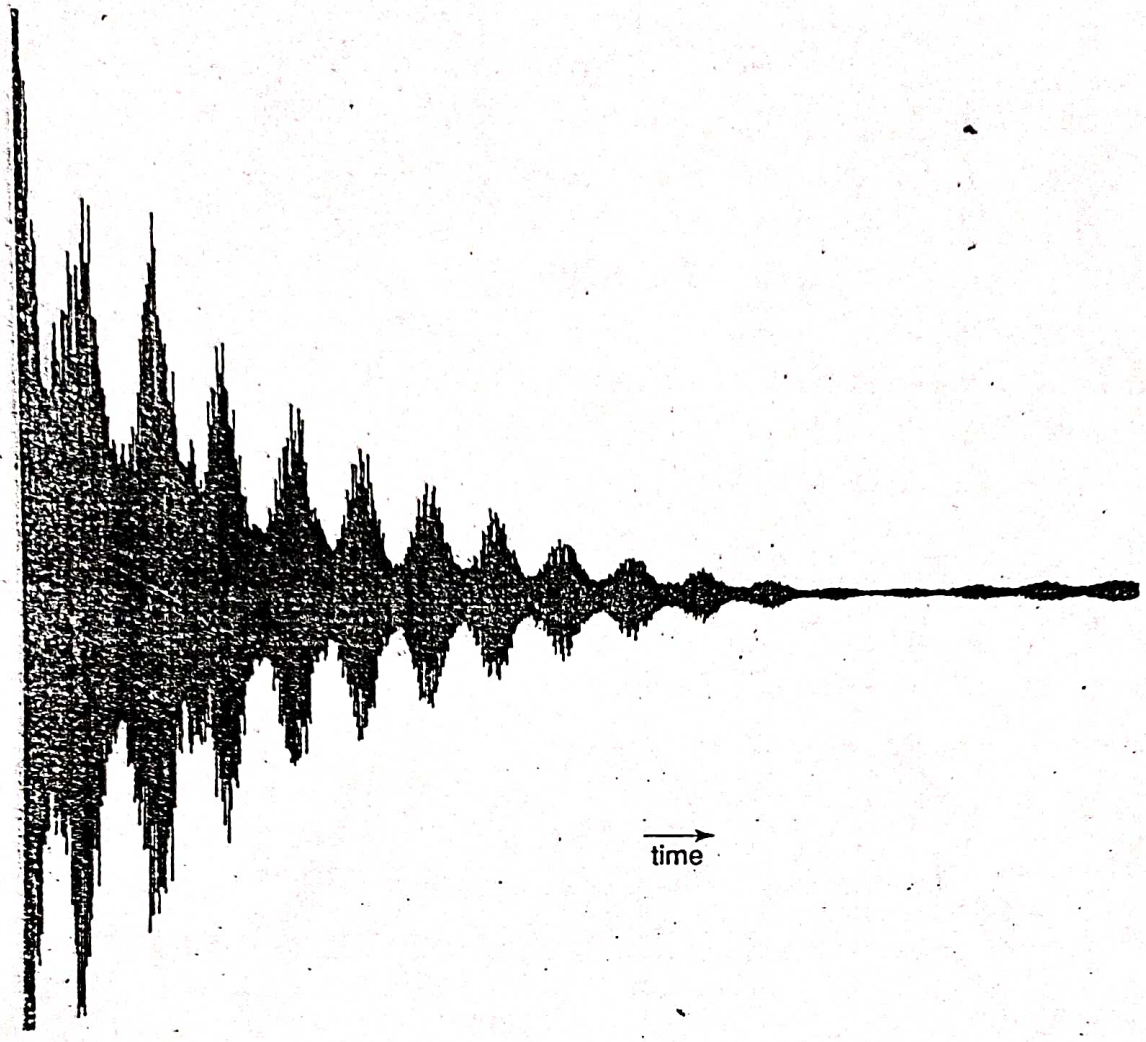


A short pulse.



Frequency content of the sample pulse.

nuclei begin to lose their excitation energy and return to their original spin state relax. As each excited nucleus relaxes, it emits electromagnetic radiation. Since the molecule contains many different nuclei, many different frequencies of electromagnetic radiation are emitted simultaneously. This emission is called a free induction decay (FID) signal. The intensity of the FID decays with time as all the nuclei eventually lose their excitation. The FID is a superimposed combination of all the frequencies emitted and can be quite complex. The individual frequencies of different nuclei are extracted using a computer and a mathematical



The ^1H free-induction decay (FID) signal of ethyl phenylacetate (300 MHz).

method called a Fourier transform (FT) analysis. The FID signal decays exponentially with time as the nuclei relax and their signal diminishes. The horizontal axis in FID signal is time axis and hence FID signal sometimes called as time-domain signal. As mentioned, the FID signal is the superimposition of many different frequencies. The mathematical method called Fourier Transform, separates each of the individual components of the FID signal and convert them from time domain to frequency domain.

A conventional modern ^1H NMR spectrum has a frequency axis and an intensity axis. However, a 2D-NMR spectrum will have two frequency axes and intensity axes. The 1D-NMR is a single pulse experiment, in which the equilibrium spin system is subjected to a single radio frequency pulse before the acquisition of FID.

A 2D NMR spectrum is actually a three-dimensional plot because it consists of two frequency axes and an imaginary intensity axes. In 2D NMR, multiple pulse techniques are applied on simple spin systems. A two-dimensional NMR experiment involves several time periods: preparation, evolution, mixing and detection.

Preparation:

This involves in establishing some well-defined state for the occurence of nuclear spins, ie. nuclear spins 'are prepared' for the experiment. The development of well-defined state is required because, all the multi-dimensional NMR methods require multiple separate NMR experiments. Initially, the spins of the nuclei of one type nucleus are randomly oriented. In this preparation period, a delay (time) sufficient is provided to give equilibrium magnetization for all nuclei. The final part of the preparation period usually involves one or more pulses that place magnetisation(s), ie. magnetisation vectors, at right angles to the direction of the ~~magnetic~~ magnetic field axis.

Evolution:

Generally, the nuclear moments precess around the direction of a magnetic field, much like a top precesses with in the gravitational field of the earth. Nuclei in different chemical environments precess at different rates. These nuclei react differently to a well defined environment. The construction of well defined environment involves magnetic field gradients, radiofrequency (rf) fields, magnetic fields, nuclear spin interactions such as J-couplings. The magnetizations induced by the last part of the preparation period are permitted to evolve over a fixed period of time (ie. t_1) under a well-defined magnetic and radio frequency environment.

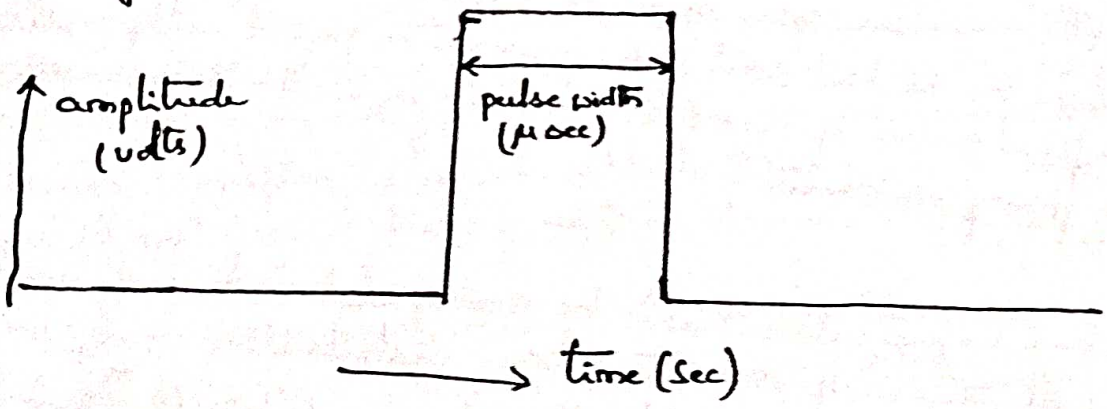
Mixing:

The nuclear magnetisation has to be ~~redo~~ redistributed among the spins (nuclei) at the end of evolution. This distribution may involve the use of pulses and rf time periods. The idea of distribution is to allow spin communication for a fixed period. The communication mechanism present will ~~defi~~ determine the way in which data can be interpreted. ~~The~~ Two examples of mechanisms of spin communication are J-coupling and dipolar relaxation.

Detection:

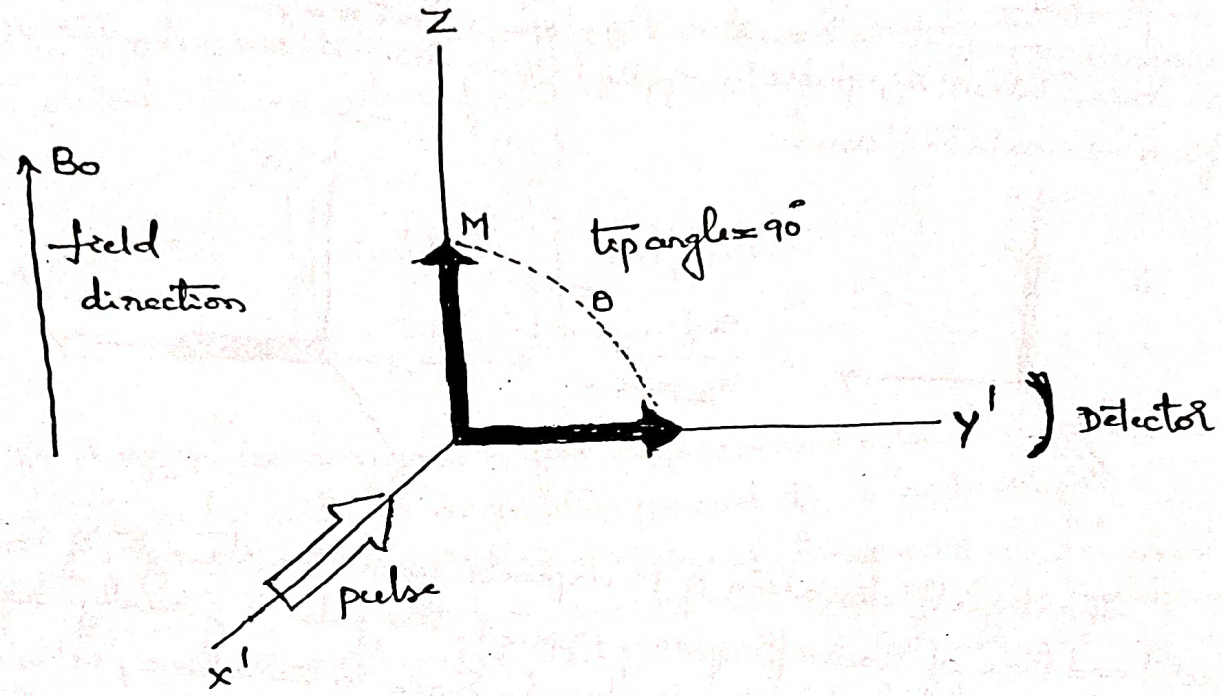
Finally, the NMR spectrum of these nuclei is monitored & recorded in the form of a normal chemical shift pattern. The appearance of the spectrum will usually differ in intensity & phase from the ordinary spectrum. These phase and/or intensity variations can be brought about by systematically and regularly varying the evolution time (t_1) from zero to some appropriate limit.

The ~~Computer~~ Computer that is built into modern FT-NMR instruments is very versatile and enables to develop more complex and more interesting pulse sequences. The use of such instruments will facilitate, the transmission of second and even third pulses, along any of the cartesian axes. The pulses can be transmitted with varying durations, and a variety of times can also be programmed into the sequences. As a result of these pulse programmes, nuclei may exchange energy, they may affect each other's relaxation times, & they may provide information about spin coupling from one nucleus to another. Generally, a radio frequency pulse of a very short duration - Typically of the order of 1 to 10 microseconds (μsec) is transmitted into the sample. The pulse can be applied along either the x' or the y' axis and in either the positive and negative direction. The shape of the pulse, expressed as a plot of DC voltage versus time, looks like the one below.



A square wave pulse.

In a typical experiment, the duration of the pulse is selected to cause a specific tip angle of the bulk magnetization vector (the resultant vector of all of the individual vectors), and a pulse duration (known as pulse width) is selected to result in a 90° rotation of the bulk magnetisation vector. Such a pulse is known as 90° pulse.



The effect of a 90° pulse (M is the bulk magnetization vector for the sample)

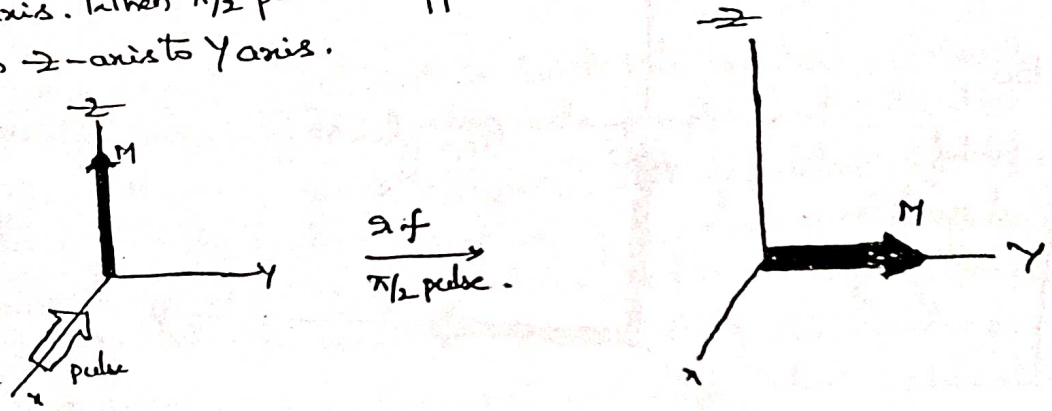
In the laboratory frame, the y' component corresponds to a magnetisation vector rotating in the XY plane. The magnetization vector rotates in the XY plane because the individual nuclear magnetization vectors are processing about Z (the principal field axis). Before the pulse, individual nuclei have random precessional motion and are not in phase. The pulse causes phase coherence to develop so that all of the vectors precess in phase.

~~Advd~~

Pulse Techniques:

The pulse techniques are simple and which involves the terms of magnetic moment of nuclei and axis along which the magnetic field is applied.

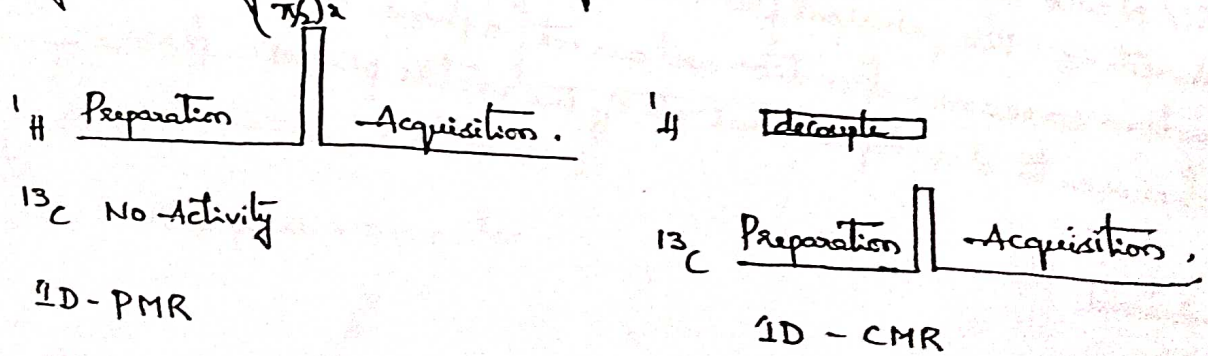
Single pulse: The application of strong magnetic field, in Z-direction, makes the magnetic moment to exist in Y-Z plane and net magnetisation vector along -z-axis. When $\pi/2$ pulse is applied then the net magnetisation vector displaces from Z-axis to Y axis.



$\pi/2$ pulse.

The angle of deviation of 'M' depends upon the strength of magnetic field and time of radio frequency applied. The pulse is generally represented as angle through which 'M' is displaced. Since in the above, 'M' is displaced from Z to Y i.e. 90° and hence the pulse is known as $\pi/2$ or 90° pulse.

In a normal 1D-NMR, a $\pi/2$ pulse (single) is used. In PMR spectra, the ^{13}C nuclei are inactive and in CMR spectra protons are active and hence spin-spin interactions between ^{13}C and ^{1}H are possible. It is possible to record proton decoupled ^{13}C NMR spectrum. The entire process can be diagrammatically represented as follows.

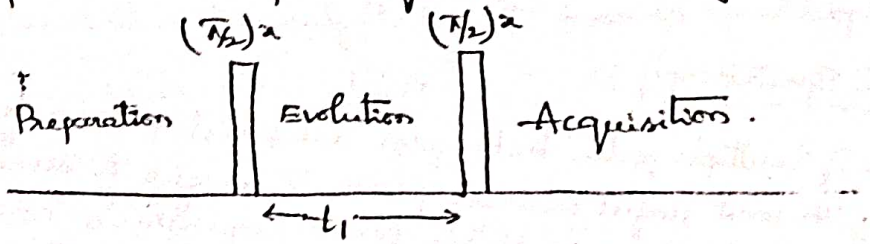


1D-PMR

1D - CMR

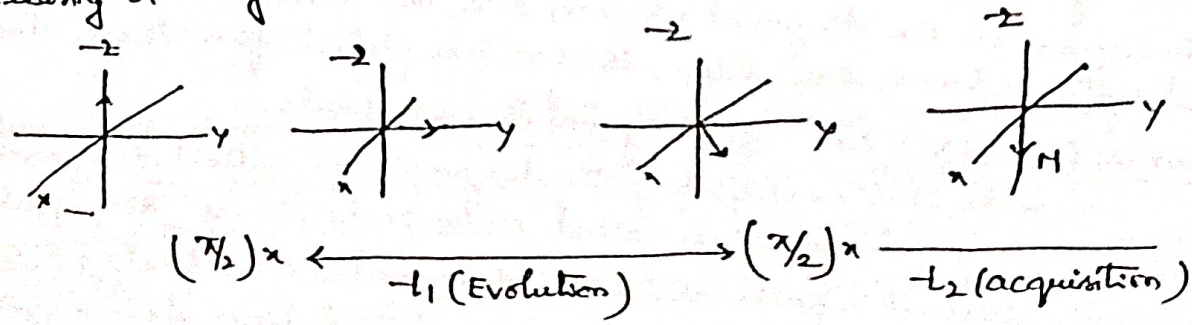
When relaxation is completed, 'M' reverses to -z axis and another pulse is applied. The number of pulses applied depends on the nature of data required.

Multiple pulse: In this the equilibration period is followed by multiple pulse sequence with an intervening time interval, the final pulse being the $(\pi/2)_x$ acquisition pulse. This is the basis of 2D-NMR and the introduction of the evolution period between pulses facilitate recording the FID's into one experiment.



In A $\pi/2$ pulse sequence is first applied and after a time interval t_1, a second $\pi/2$ pulse with an acquisition period t_2 is applied. This gives the FID and the experiment is repeated several times with an increased t_1 interval. The signals are collected immediately after the pulse is stopped and continued till the next pulse. This increases the resolution of the signals.

This ~~repetition~~ repetition of the experiment generally includes increasing t_1 many times and as a result, several FID's are obtained.

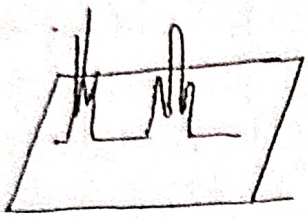


In these techniques, the signals is detected as a function of 't_2' and is modulated by 't_1'.

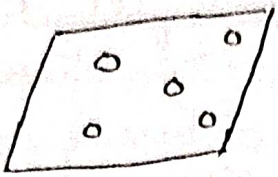
Representation: The 2D plots are generally represented in two ways.

① Stacked plot: These are 'mountain-like' spectra and are not much used in practice to study a compound (3D photograph of a geographical model consisting of plains and mountains).

② Contour plots: The plot due to top-down view of mountains. Here plotting bands in this the slice of each mountain is represented by contour circle.



Stacked plot



Contour plot.

COSY (Correlation Spectroscopy):

A number of multiple pulse techniques are used in 2D NMR spectroscopy. Among them, one of the most useful multiple pulse technique to reveal the coupling relations in a molecule by using suitable pulse sequence is COSY (Correlated Spectroscopy). The COSY signals are plotted in a 3D-plot either in stacked form or contour form.

The application of pulse sequence in COSY results in the evolution of magnetisation with one frequency during t_1 and with a different frequency during t_2. This results in two peaks with different frequencies ν_1 & ν_2.

Generally, COSY spectrum appears on the diagonal. Those components of the spin system that remain unperturbed by coupling even in a spin coupled system appears on the diagonal where ν_1 & ν_2 are identical. But in a coupled system, where ν_1 & ν_2 differ, the contours plotted from these unequal frequencies (i.e. $\nu_1 \neq \nu_2$) are off-diagonal or cross peaks.

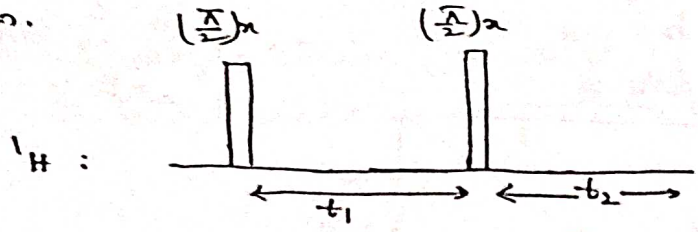
The peaks that occur along the diagonal are called diagonal peaks. The peaks outside the diagonal are called cross peaks. These cross-peaks are symmetrically disposed in pairs about the diagonal and indicate which protons are coupled to which, as shown by dotted lines drawn vertically and horizontally from either one of the paired cross peaks of the diagonal.

The two axes ν_1 and ν_2 are different from each other and the axis ν_2 represents the same nucleus (protons) and ν_1 may represent same or different nuclei (either ^1H or ^{13}C) or a coupling constant.

The plotting of chemical shifts of protons on both the axes results in HOMO COSY and if chemical shifts of protons on one axis and that of ^{13}C nucleus on other axis results in HETERO COSY.

The pulse Technique involves the application of a broad strong $\pi/2$ pulse and the nuclei are allowed to relax for a short time 't_1'. Then a second $\pi/2$ pulse is applied and recording is started and continued till relaxation is complete. The process is repeated by changing 't_1'.

The data is then subjected to FT operation to obtain frequency domain.

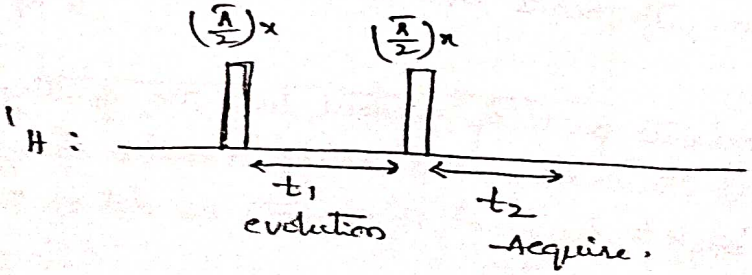


Importance of COSY

1. COSY is an excellent technique for analysing the coupling nuclei even though there are several nuclei
2. The spectrum is helpful to analyse the no of protons to different carbon atoms due to high resolution of cosy.

HOMO COSY : Homonuclear correlated Spectroscopy [^1H-^1H COSY]

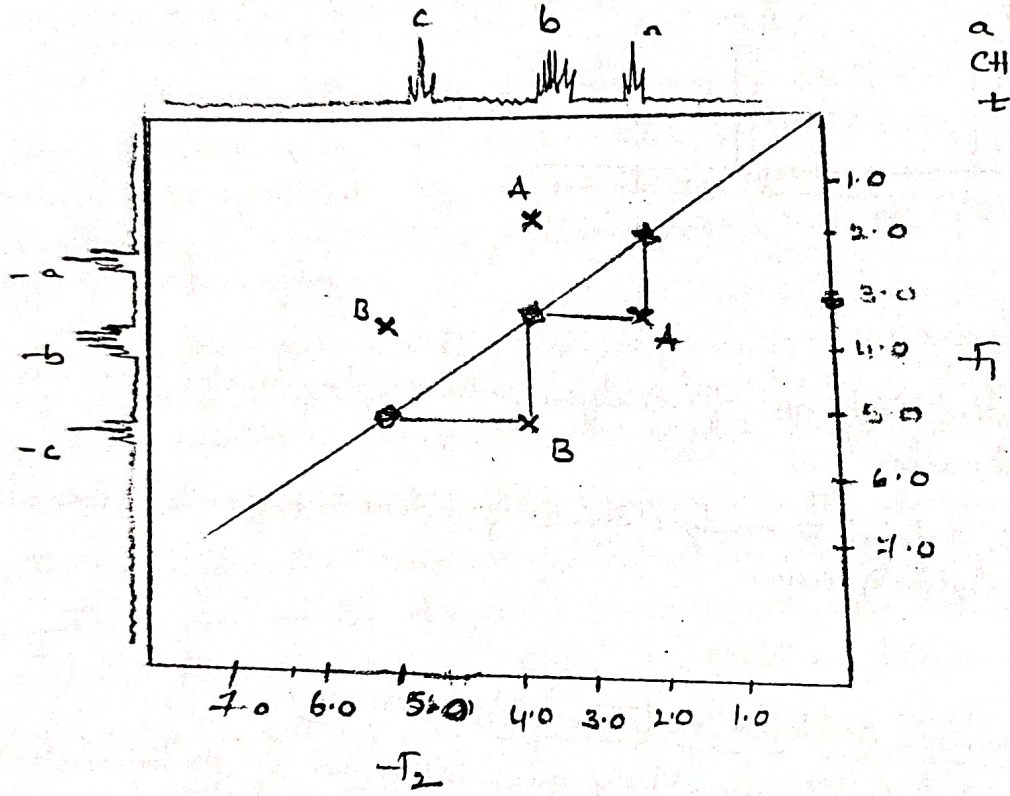
The correlated spectroscopy which gives information of proton nuclei that are coupled to each other is known as ^1H - ^1H COSY. The basic pulse sequence involves the two $\pi/2$ proton pulses which are separated by required evolution period 't_1' and acquisition period t_2.



The proton spectrum appears along the diagonal as contour representing peak intensities. The off-diagonal contours are called cross-peaks. A horizontal line drawn from a ~~Contour~~ cross-peak will intercept a contour on the diagonal and a vertical line from the same cross-peak will intercept another contour on the diagonal with which the diagonal contour is correlated i.e. coupled.

Eg 1. Nitropropane

The HOMOCOR Spectrum of 1-nitropropane is :—



a b c
CH_3 CH_2 CH_2NO_2
t multiplet t

The usual one-dimensional ^1H NMR spectrum is plotted on both x and y axes. To analyse this, a diagonal is drawn through the peaks that bisects the spectrum. The peaks that are not on the diagonal are called cross peaks.

Cross peaks indicate pairs of protons that are splitting each other. For example, if we start at the cross peak labelled 'A' and a straight line is drawn parallel to y-axis back to diagonal, it connects the dot on the diagonal at ~2.0 ppm produced by the protons.

If we then go back to 'A' and a straight line parallel to x-axis is drawn back to the diagonal, it connects the dot on the diagonal at ~3.0 ppm produced by the protons. This indicates that cross peak 'A' shows correlation between Ha and Hb protons and hence we can say that Ha and Hb protons are coupled.

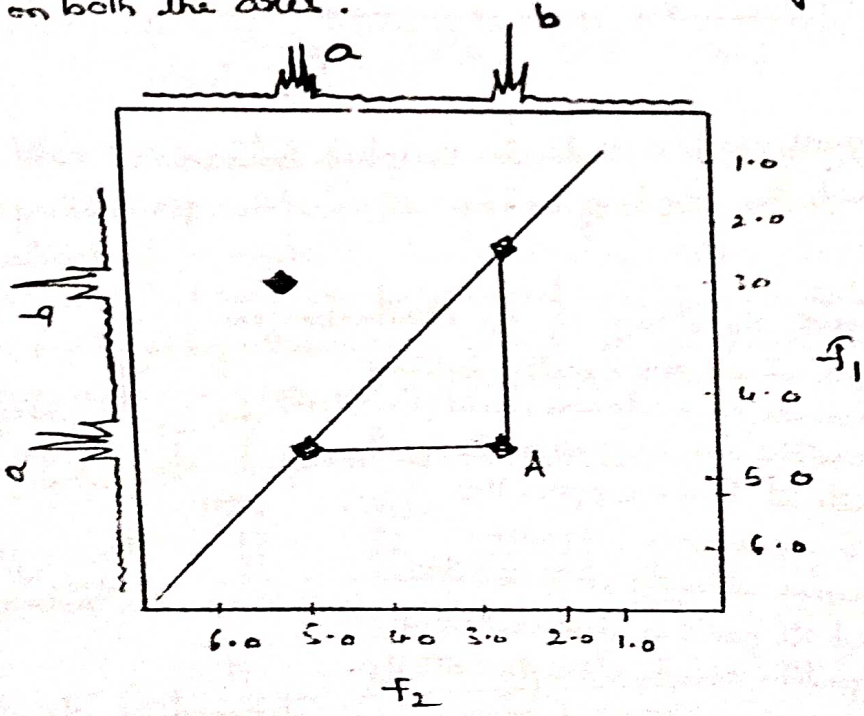
Similarly cross peak 'B' shows that Hb and Hc protons are coupled. Cross peaks are symmetrical on both side of the diagonal. So the cross peaks above the diagonal gives the same information as those below the diagonal.

Example: 2. ethyl Ethane [CH_3CH_2Cl]

The methyl protons are designated as 'b' and methylene protons as 'a'.

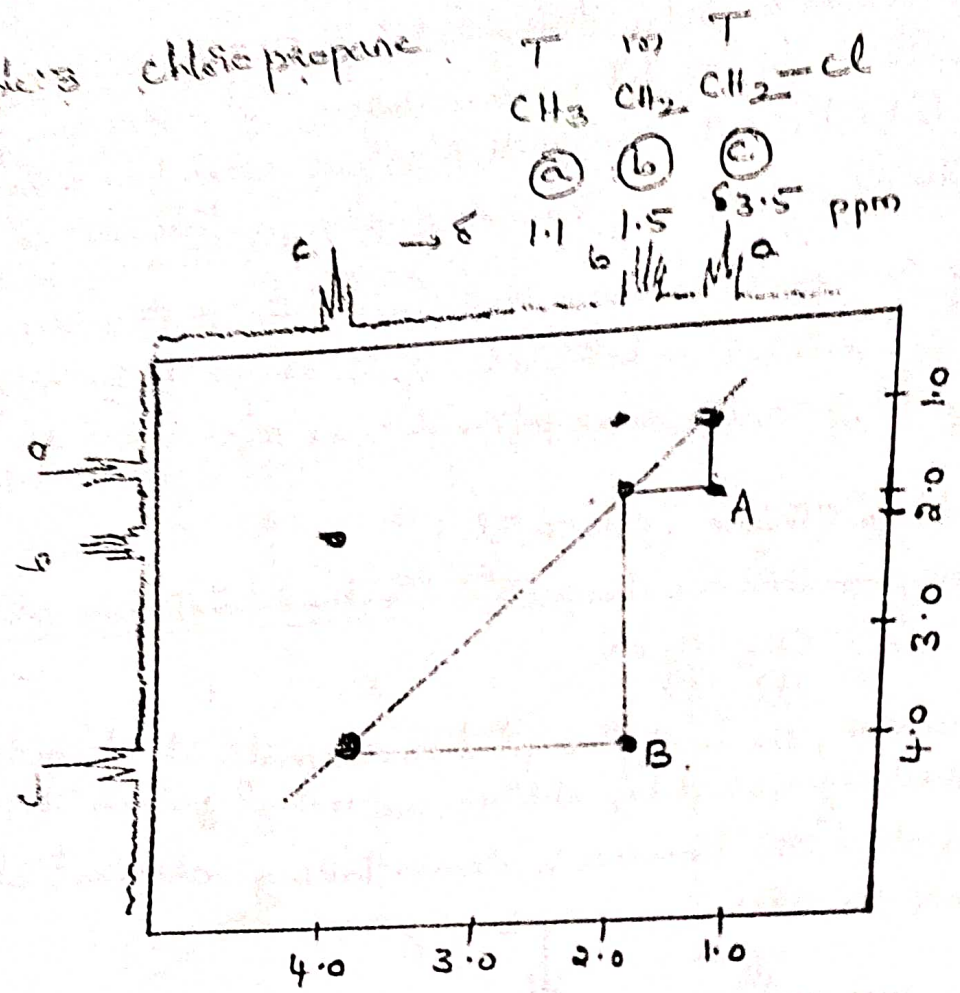
i.e. $CH_3\ CH_2Cl$
(b) (a)

In one 1D NMR, the methylene protons resonate at down field & high δ due to deshielding caused by chlorine and methyl protons resonate at slightly up field. The Homocor is drawn taking chemical shifts of protons on both the axes.



$CH_3\ CH_2Cl$
b a
T Q

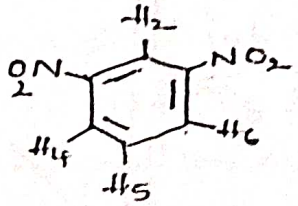
Example 13 Chloropropene



The cross peak 'A' indicates coupling between H_a and H_b protons and cross peak 'B' indicates coupling between H_b and H_c protons.

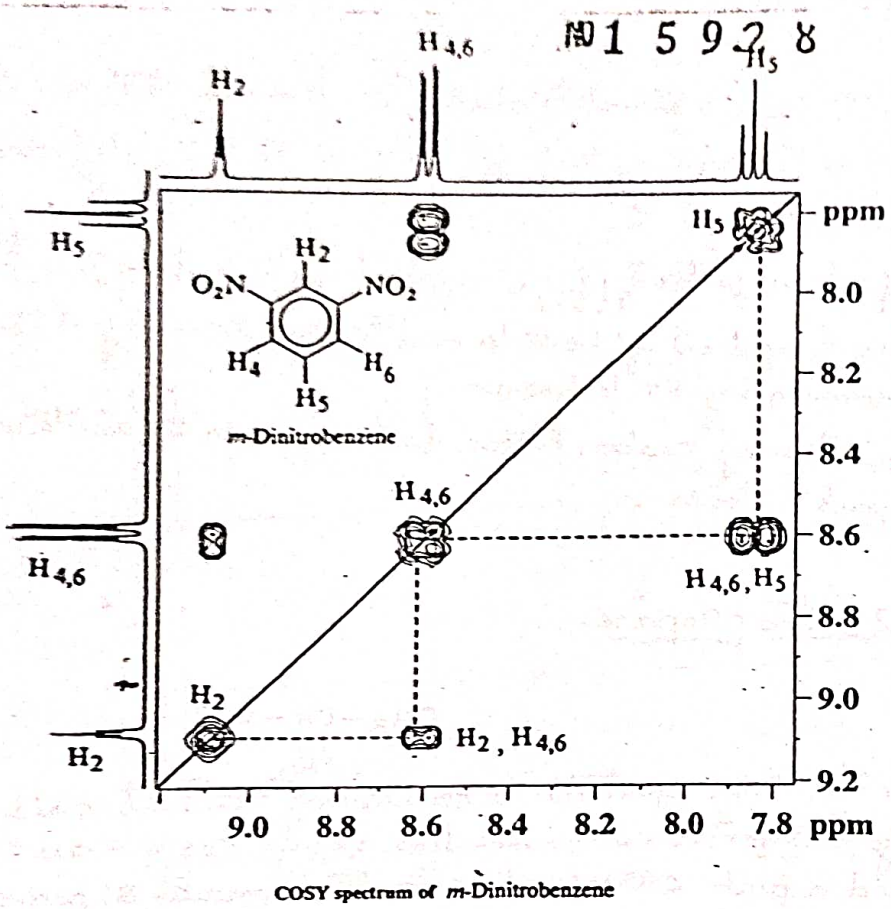
Example 14 Homocor Spectrum of m-dinitrobenzene

The H_2 protons, which is existing between two nitro groups, resonate at a down field ($\delta \sim 9.1$ ppm) The H_4 and H_6 protons are in similar chemical environment and resonate at $\delta \sim 8.6$ ppm. H_5 proton resonates at $\delta \sim 7.8$ ppm.



m-dinitrobenzene.

H_5 proton couples strongly with two ortho protons H_4 and H_6 and its peak is observed as a triplet. H_4 and H_6 protons couple strongly with H_5 and weakly with meta H_2 proton and its peak is observed as doublet of doublets. The H_2 couples weakly with H_4 and H_6 protons.

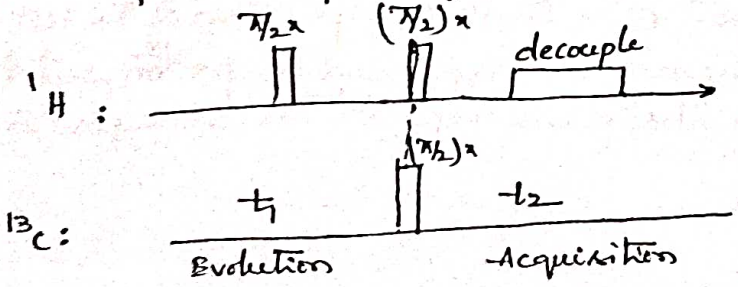


COSY spectrum of *m*-Dinitrobenzene

$^1H - ^{13}C$ COSY: HETCOR – Heteronuclear Correlated Spectroscopy & chemical shift correlation map.

The 2D NMR spectra that show $^{13}C - ^1H$ shift correlations are called HETCOR spectra. HETCOR spectra gives information about the specific protons that are attached to each ^{13}C. The proton spectrum (^1H) is presented on the vertical axis and ^{13}C broad band decoupled spectrum on horizontal axis. The $^1H - ^{13}C$ correlation is shown by a cross peak contour at the intersection of a horizontal line drawn from a proton peak and a vertical line drawn from ^{13}C peak.

The pulse sequence for HETCOR is :-



The cross peaks give information about $^{13}C - {}^{1}H$ nuclei that are coupled. The pulse sequence is same as that for HOMCOR but the difference is that there is no first pulse for ^{13}C.

At first, a pulse of $\left(\frac{\pi}{2}\right)$ is applied for ^{1}H and after a time period of 't', another $\left(\frac{\pi}{2}\right)$ pulse is applied for both ^{1}H and ^{13}C. The resulting FID is converted into frequency domain by FT technique.

Quaiternary carbon atoms donot appear in the spectrum because they have no cross peak contours.

Example 1: 2-nitro propane

$$CH_3 - \underset{\displaystyle NO_2}{\underset{|}{CH}} - CH_3$$

Generally, the PMR spectrum is taken along vertical axis and ^{13}C-NMR spectrum along horizontal axis. The two methyl carbons are in similar enviroments and a peak corresponding to these appear at 21 ppm and a peak corresponding to methine carbon appear at 79 ppm.

The two methyl protons are in similar chemical enviroment and a doub corresponding to these protons due to coupling with methine proton is observed at 1.56 ppm and a septet for the methine proton at 4.6 ppm.

A vertical line from the methyl peak of the carbon spectrum (21 ppm) and a horizontal line from the methyl peak of the proton spectrum (1.56 ppm the two lines would intersect at the exact point 'A' on the two dimensional plot where a spot (cross peak) is observed. This spot indicates that the protons at 1.56 ppm and the carbons at 21 ppm represent the same position in the molecule. That is, the hydrogens are attached to the indicated carbon In the same way, the spot B in the lower left corner of the HETCOR plot correlates with the carbon peak at 79 ppm and the proton septet at 4.6 ppm indicating that these two absorptions represent the same positions in the molecule.

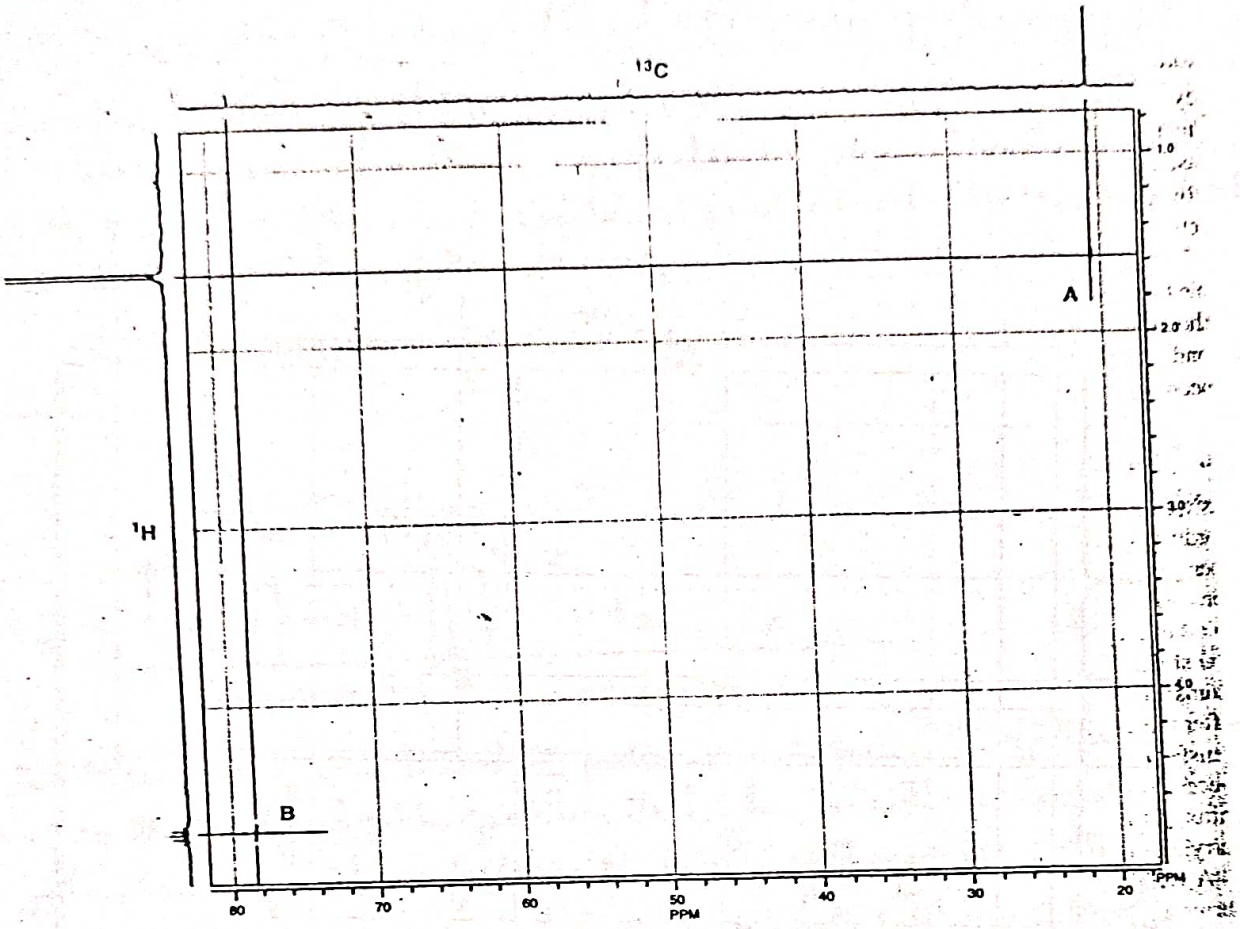
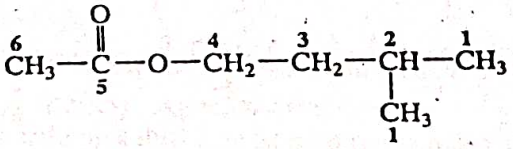


Figure *HETCOR spectrum of 2-nitropropane.*

Example: 2.

Isopentyl Acetate. A second, more complex example is isopentyl acetate. [redacted]



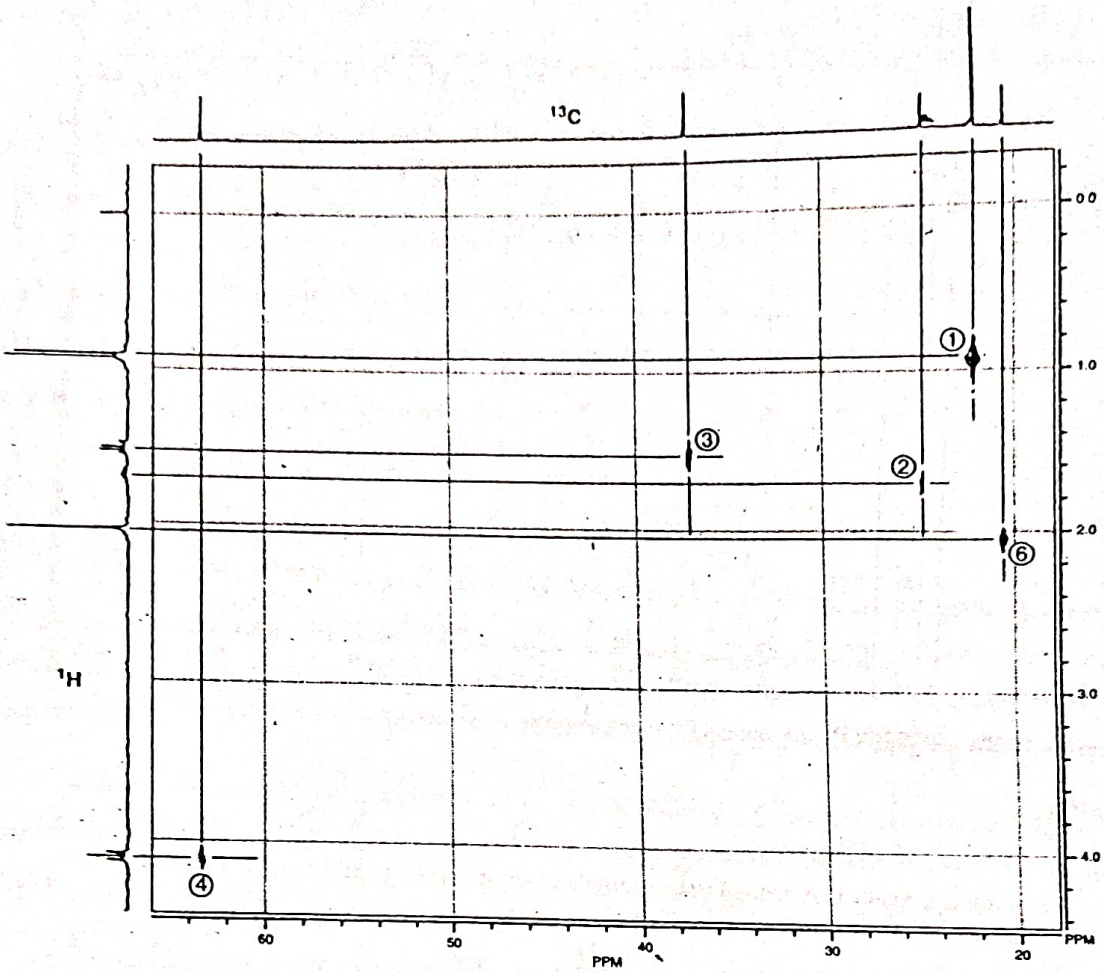


Figure [illegible] *HETCOR spectrum of isopentyl acetate.*

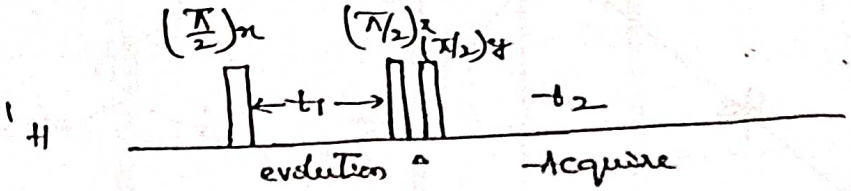
Each spot on the HETCOR plot has been labeled with a number, and lines have been drawn to help you see the correlations between proton peaks and carbon peaks. The carbon peak at 23 ppm and the proton doublet at 0.92 ppm correspond to the methyl groups (1); the carbon peak at 25 ppm and the proton multiplet at 1.69 ppm correspond to the methine position (2); and the carbon peak at 37 ppm and the proton quartet at 1.52 ppm correspond to the methylene group (3). The other methylene group (4) is deshielded by the nearby oxygen atom. Therefore, a spot on the HETCOR plot for this group appears at 63 ppm on the carbon axis and 4.10 ppm on the proton axis. It is interesting that the methyl group of the acetyl function (6) appears downfield of the methyl groups of the isopentyl group (1) in the proton spectrum (2.04 ppm). We expect this chemical shift since the methyl protons should be deshielded by the anisotropic nature of the carbonyl group. In the carbon spectrum, however, the carbon peak appears *upfield* of the methyl carbons of the isopentyl group. A spot on the HETCOR plot that correlates these two peaks confirms that assignment.

[illegible]

DQF COSY (Double Quantum filtered correlated Spectroscopy):

The normal COSY spectrum includes the application of two $\pi/2$ pulses with a time interval of t_1. If a third $\pi/2$ pulse is applied immediately following the second $\pi/2$ pulse, a DQF COSY spectrum is obtained. Hence, this technique is known as double quantum filtered COSY.

The pulse sequence for DQF cosy is



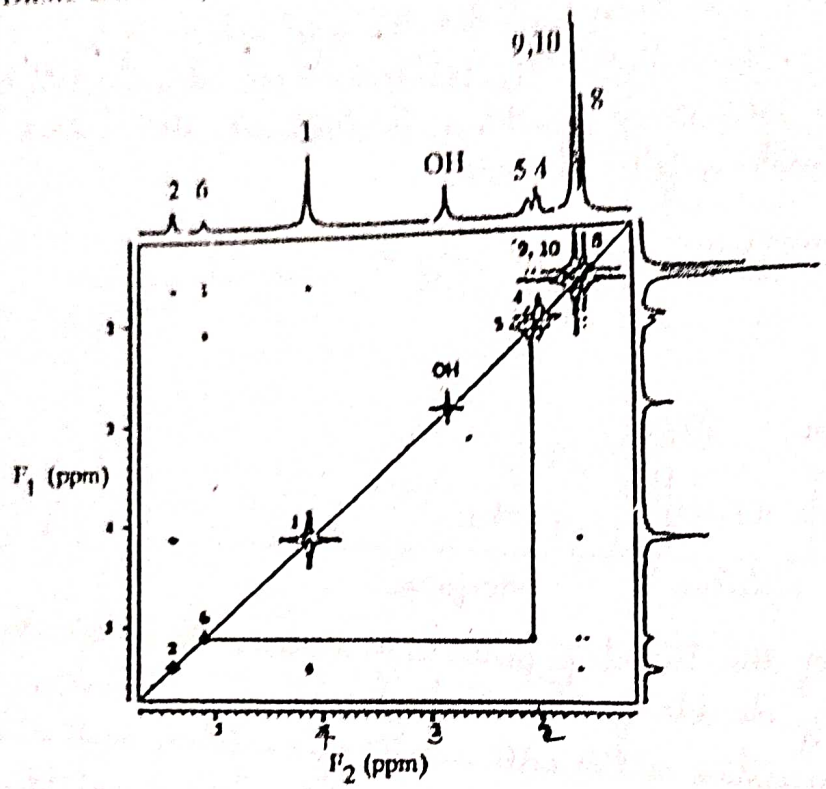
The purpose of the third $\frac{\pi}{2}$ pulse is to remove or filter single quantum transitions so that only double quantum or higher transitions remain.

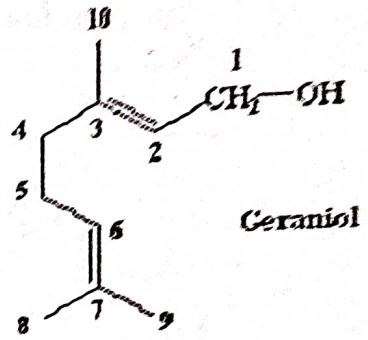
The double quantum filter will select for systems with atleast two spins (AB or AX). The non-coupled methyl singlets will be greatly reduced.

The basic COSY however results in considerable overlap along the diagonals and thus it is difficult to make assignments. This ~~could~~ difficulty can be minimised by a double quantum filtered COSY (DQF COSY). The intense singlets of non-coupled methyl groups in particular are greatly reduced. The advantage of DQF COSY over normal COSY can be explained by considering the case of Geraniol. The normal cosy spectrum consists of signals for three methyl groups which are severely overlapped and those for two methylene groups.

In the DQF COSY spectrum, the H_8 and H_9 methyl proton signals are clearly separated and the small allylic coupling to H_6 is apparent through off-diagonal cross peak. The long range coupling of the H_8 and H_9 methyl groups with one another is apparent from DQF COSY. The basic COSY lacks the H-1, H-4 and the H-6, H-8 couplings that are present in the DQF COSY. But separation of H-4 and H-5 along the diagonal is better in basic COSY than DQF COSY.

1. Basic COSY spectrum of geraniol, in $CDCl_3$ at 500 MHz

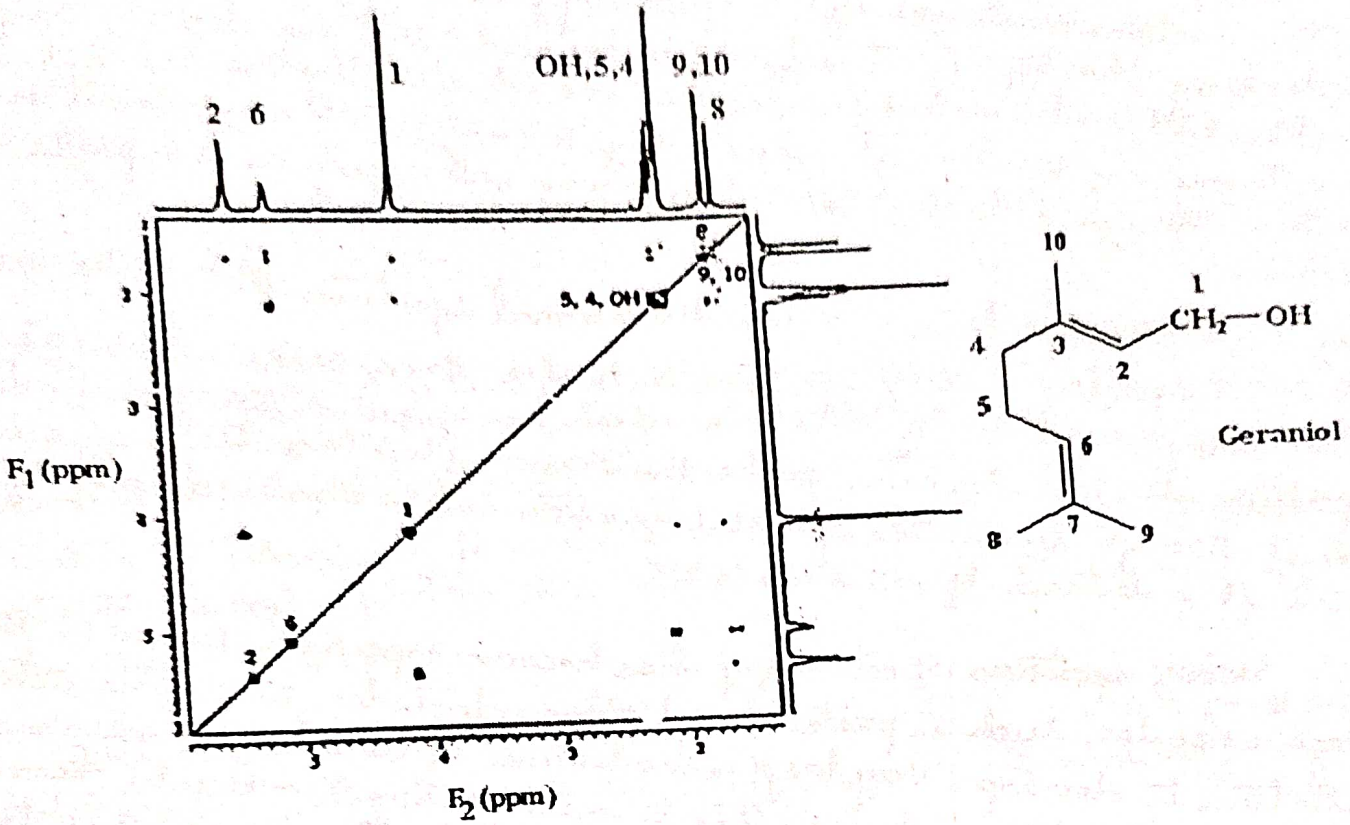




● From the basic COSY spectrum, we can see that H-5 and H-6 are coupled by each other. However, the signals for 3 methyl groups at C-8, C-9, and C-10 are severely overlaped, as are those for the 2 methylene groups at C-4 and C-5. Moreover, it lacks of the H-1----H-4 and H-6----H-8 couplings, and the differentiation between H-8 and H-9 is uncertain.

● These problems can be less by using a double quantum filtered COSY (DQFCOSY); the intense singlets of noncoupled methyl groups are greatly reduced.

2. The DQFCOSY spectrum of geraniol, in $CDCl_3$ at 500 MHz



● In the DQFCOSY spectrum, we can see that the H-8 and H-9 methyl proton signals are clearly separated. The long-range coupling of H-8 and H-9 methyl groups with one another and the H-1----H-4 and H-6----H-8 couplings are present. However, the differentiation between H-8 and H-9 is still uncertain.

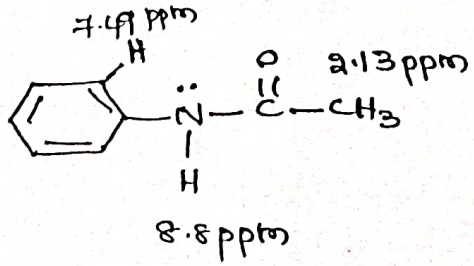
NOESY (Nuclear Overhauser Exchange Spectroscopy) Spectrum

A 2D NMR experiment that takes advantage of the nuclear Overhauser effect is nuclear overhauser effect spectroscopy of nuclear overhauser exchange spectroscopy. This type of interaction includes nuclei that are directly coupled to one another, but it also includes nuclei that are not directly coupled but are located near to one another through space. Any ^1H nuclei that may interact with one another through a dipolar relaxation process will appear as cross peaks in a NOESY spectrum.

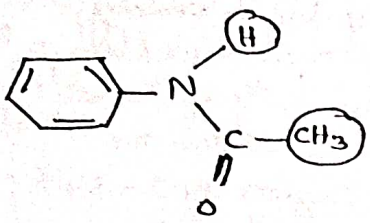
NOESY spectrum is a two-dimensional spectrum that looks very much like a COSY spectrum. COSY spectrum includes cross-peaks which arise due to interactions between ^1H-^1H & ^{13}C-^1H which are connected through bonds. NOESY spectrum, also includes cross-peaks that arise from interactions of nuclei that interact through space. The spatial interaction can be observed only if the nuclei exists at a distance of ~5 Å in space.

NOESY spectroscopy has become especially useful in the study of large molecules, such as proteins and polynucleotides. NOE interactions take more time to develop. Very large molecules tend to tumble more slowly in solution and hence interactions take more time to develop. Small molecules tumble more quickly in solution; the nuclei move past one another too quickly to allow a significant development of dipolar interactions. The result is that NOESY cross peaks may be too weak to be observed.

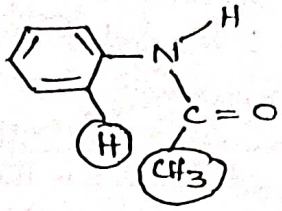
As cross peaks in NOESY spectra arise from spatial interactions and this spectroscopy is very useful in the steady of configurations and conformation of molecules. The purpose of NOESY experiment can be understood from the example of acetanilide. The structural formula with the proton NMR chemical shifts of the relevant protons is indicated below.



Two possible conformation are available for acetanilide and from the NOESY spectrum, it is possible to decide which conformation is more probable.



A



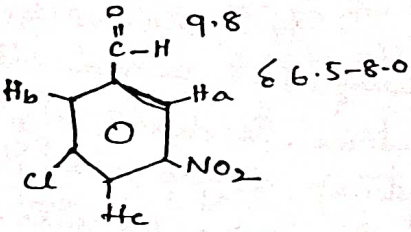
B.

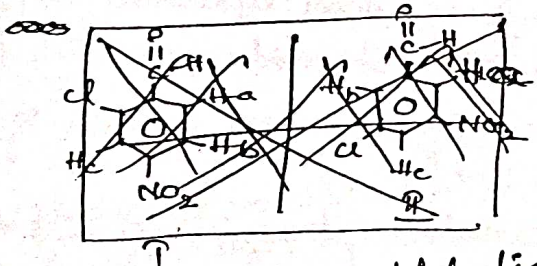
If Conformation A is true, then a cross peak in the NOESY spectrum can be observed which correlates to N-H peak at 8-8 ppm with C-H peak at 2.13 ppm as the N-H hydrogen is close to the methyl C-H hydrogens. Conformation B corresponds to a cross peak in NOESY spectrum which correlates to the methyl protons at 2.13 ppm with the ortho proton of the aromatic ring at 7.49 ppm.

The actual NOESY spectrum consists of a weak cross peak that links the 8.8 ppm peak with the 2.13 ppm peak. This clearly indicates that the preferred conformation for acetanilide is A.

Example: 2

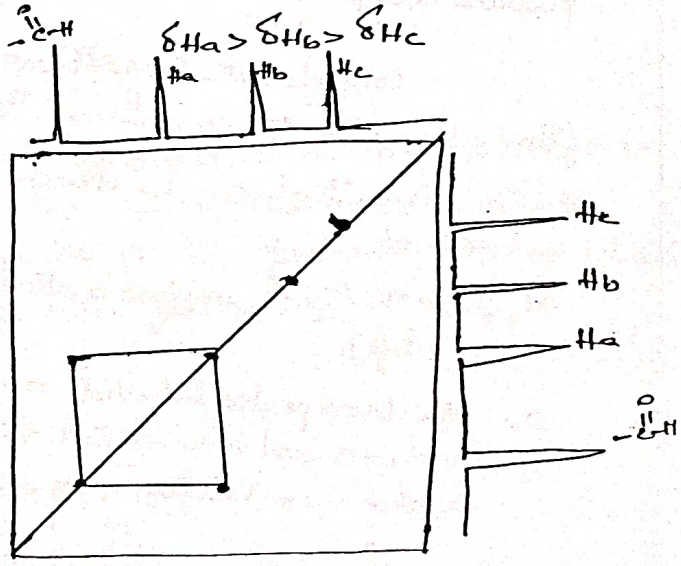
The NOESY spectrum of this molecule is useful in establishing the actual structure of this molecule.





I

The cross peak for aldehydic proton and Ha indicates a spatial interaction between them.



INADEQUATE (Incredible Natural Abundance Double QUAntum Transfer Experiment)

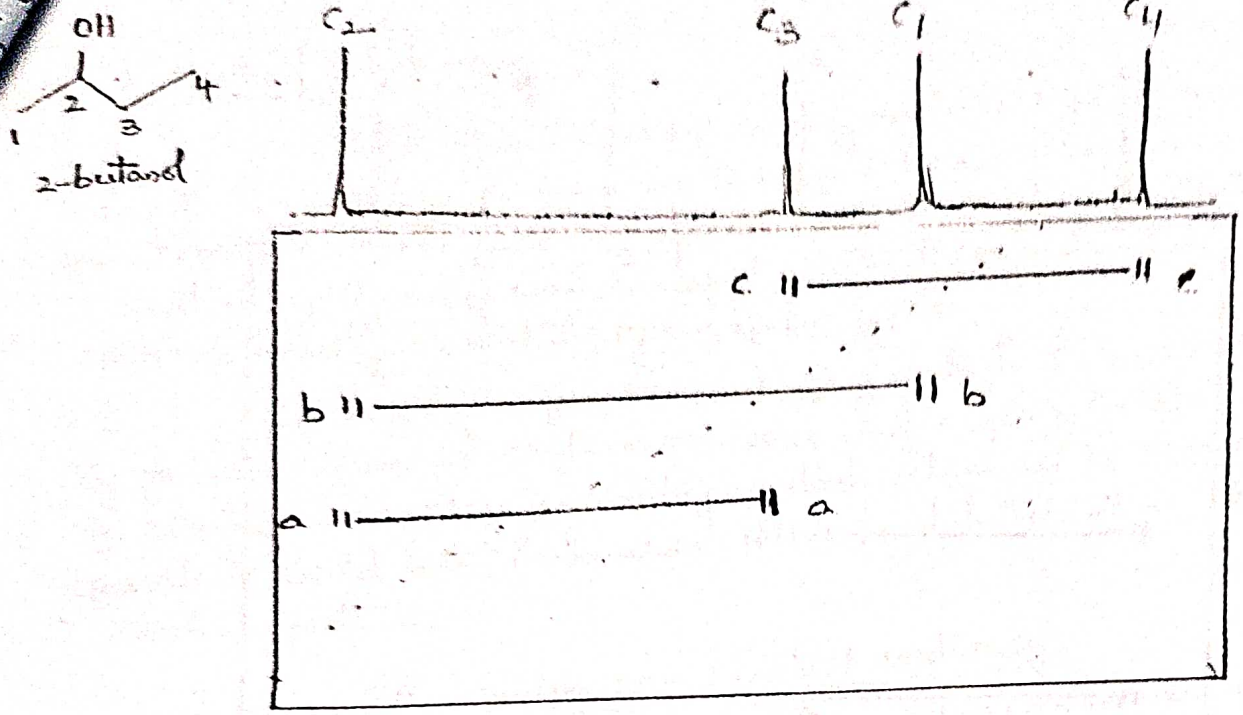
The 1D INADEQUATE experiment is used to measure $^{13}C-^{13}C$ coupling constants and for determining carbon-carbon connectivity by determining coupling magnitudes which are common to two carbon atoms. There exists a problem in the determination of carbon-carbon connectivity due to low sensitivity of ^{13}C nuclei and also due to the similarity of many $^{13}C-^{13}C$ couplings. There all the one bond carbon-carbon couplings in cyclooctanol fall in the narrow region 34.2 - 34.5 Hz, except for C_1 and C_2, which is 37.5 Hz. This problem is solved by recording the 2D NMR spectrum.

In an INADEQUATE experiment, it is intended to detect $^{13}C-^{13}C$ nuclei attached to each other. The number of ^{13}C nuclei existing will be very minute (i.e. about 1 in 10,000). To record an INADEQUATE experiment a more powerful pulse sequence is required. This spectrum gives information regarding direct carbon-carbon connectivities and is thus helpful in establishing the carbon skeleton. Actually, the vicinal proton-proton couplings help to elucidate the components which make up the chain of carbon atoms. Difficulties will arise, e.g., when there is an intervening quarternary carbon, and with no protons on it to couple with the adjacent protons, the chain is interrupted.

The INADEQUATE experiment involves a pulse sequence - a double quantum filtering which removes all single spin interactions which correspond to isolated ^{13}C atoms. It detects only transitions from systems with two spins (AB and AX systems). A gap caused due to the presence of a hetero atom C-X-C will prevent mapping the entire molecule.

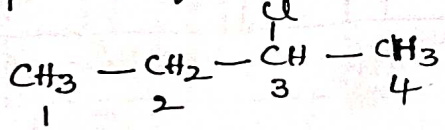
Consider the INADEQUATE spectrum of 2-butanol represented in contour form along with the conventional ^{13}C NMR spectrum and is represented below. The conclusion that can be drawn from the 2D INADEQUATE spectrum of 2-butanol

1. In 2-butanol, oxygen is attached to C-2 carbon and resonates at high δ (the far left).
2. The cross-peaks labelled a and b represents the connectivities between C-2 and C-3 and between C-2 & C-1, respectively. The cross-peaks C indicates the connection between C-3 and C-4.

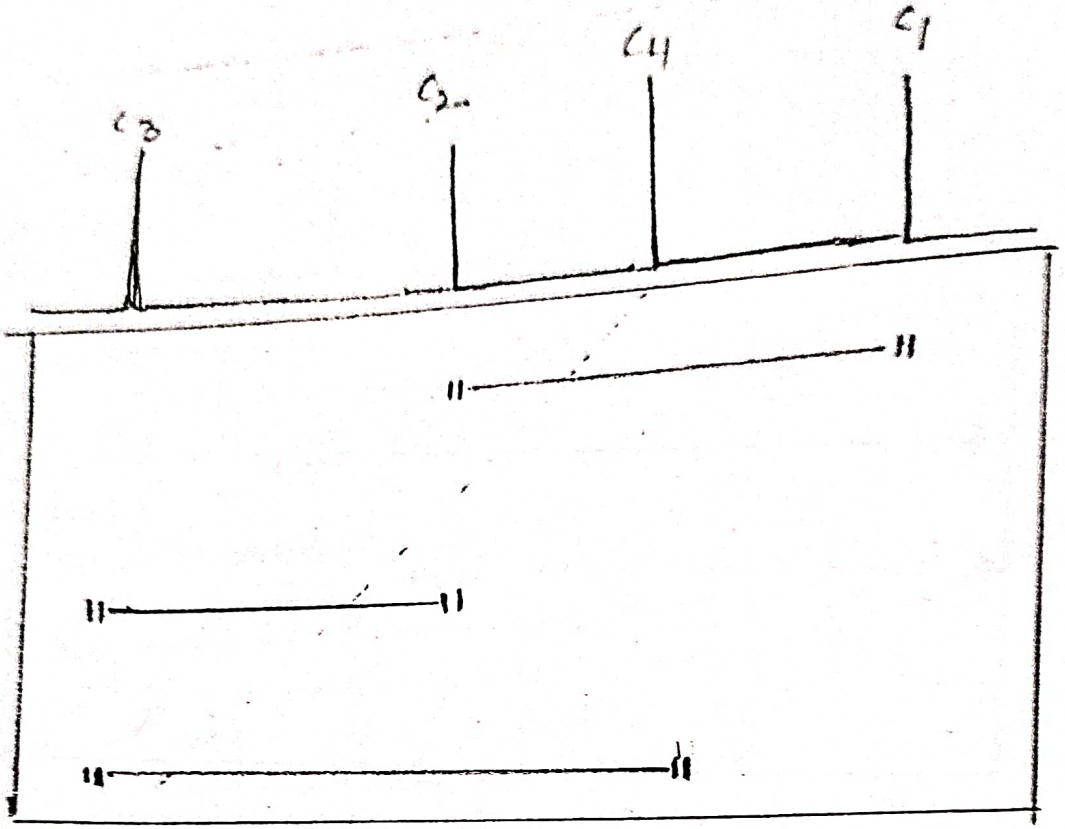


3. The dashed line bisects the mid-point between each of the pairs of Cross-peaks.
4. A connectivity is established by making a horizontal correlation between Cross peaks which are symmetrically placed with respect to the dashed line which is followed by vertical correlation at either end to other Cross peaks.

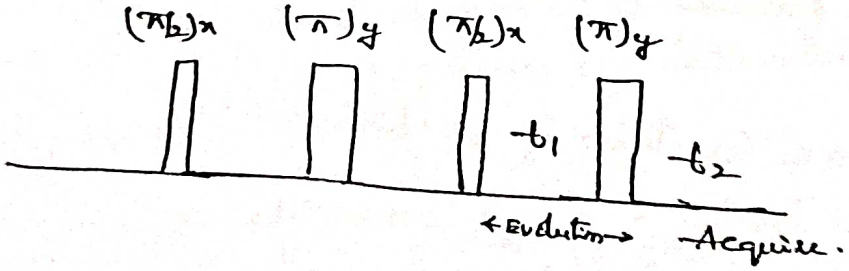
The INADEQUATE Spectrum of 2-chloro butane.



The peak corresponding to C_3 carbon is observed at down field as it is deshielded by chlorine attached to it. The carbon-carbon connectivities can be established by observing the Cross-peaks in 2D INADEQUATE spectrum. The chemical shift values will be in the order $\delta C_3 > \delta C_2 > \delta C_4 > \delta C_1$



The pulse sequence of INADEQUATE can be represented as follows.



J- Resolved Spectroscopy

A valuble information about coupling nuclei and the carbon skeleton of a molecule can be known from 2D-techniques like COSY, NOESY and INADEQUATE These techniques do not give any information about coupling constants values. So J-resolved spectroscopy was developed which helps in the simplifcation of spectra in heavily crowded regions of the spectra.

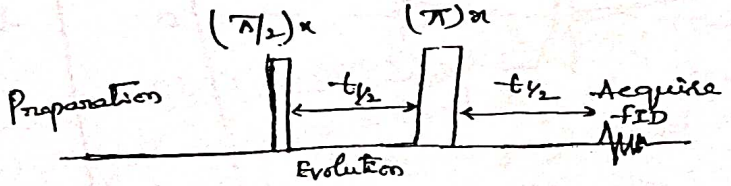
In this Techniqee the overlapping multiplicities can be resolved by presenting chemical shifts on one axis and coupling constant values (J) on the other axis. Thus the coupling constants values can be known. It is possible to record both, homonuclear ($^1H-^1H$) and heteronuclear ($^{13}C-^1H$). J -resolved spectrum.

HOMO 2DJ - Homo nuclear 2D-J resolved spectroscopy

In this, the proton chemical shifts are taken on one axis and the $^1H-^1H$ coupling constants on the other. This inolves in the resolution of ^1H signals which are betwees same nuclei. Hence this is called Hom 2DJ.

This is most useful when overlapping multiplicities are not coupled to each other of weakly coupled.

The pulse sequence for Hom 2DJ is as follows

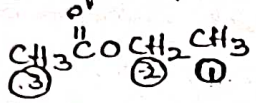


In J-resolved homo nuclei, after a preparation period of 'T' a π/2 puls is applied followed by a π-pulse with an interval $t_{1/2}$.

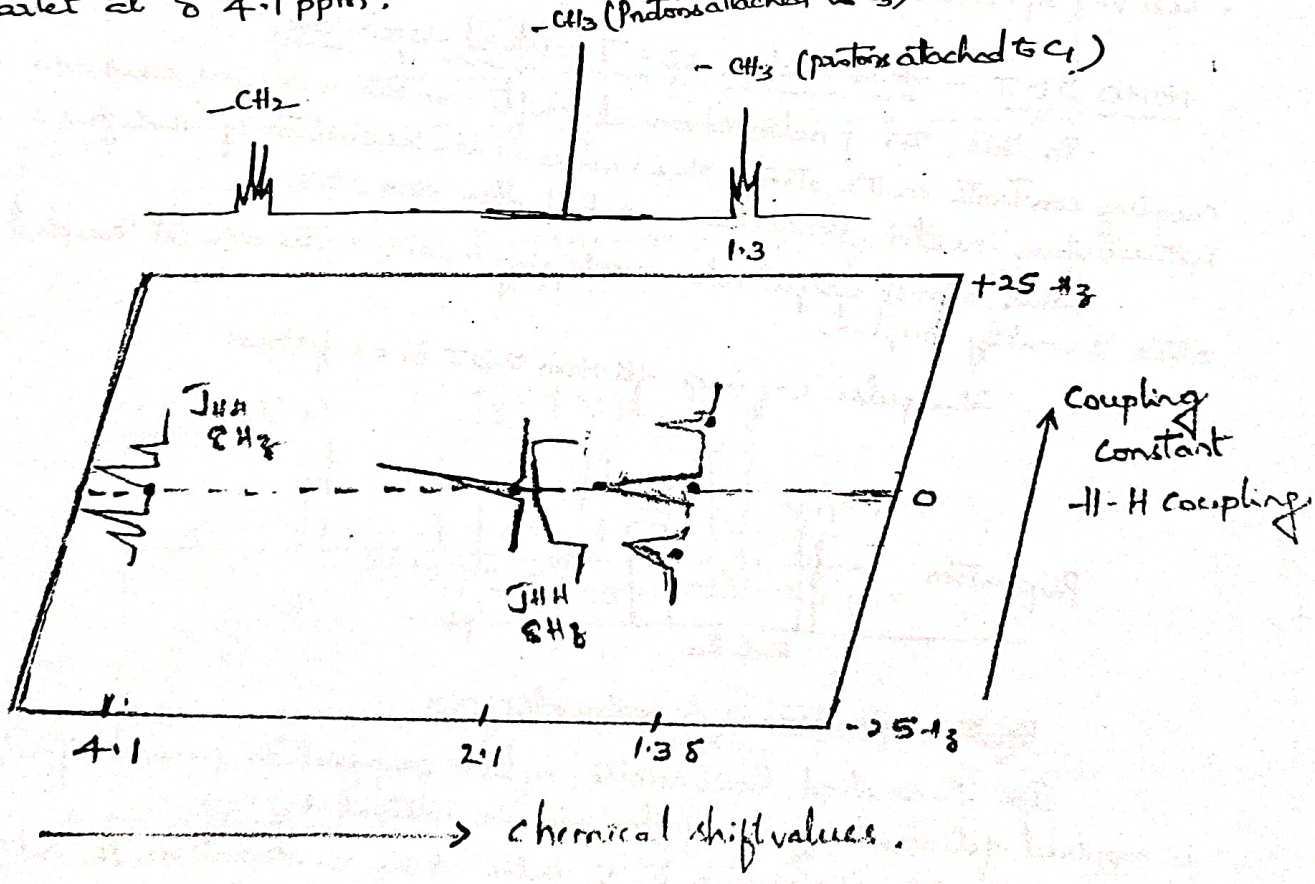
By the application of π/2 pulse along x-direction, the net magnetic moment M_0 tilts to Y-Component. In the evolution time $t_{1/2}$, the magnetic moment vector undergoes precessional motion in XY plane. Then a π pulse is applied in x-direction. If any neighbouring nuclei are present, then the spin-spin Coupling interactions take place and inversion of rotating frame occurs.

After $t_{1/2}$ time the spin-spin coupling interactions may be removed and transverse magnetisation takes place. In acquisition period - FID is obtained. From this, a 2-dimensional data is obtained which relates to J and δ.

Consider the case of ethylacetate for 1Hnm 2DJ resolved spectrum.



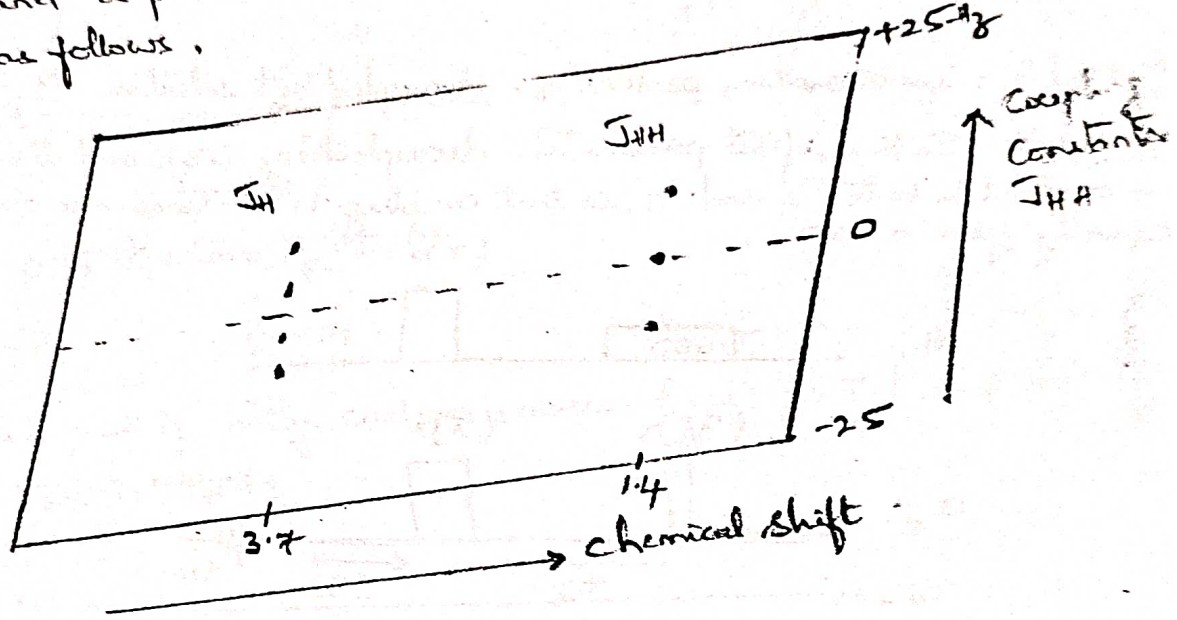
The normal PMR spectrum of ethylacetate can be shown as follows. The peak corresponding to methyl protons (attached to C_1 carbon) is observed as a triplet at δ 1.3 ppm and the peak corresponding to another methyl protons (attached to C_3 carbon) is observed as a singlet at δ 2.1 ppm. The peak corresponding to CH_2 protons, which are deshielded to a greater extent, is observed as a quartet at δ 4.1 ppm.



Consider the case of CH_3CH_2Cl for Hom 2DJ resolved spectrum.

$CH_3 - CH_2 - Cl$
a b
T Q
$\delta 1.4$ $\delta 3.7$

The b protons in the molecule are observed as quartet at δ 3.7 down field and a protons are observed as triplet at δ 1.4. The J-resolved spectrum is as follows.

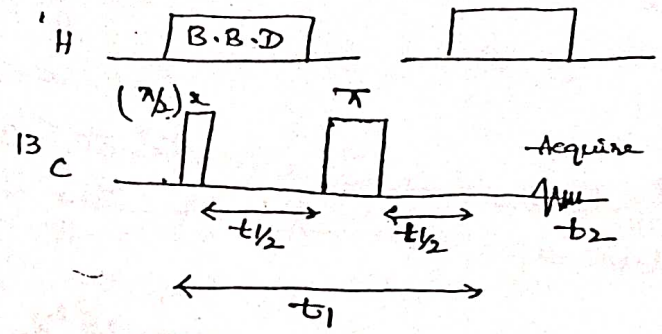


Hetero nuclear 2D-J resolved spectroscopy (Het 2DJ)

This technique is used to resolve $^{13}C-^{1}H$ coupling i.e. heteronuclear. The chemical shifts of ^{13}C nucleus are presented on horizontal axis and $^{1}H-^{13}C$ coupling constants (J_{CH}) on vertical axis. The result of HET 2D-J spectrum is equivalent to that available from a ~~non-decoupled carbon~~ non-decoupled ^{13}C spectrum but without severe overlap of the latter. HET-2D J is used to know the multiplicity of protons on each carbon and also $^{1}H-^{13}C$ coupling constants. It permits the distinction among Sp^3, Sp^2 and Sp hybridised car and indicate attachment of electronegative atom.

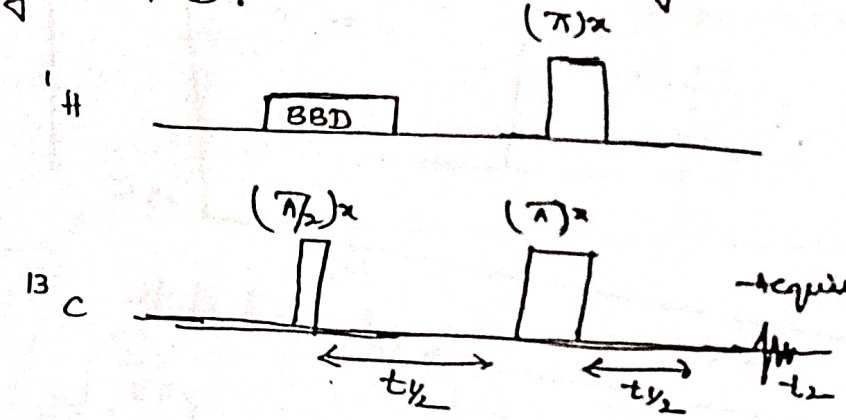
In this technique, three methods are used basing on the modulation of J_{C-H}.

Method-I : The protons are decoupled by using broad band decoupling techniq
the carbons are applied with the pulse. Later coupling is allowed before acquisit



Method-II : In this also, protons are decoupled but selective decoupling is applied.

Method-III : In this, first protons are decoupled by BBD and then a π pulse is applied to both ^{13}C and ^1H, so that coupling interactions are observed which finally give a FID.



HET 2DJ does not give $^1H-^{13}C$ correlations, it is used less frequently than the more powerful HETCOR.

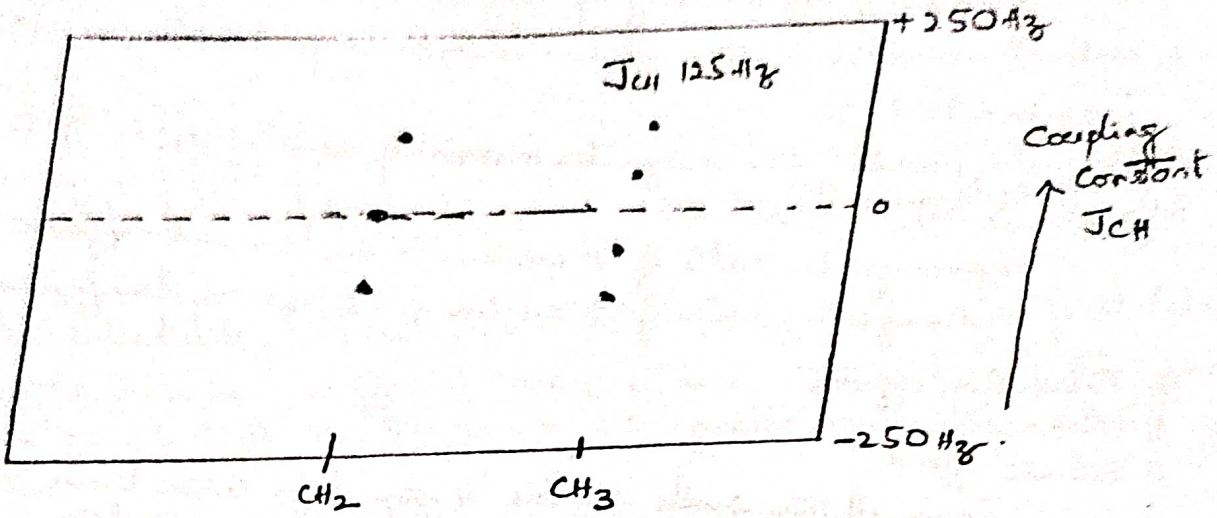
Consider the case of Ethyl chloride HET 2D-J resolved spectrum.

CH_3-CH_2-Cl

(2) (1)

The first carbon peak is observed as a triplet due to spin-spin coupling with two protons attached to it. The peak of second carbon is observed as quartett due to spin-spin interaction with three protons attached to it.

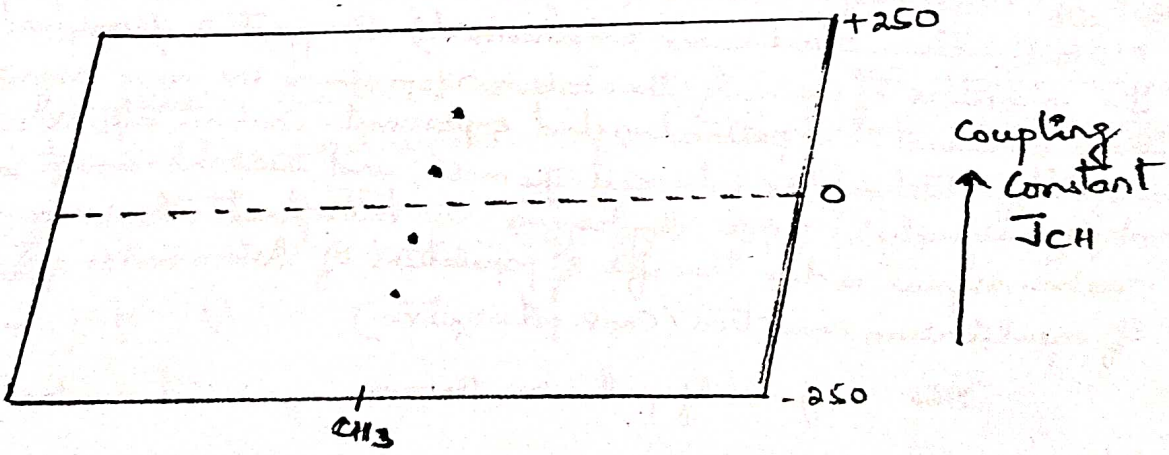
$\delta_{CH_2} > \delta_{CH_3}$



HET 2D-J spectrum of CH_3OH.

CH_3OH

The peak of methyl carbon is observed as quartet and J_{CH} Coupling constant is 125 Hz.



INEPT (Insensitive Nuclei Enhanced by Polarisation Transfer) Spectrum

Generally the intensity of signal in NMR depends on the following factors

1. Natural abundance: The natural abundance proton (^1H) is 99.9% and that of ^{13}C carbon is 1.1%
2. Magnetic moment: The magnetic moment of proton (μ_H) is 2.29 and that of carbon (μ_C) is 0.79.

   Gyromagnetic ratio of ^1H nucleus is 26,753 radians/gauss

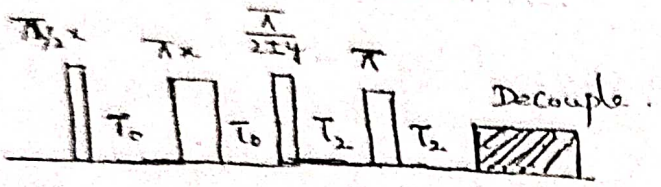
   Gyromagnetic ratio of ^{13}C nucleus is 6,728 radians/gauss.
3. Relaxation effects
4. Polar effects
5. Solvent effects.

From all these factors ^1H signal is 5,700 times intense (&) sensitive than ^{13}C signal. Hence, powerful pulse as is used to record ^{13}C-NMR spectrum.

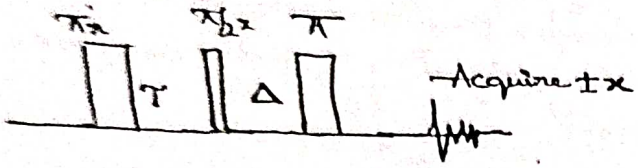
Basing on the above considerations, generally carbon (^{13}C) is called insensitive nuclei and hydrogen is called sensitive nuclei. Magnetic momentum, gyromagnetic ratio, precessional frequency of hydrogen are nearly 4 times greater than that of carbon (^{13}C) nucleus.

The INEPT method involves simultaneous application of a programmed sequence of pulses to the proton spectrum and to the spectrum of a heteronucleus (^{13}C). The principle involved in INEPT is more & less similar to the one observed in NOE (Nuclear Overhauser Enhancement). In proton-decoupled ^{13}C NMR spectrum, the intensities of many of the carbon resonances increase significantly above those observed in a proton coupled experiment. Carbon atoms with hydrogens atoms directly attached are enhanced the most, and the enhancement increases (but not always linearly) as more hydrogens are attached. The increase in ^{13}C intensity of carbon signal is due transfer of population of heteronuclei for the maintenance of equilibrium condition (Cross polarization).

The sequence of pulse in INEPT experiment can be represented as follows.



Experiment
INEPT
2.35 Tesla (Field strength)
100 MHz (Precessional frequency)



25 MHz (Precessional frequency)

Where T is called delay time which is constant $[T = \frac{1}{2} J_{CH}]$. Δ is final delay. The variation of Δ results in different CMR spectrum. The editing of these give clear infomation about CMR. In this process all spectra are compared with BBD spectrum.

The results can be explained as follows

1. If the final delay time is maintained as such $\Delta = \frac{1}{4} J_{C-H}$ then the CMR spectrum consists of peaks corresponding to CH_3, CH_2 & CH but not 4° carbon i.e. (Quaternary carbon)

   i.e. Peaks are observed for CH_3, CH_2 & CH only and not for carbon to which no hydrogen is attached.

2. If the final delay time is maintained as such $\Delta = \frac{1}{2} J_{C-H}$ then signals are observed for only CH group

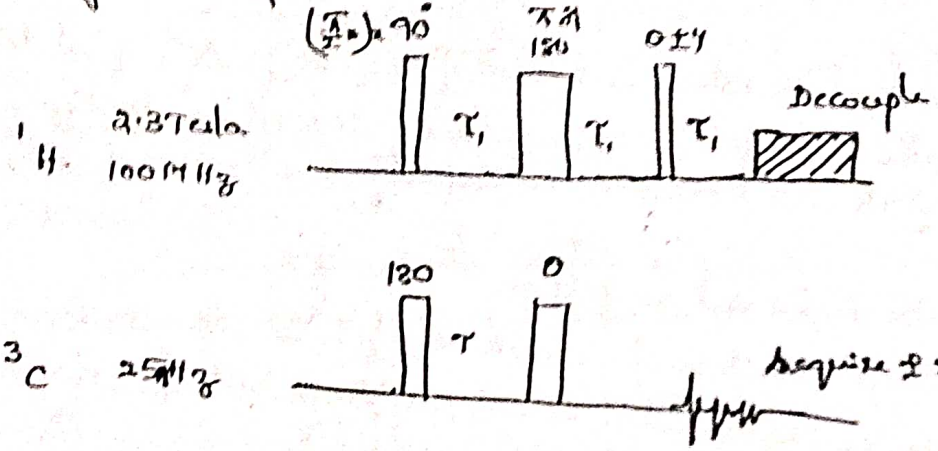
   i.e. Peaks of CH groups are only observed and not for CH_2 & CH_3

3. If the final delay time is maintained as such $\Delta = \frac{3}{4} J_{C-H}$, then positive signals are observed for CH & CH_3 and negative signals are observed for CH_2 group

The polarisation transfer Techniques enhance the intensity of the signals pertaining to protonated atoms in heteronuclear spectrum by a factor ν_H/ν_x (i.e. a factor of 3.95 for ^{13}C and about 10 for ^{15}N)

DEPT Spectrum (Distortionless Enhancement by Polarisation Transfer spectra)

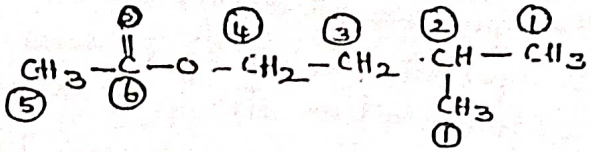
A very useful pulse sequence in ^{13}C spectroscopy is employed in the DEPT experiment. This method has become one of the most important techniques available for determining the number of hydrogens attached to a given carbon atom. The technique involves the a complex programme of pulses and delay times in both the ^1H and ^{13}C channels. The application of pulse sequence can be diagrammatically represented as follows.



τ is delay time which is constant $\tau = \frac{1}{2} J_{C-H}$.

The use judicious variation of the pulse angle θ in the DEPT pulse sequence has results in different types of CMR spectra. The resultant spectra are useful in determining the number of hydrogens attached to carbon atom in the molecule. In one experiment, called a DEPT-45 ($\theta = 45°$), only carbon atoms that bear any number of attached hydrogens will produce a peak. With a slightly different delay, a second experiment (called a DEPT-90 i.e. $\theta = 90°$) shows peaks only for those carbon atoms that are part of a methine (CH) group. With an even longer delay, a DEPT-135 spectrum is obtained. In a DEPT-135 spectrum, methine and methyl carbons (i.e. CH & CH_3) give rise to positive peaks, whereas methylene carbons appear (i.e. CH_2) as inverse peaks. Quaternary carbons, which have no attached hydrogens, give no signal in DEPT Experiment. In many instances, a DEPT spectrum makes spectral assignments easier than does a proton-decoupled ^{13}C spectrum. These spectra are always compared with BBD spectrum to get exact information.

One common method of presenting the results of a DEPT experiment is to plot four different subspectra. Each subspectrum provides different information. A sample DEPT plot for isopentyl acetate is given as follows.



The last spectrum in the figure is the usual broad-band-decoupled ^{13}C spectrum. The second one from the bottom is the result of a pulse sequence (called a DEPT-45) in which the only signals detected are those that arise from protonated carbons. The peak corresponding to carbonyl carbon (labelled 6) is observed at 172 ppm in BBD spectrum and was not observed in the second one. The solvent peaks arising from $CDCl_3$ (77 ppm) are also not seen. The third one from the bottom resulted from a slightly different pulse sequence (called a DEPT-90). In this spectrum, the peaks of carbons that bear a single hydrogen are seen i.e. only peak at position 2 (25 ppm) is observed.

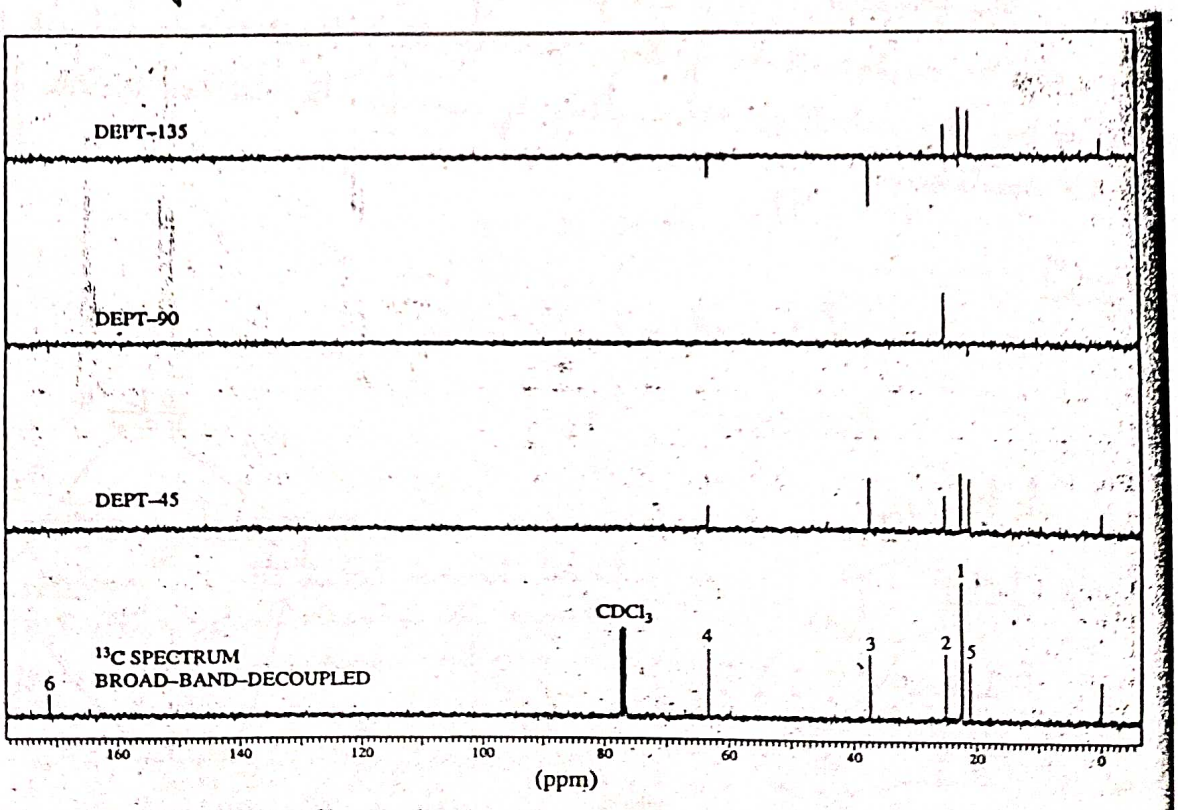


Figure 4.9 *DEPT spectra of isopentyl acetate.*

The uppermost one is more complicated than the previous subspectra. The pulse sequence that give rise to this subspectrum is called DEPT-135. In this, all carbons that have an attached proton provide a signal. Signals arising from CH & CH_3 groups will give positive peaks, while signals arising from CH_2 groups will form negative (Inverse) peaks. The positive peaks at 21 and 22 ppm must represent CH_3 groups as those peaks are not represented in the DEPT-90 subspectrum. The original BBD spectrum consists of the same two peaks at 21 & 22 ppm and the peak at 21 ppm is not as strong as the peak at 22 ppm. It can be concluded that the peak at 21 ppm must come from the CH_3 carbon at position 5, while the stronger peak at 22 ppm comes from the pair of equivalent CH_3 carbons at position 1. The inverse peak at 37 ppm is due to a CH_2 group C at the 3rd position. The inverse peak at 63 ppm is clearly caused by the CH_2 carbon at position 4, deshielded by the attached oxygen atom. Finally, the downfield peak at 172 ppm is due carbonyl carbon at 6th position. This peak appears only in the ^{13}C BBD spectrum; therefore, it must not have any attached hydrogens.

The formation of negative peak by CH_2 (i.e. methylene group) in DEPT-135 spectrum can be explained as follows theoretically. Population manipulation of spin states by the selective irradiation of specific transition is the basis for DEPT spectroscopy

Figure 1

4. ββ ↓↓ (0)

3. βα ↓↑ : : (4)

2. αβ ↑↓ : : : : : : : : (16)

1. αα ↑↑ : : : : : : : : : : (20) population.

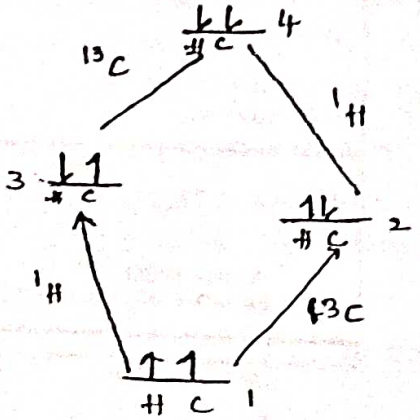


Figure 1 shows the polarisation of the ^{13}C & ^{1}H nuclei in a sample. The spin states labelled 1, 2, 3, 4 are produced by the spin-spin coupling of the carbon and hydrogen. The population difference between 1 & 2 is 4 units, as same as that between 3 & 4. The population difference between 2 & 4 is 16 which is the same as the difference between 1 & 3.

The signals due to hydrogen are generally formed from transition $2 \rightarrow 4$ & $1 \rightarrow 3$ and carbon signals arise due to $3 \rightarrow 4$ & $1 \rightarrow 2$. If the system is irradiated with a radio frequency that just matches the value of ΔE corresponding to $1 \rightarrow 3$ transition. Some of the nuclei of state 1 changes their spin and absorption occurs until saturation state is reached. i.e. population of 1 and 3 states become same (let the population of state 1 & state 3 be 12 units). Figure-2 shows the spin states that results when the population of 1 & 3 become equal. At this stage the population of state 2 is more than that of state 1. If a spectrum was to record corresponding to ^{13}C transition the $2 \rightarrow 4$ transitions remain unchanged. The intensity of 1,3 transition has dropped to zero. The $1 \rightarrow 2$ transition produces a negative peak. The signal for $3 \rightarrow 4$ transition has become stronger i.e. enhanced by polarisation transfer.

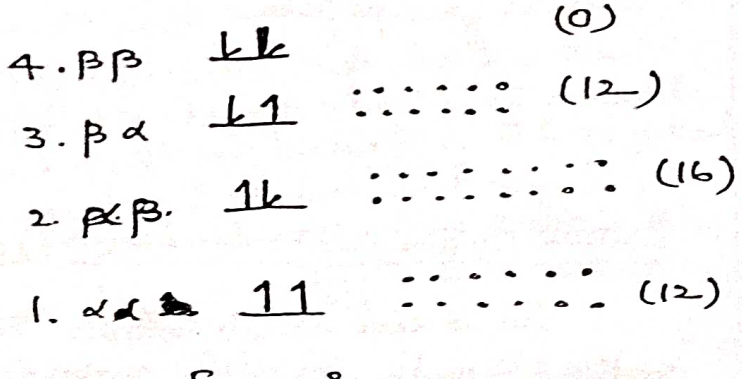
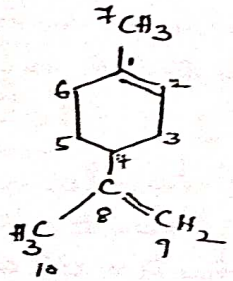


figure-2

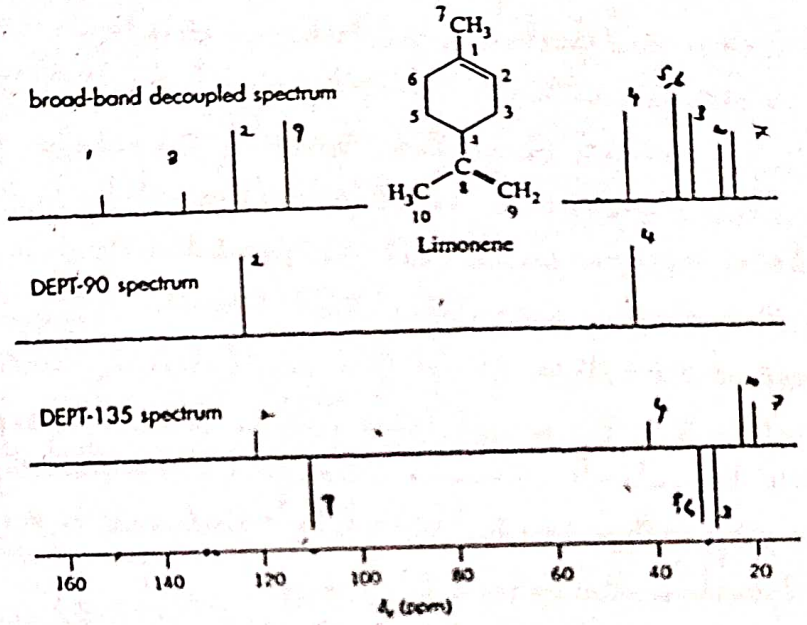
DEPT Spectrum of Limonene:

The spectrum 1 is the usual broad-band decoupled spectrum which displays the expected number of 10 lines for ten carbons and groups them into six alkyl carbons signals at high field (20-40 ppm) and four alkenyl carbon signals at low field (108-150 ppm). The spectrum 2 is easy



Limonene

DEPT-90 spectrum in which only two CH signals for carbon C-2 and C-4 appear Spectrum 3 is DEPT-135 spectrum which displays positive absorptions for two CH_3 groups (C-7 and C-10) and for two CH groups (C-2 and C-4), moreover it shows negative peaks for four CH_2 groups (C-3, C-5, C-6 and C-9) while no signals for C, C-1 and C-8.

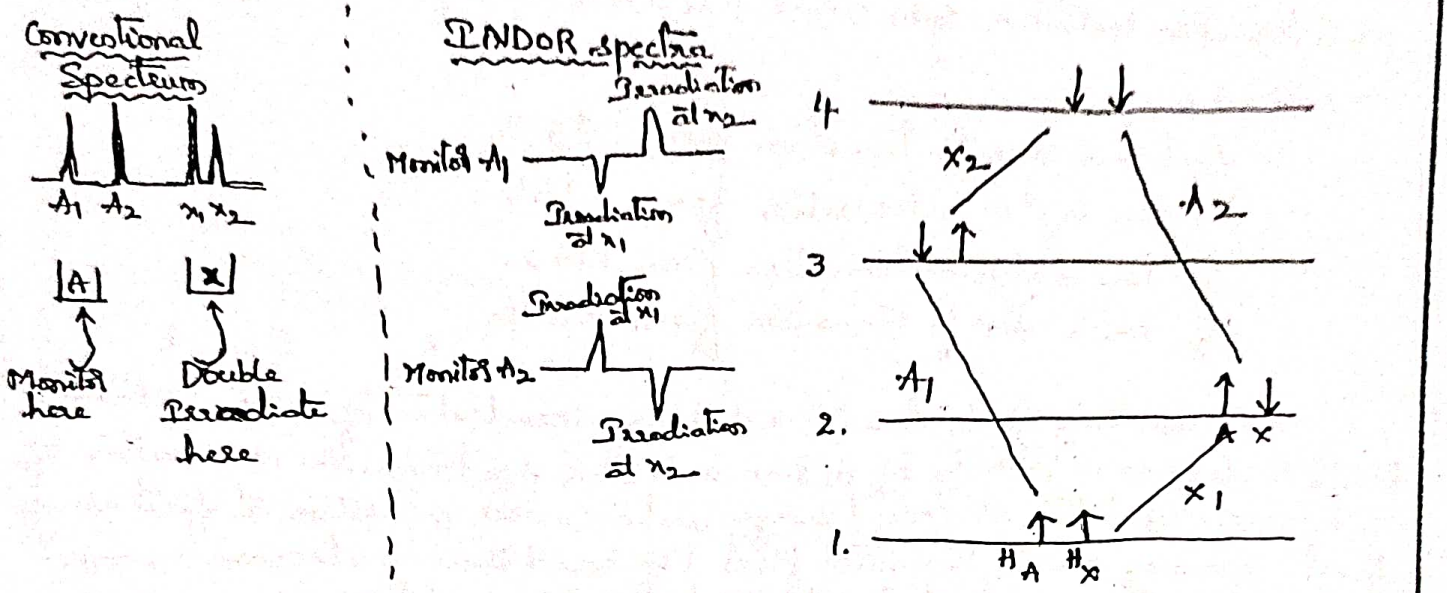


INDOR (Internuclear Double Resonance) and SPI (Selective Population Inversion)

In a real sample molecule the nuclear energy levels will be populated with millions of nuclei. The relative number of nuclei at equilibrium in each level can be calculated from Boltzmann distribution theory. These equilibrium populations will be disturbed if the system is doubly irradiated with radio frequency corresponds in energy to that of any of the transitions, and this, in turn, will affect the probability of other transitions arising.

Consider a molecule consisting of AX type protons. The spin-spin coupling of these will split the peak corresponding to A into a doublet and similarly the peak due to x will also split into a doublet. This situation can be easily understood in simple molecules (where first order spectrum is possible) and it is not so

easy to interpret in complex molecules. In such complex molecules, whether such spin-spin interaction is possible or not, can be known from its INDOR spectrum.



In the INDOR experiment, applied to simple AX systems, we monitor the line intensities of the signal A and sweep a weak radiofrequency source through the X frequencies; the stimulation of X frequencies will alter the line intensities of A signal and it is possible to plot this change in intensity onto a recorder. The perturbing irradiation used in INDOR is about one-twentieth of that required for complete decoupling. The perturbed signals and the monitored signals can be very much close on the spectrum than is true in decoupling. In INDOR only the population of spin states are altered - no change in energy levels is induced.

A simple INDOR experiment is illustrated in the above figure for a four-line AX spectrum. The line intensity (Peak height) of A_1 peak is monitored continuously, sweeping the perturbing irradiation through X_1 and X_2. The perturbation at X_1 causes a decrease in the line intensity of A_1, while at X_2 it causes an increase. If line A_2 is monitored and irradiation of X lines is carried out this perturbation at X_1 causes an increase in the intensity of A_2 line and decrease in intensity of A_2 due to perturbation (Irradiation) at X_2.

The energy diagram in the above figure is useful to explain why line intensities in the A signal change during double irradiation of the x signal. In the above energy level diagram, each line on the spectrum is represented as a transition between spin states 1, 2, 3 & 4. (noting that energetically $A_1 > A_2 > x_1 > x_2$).

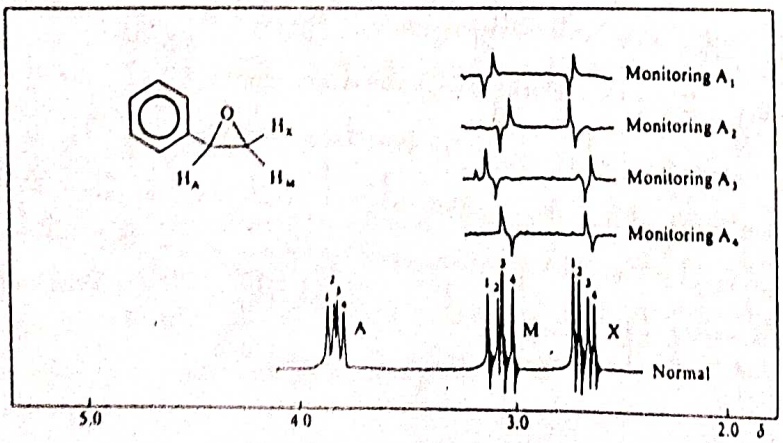
i.e. A_1 line is due to transition from 1 to 3
A_2 line is due to transition from 2 to 4
X_1 line is due to transition from 1 to 2
X_2 line is due to transition from 3 to 4.

The monitoring of A_1 line ($1 \rightarrow 3$) during irradiation of X_1 (due to $1 \rightarrow 2$) leads to decrease in intensity of A_1 line in INDOR spectrum. The irradiation of X_1 increases population at second energy and decreases population at first energy level. A_1 arises due to transition from 1 to 3 and there is decrease in population at first energy level due to irradiation of X_1 and hence A_1 peak is negative.

The irradiation of X_2 causes a decrease in population at 3 and increase in population at 4. A_1 is due to transition from 1 to 3, the decrease in population at 3, increases the intensity of A_1 line.

In case of A_2 monitoring and irradiation of X_1 and X_2, it is possible to explain the increase and decrease in intensities can be explained in a similar way. This regression and progression in peak intensities of A_1 & A_2 during irradiation of X_1 and X_2 indicates that A and X are spin-spin coupled.

Eg: Indor spectrum of styrene oxide (epoxide ring protons only).



Advantages of 2D NMR:

The two dimensional NMR leads to the development of chemical shifts in two dimensions and to resolve and overlap of resonances which enables the correlation of interacting nuclei to be be determined.

It can be applied to complex spectra which are difficult to be analyzed by conventional ~~spectroscopic~~ methods.

It is helpful to determine the protons that are coupling and also C-C and C-H bond connectivities of molecules.