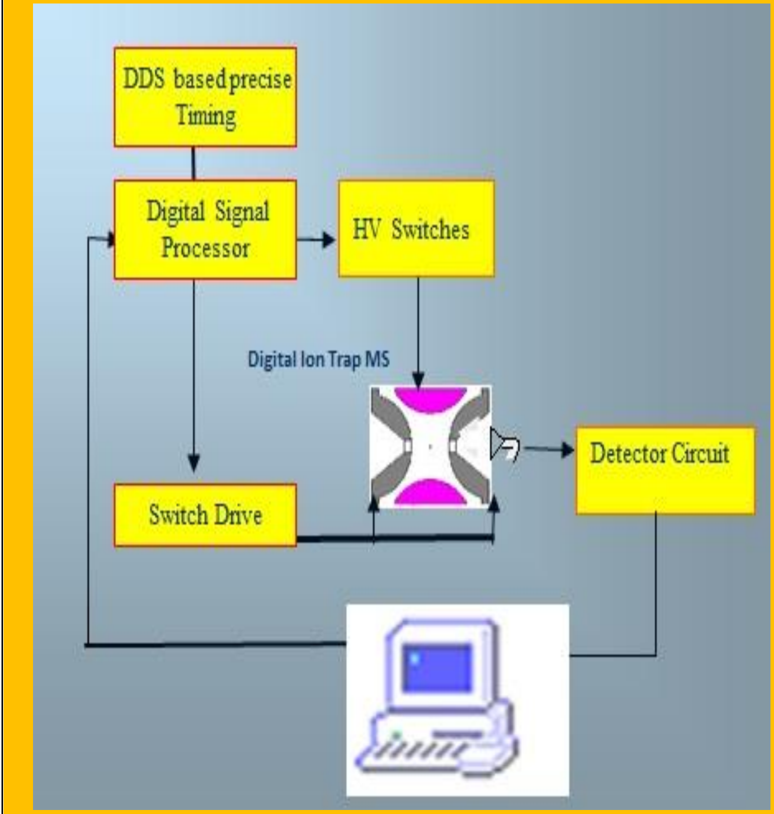
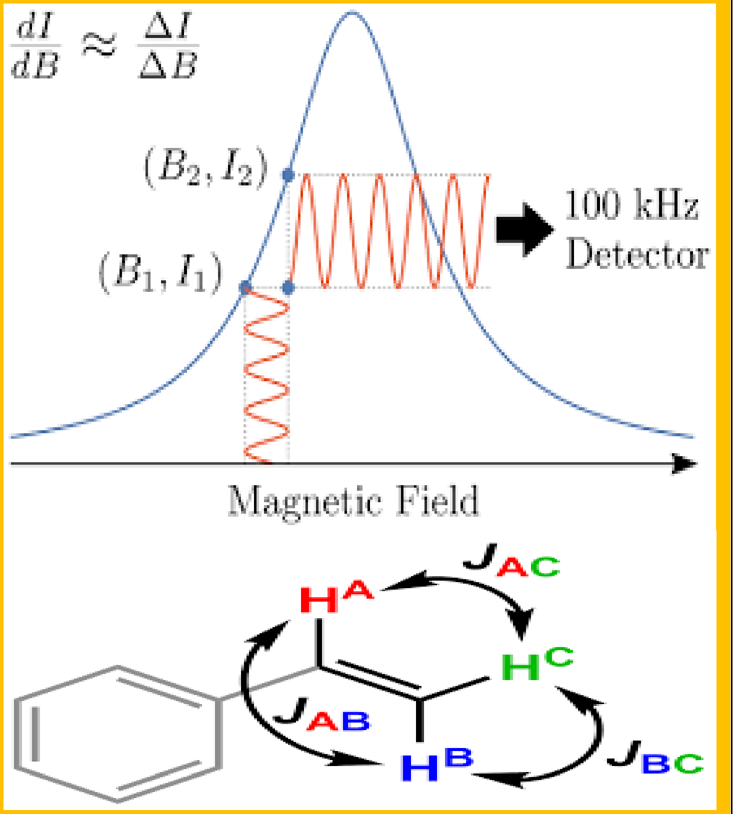
MSCCH-602
M.Sc III Semester
SPECTROSCOPY -II





SCHOOL OF SCIENCES
DEPARTMENT OF CHEMISTRY
UTTARAKHAND OPEN UNIVERSITY,HALDWANI (NAINITAL)

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Spectroscopy-II

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DEPARTMENT OF CHEMISTRY

UTTARAKHAND OPEN UNIVERSITY,HALDWANI (NAINITAL)

Phone No. 05945-26112, 261123

Toll free No. 18001804025

Fax No.05946-264232. Email info2uou.ac.in

Htpp://uou.ac.in

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Academic Consultant
Department of Chemistry
School of Science
Uttarakhand Open University, Haldwani

PROGRAMME COORDINATOR

Dr. Shalini Singh
Assistant Professor
Department of Chemistry
Uttarakhand Open University
Haldwani

UNIT WRITTEN BY	UNITNO.
1. **Dr- Rohit Verma, Gopeshwer** Assistant Professor Department of Chemistry Gov . P.G.Collage Gopeswer	**01,02,03,04**
2. **Dr. Girdhar Joshi** Assistant Professor Department of Chemistry Gov . P.G.Collage Gopeswer	**05,06**
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COURSE EDITOR

Dr. Shalini Singh & Dr. Ruchi Pandey

School of Sciences , Department of Chemistry

Uttarakhand Open University , Haldwani, Nainital

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CONTENTS

BLOCK I

BLOCK II

Unit 1: ^{1}H NMR Spectroscopy-II

1.1 OBJECTIVE :

NMR is an important analytical tool used specially in qualitative analysis of molecular structure. In this unit you will learn about:

- Condition of resonance and absorption of radiowaves leading to NMR spectrum.
- Natural isotopic abundance and isotopic ratio.
- Nuclear spin quantum number and NMR (magnetically) active nuclei.
- Fundamental NMR equation and principle of NMR spectroscopy.
- Chemically and magnetically equivalent protons.
- Determination of NMR signals of a compound.
- Definition of Chemical shift and concept of shielding and deshielding.
- Chemical shift range for ^{1}H-NMR.
- Intensity of signal, peak area and integration.
- Introduction of FT-NMR and its advantages over NMR spectroscopy.
- Spin-spin coupling, Coupling constant J and splitting of NMR signal.
- Types of coupling interactions i.e. ^{2}J, ^{3}J, etc.
- Long range coupling interactions.
- First and non-first order (second order) NMR spectra and their characteristic features.
- Use of shift reagents in simplification of NMR spectrum.
- Decoupling experiments in NMR to attain simplified spectra.
- Nuclear Overhauser Effect and its implication in stereo chemical studies.

1.2 INTRODUCTION :

In analytical chemistry Nuclear Magnetic Resonance (NMR) is one of the important techniques (along with IR spectroscopy and Mass Spectrometry) for structure elucidation of compounds. NMR technique is based on the property of nuclear spin of nuclei and it is due to this property some nuclei gives NMR spectrum and some other does not. Nuclear spin (I) is fundamental property of atomic nuclei and the nuclei with non-zero nuclear spin (I) have degenerate spin states. In presence of external magnetic field, the degeneracy of theses spin states is lost, i.e. these spin states now have different energies and this energy difference is equivalent to that of radiowaves. This energy difference depends mostly on the applied field and electronic surrounding. Thus the energy difference between different spin states varies with any slight change in electronic environment. On irradiation of radio waves, nuclear spin state transition takes place from low energy spin state to that of high energy. Also a nucleus in high energy spin state may revert back to low energy spin state and this will results in emission of radiowaves. Thus absorption or emission of radio waves takes place and these absorbed or emitted radio waves are recorded as signal by the instrument.

In a given sample, different nuclei are in different electronic environment and therefore the energies of nuclear spin states vary with electronic environment. Thus the energies or frequencies of radio waves absorbed (or emitted) are different for nuclei having different electronic surrounding. This can be simply concluded as ‘nuclei with different electronic environment absorbs (or emits) radio waves of different frequency’. This is helpful in ascertaining different types of nuclei which possibly helps in understanding the structural elucidation of compound under study.

Nuclear Magnetic Resonance is an analytical approach based on interaction of radio waves with nuclei, in presence of applied magnetic field. NMR has profound applications in various qualitative-structural analyses in various fields and therefore considered as a scientific discipline of its own.

1.3 PRINCIPLE OF NUCLEAR MAGNETIC RESONANCE :

NMR involves the interaction between oscillating magnetic field of electromagnetic radiation (radio waves) and the magnetic energy of the nuclei when placed in external static magnetic field. The nuclear magnetic resonance technique was first developed by **E. M. Purcell** and **Felix Bloch** (1946). The electromagnetic radiations used are low energy radiowave radiations. These radiations do not have enough energy to rotate or vibrate molecule or excite electron in the molecule. But the energy of the radiowaves is sufficient to affect the nuclear spin of the magnetically active nuclei in the molecule.

As you know all nuclei carry a charge, so they will possess spin angular momentum and therefore have nuclear magnetic moment. This nuclear magnetic moment (spin angular momentum) of various nuclei depends on the nuclear spin quantum number. On the basis number of number of protons and number of neutrons in the nucleus different nuclei have different values of nuclear spin quantum number (I). In other words, the nuclear spin quantum number I is associated with mass number and atomic number of the nuclei. The nuclei with zero spin quantum number (i.e. $I = 0$), does not have any spin angular momentum and therefore remains unaffected by external applied field. But for nuclei having non-zero spin quantum number ($I > 0$) have nuclear magnetic moment (spin angular momentum) and thus get affected by the presence of applied field, such nuclei are known as **magnetically active nuclei**.

Now it is important to understand that how external magnetic field influences the magnetically active nuclei. The magnetically active nuclei can have multiple spin state depending on its spin quantum number (I). The number of spin states is given by '$2I + 1$' which depends upon the value of I. These spin states can be represented as +I to –I, with all possible (equally-spaced) values obtained from ($2I + 1$). For example, hydrogen has the nuclear spin quantum number $I=1/2$, so using the expression ($2I + 1$), hydrogen atom has two spin states, these states are represented by +1/2 and -1/2 (as $I = 1/2$). Similarly deuterium has $I = 1$, so it has three spin states which are represented by +1, 0 and -1.

In absence of external magnetic field, these states are degenerate, and equally probable to exist. In presence of applied magnetic field these spin states does not remain degenerate. Thus these nuclear spin states splits in terms of energy as these nuclear spin orientations states are unequally affected by the external magnetic field. You can understand this concept in terms of aligned or opposed-orientation of nuclei with the direction of applied magnetic field. Consider the case of hydrogen for which $I = 1/2$, so hydrogen nuclei has two spin states, which are represented by +1/2 and -1/2. In absence of any applied magnetic field these two state have same energy i.e. these two states are degenerate. But in presence of applied magnetic field one spin state get aligned with the direction of external applied field and other attain opposed orientation with respect to direction of applied (external) magnetic field.

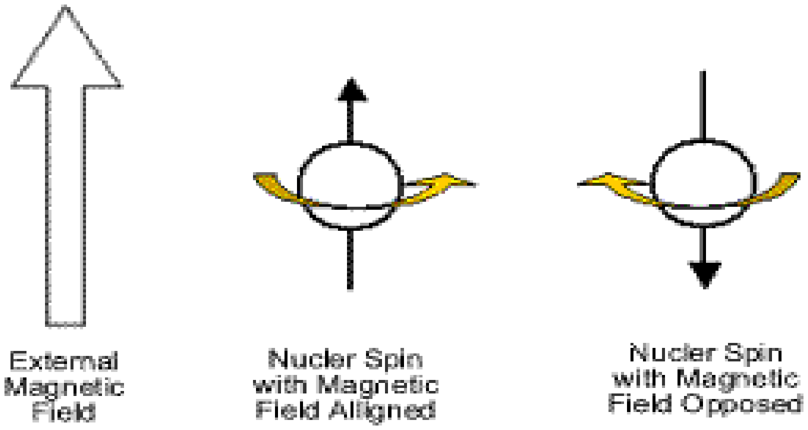


Figure 1.1: Aligned and Opposed nuclear spin states.

This means, in external magnetic field the two spin states separated by a energy difference. And this energy difference depends upon the strength of the magnetic field on the nucleus under consideration.

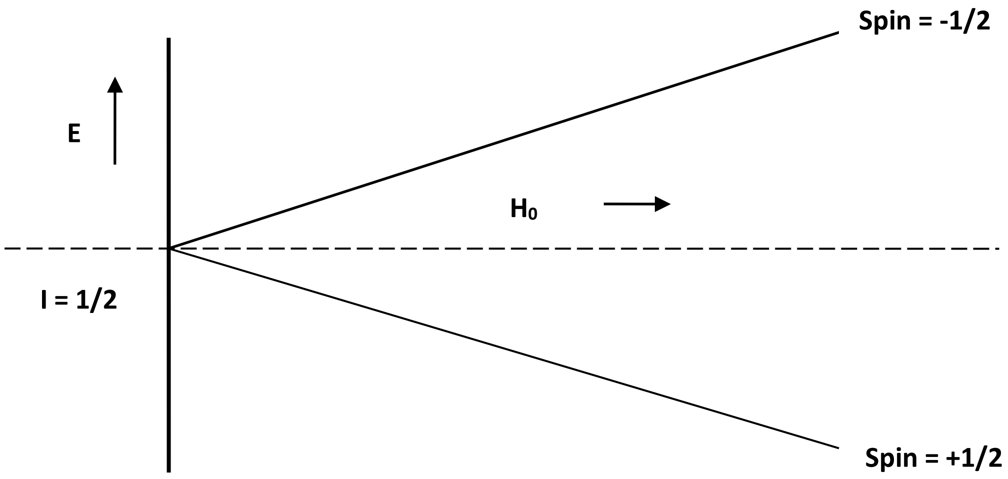


Figure 1.2: Two spin states for a proton in a magnetic field H_0

The radiowave region of electromagnetic spectrum is required to cause transitions between nuclear spin states. Thus on absorption of radio frequency, the spin orientation of the hydrogen nuclei changes from +1/2 to -1/2. This nuclear spin transition is known as **flipping**.

This concept can be described in terms of precessional motion of the nuclear magnetic moment vector associated with the nuclei. In presence of applied magnetic field the magnetic moment vector of nuclei starts precessing about the direction of external magnetic field at certain frequency. This precessional motion is known as Larmor precession. The direction of

Larmor precession depends on two spin states. The frequency of precession i.e. precessional frequency is also known as Larmor frequency of the nuclei. It is defined as number of revolution made by the magnetic moment vector of the nucleus about the external field H_o. The precessional velocity of a nucleus is given by following equation.

$$\omega = \gamma H_0 \qquad \ldots (1)$$

where, ω is precessional velocity, H_o is applied field in gauss and γ is Gyrometric ratio given by:

$$\gamma = \frac{2\pi\mu}{hI} \qquad \ldots (2)$$

where, μ is magnetic moment of the spinning bar magnet, I is spin quantum number of the spinning magnet and h is Plank's constant. Gyrometric ratio γ is characteristic of the nuclei under study and its value is constant for a particular nucleus with units in radian.gauss^{-1}.sec^{-1} (in CGS) or radian.tesla^{-1}.sec^{-1} (in MKS). When the frequency of precessional motion i.e. precessional motion matches with the radiowave frequency then absorption of radiowaves takes place and transition from lower energy spin states (aligned state) to higher energy spin state (opposed state) takes place. According to **fundamental NMR equation** which correlates electromagnetic radiation frequency 'υ' with the magnetic field is written below:

$$\omega = \gamma H_0 = 2\pi\upsilon \qquad \ldots (3)$$

The value of υ in equation (3) is frequency of radiowave radiations absorbs to cause nuclear spin transitions (i.e. to cause flipping). Thus the condition for flipping is that 'frequency of radiowave (υ) must matches with the precessional velocity (ω) of proton according to equation (3)'. So there must be a resonance between radio frequency radiation and the absorbing nucleus and therefore this technique is known as Nuclear Magnetic Resonance (NMR).

In Proton-NMR (PMR) a proton precessing in the aligned orientation can transit to the opposed orientation by absorbing energy. The transition from one energy state to another is known as flipping of the proton. The transition between the two energy states can be brought about by the absorption of the electromagnetic radiations in the radiowave region (about 60 MHz). The energy required to flip the proton depends upon the strength of external magnetic field, greater the field greater will be the tendency of the nucleus magnet to remain lined up with it and higher will be the frequency of the radiation needed to flip the proton to the higher energy state.

The proton will absorb energy from the radio frequency source only when the quantum energy υ of the electromagnetic region matches up the energy difference between the nuclear spin states at the field strength (Ho). In terms of precessional motion, when radiowave frequency matches with the Larmor frequency of the nuclear magnetic moment vector of proton then the proton and the radiowave frequency is said to be in resonance. This is the condition for absorption of radiowave by the proton i.e. this resonance leads to nuclear spin transition and signal is observed in the NMR spectrum.

The NMR spectrum can be obtained by two ways: (a) Magnetic scanning, in which the radio frequency is kept constant and the strength of magnetic field is continuously varied; (b) Continuous Wave scanning (CW-NMR), in which the radio frequency is varied and the strength of magnetic field is kept constant.

1.4 ISOTOPE RATIOS:

In nature different isotopes of various elements exists. As you know isotopes differs in the mass number (A) but have same atomic number (Z). For example the different isotopes of hydrogen are protium (^{1}H or simply H), deuterium (^{2}H or D) and tritium (^{3}H or T). The difference in mass number and atomic number for these isotopes is presented in table 1.1.

Table 1.1: Isotopes of hydrogen

Isotope of hydrogen	Mass Number (A)	Atomic Number (Z)
Protium (^{1}H)	1	1
Deuterium (^{2}H)	2	1
Tritium (^{3}H)	3	1

In other words isotope has different number of neutrons in nucleus, as protium has no neutrons, deuterium has one neutron in its nucleus and tritium has two neutrons inside its nucleus. The other important aspect of isotopes is related to their abundance of isotopes naturally found on earth. As different isotopes have characteristic half lives i.e. their stability is different so they are available on earth in different proportion. It is quite obvious that isotope which has greater half life is more stable and has greater abundance in nature and isotope with lesser half life is less stable and therefore has lesser abundance in nature. The relative amount of isotopes of an element as it occurs in nature is known as natural abundance. It is calculated as isotope ratio is the ratio of natural abundance of an isotope with respect to natural abundance of its most stable isotope. In order to understand the concept of natural abundance and isotope ratio consider the example of isotopes of oxygen. In nature oxygen exists in three isotopes i.e. ^{16}O, ^{17}O and ^{18}O and the natural abundance of these isotopes of oxygen and isotope ratios are presented in table 1.2.

Table 1.2: Natural Abundance and Isotope ratio of Isotopes of oxygen

Isotope of Oxygen	Natural Abundance (in %)	Isotope ratio
^{16}O	99.759	1
^{17}O	0.037	$\sim 10^{-4}$
^{18}O	0.204	$\sim 10^{-3}$

The natural abundance of ^{17}O is 0.037 % and that of ^{16}O is 99.759 %, this represents the relative ratio of these oxygen isotopes in a given sample of oxygen taken from nature. In terms of isotope ratios this consideration is more simple, as you know the isotope ratio of ^{16}O is 1 and that of ^{17}O is approximately 10^{-3}, this simply means for every thousand oxygen ^{16}O isotope a sample have one ^{17}O isotope. In same way for every ten thousand ^{16}O isotope a sample have one ^{18}O isotope. Thus it can be concluded that isotope ratio is relative abundance of an isotope with respect to the most abundant isotope of an element.

Now question arises that in NMR spectroscopy what is the relevance of isotope ratio or relative natural abundance. This can be explained by considering the example of hydrogen and carbon. First consider the example of hydrogen atom in organic compounds, the relative abundance of its two isotopes for ^{1}H (protium) and ^{2}H (deuterium) are 99.98 % and 1.5×10^{-2} respectively. Depending on the values of magnetic moment (μ) and gyrometric ratio of these isotopes absorbs different radiowave frequencies in the NMR spectrum. As the NMR signals depends on the number of absorbing nuclei, therefore signal intensity of the isotopes is also different. So the two isotopes for ^{1}H (protium) and ^{2}H (deuterium) gives signals with different absorbing frequencies and intensities which are directly related to their isotope ratio. Carbon has two important isotopes i.e. ^{12}C and ^{13}C, the natural abundance of ^{12}C is 98.9 %

but nuclear spin quantum number I for ^{12}C is zero (I = 0), so it does not show any absorption (signal) in NMR spectrum. The natural abundance of ^{13}C is 1.1 % and nuclear spin quantum number I for ^{13}C is 1/2 (I = 1/2). This means ^{13}C in magnetically active and gives show characteristic radiowave absorption. Due to low abundance of ^{13}C it is difficult to have more than one ^{13}C in a molecule. Thus low abundance of ^{13}C drastic affects on detection and recording of its NMR signal.

Thus it can be concluded on the basis of above discussion that 'the isotopic ratio/abundance is important in order attain or detect NMR signal of an isotope. It may be possible that isotope has nuclear spin quantum number I greater than zero (I > 0), i.e. it may be magnetically active but if its relative abundance or isotope ratio is low then possibility of its presence in the sample under study is lesser. So though its nuclei may interacts with external magnetic field but due to low isotope ratio its signal may not be detected. In this way isotope ratio is related to sensitivity of the NMR method. Nuclei (having I > 0) with higher isotope ratio gives characteristic signals which are easy to detect and record, while nuclei with lower isotope ratio are rare to be present in the sample and therefore it is difficult to attain their signals and to undertook NMR studies on the basis of such nuclei.

1.5 NUCLEAR SPIN:

The total angular momentum of a nucleus is represented by a symbol I and it is commonly known as nuclear spin. Nuclear magnetic moment is associated with nuclear spin I, which interacts with the external magnetic field. The origin of nuclear magnetic moment is related to magnetic field associated with nucleons constituting the nucleus. As nucleus contains charge particles, the spin of charge particles gives rise to a magnetic field. This magnetic field is analogous to the magnetic field produced by an electric current when passed through a coil of wire. This nuclear magnetic field is measured in terms of a resulting nuclear magnetic dipole μ which is characteristic of the nuclei and which is oriented along the axis of spin.

When such a nucleus is placed in an external magnetic field, it tends to get oriented such that the direction of magnetic dipole i.e. spin axis is parallel to the external magnetic field. The potential energy of the nuclei is dependent on the orientation of the dipole with respect to the applied field. The potential energy of such nuclei is given by the following equation:

$$E = -\mu_H H_o \qquad ...(4)$$

where μ_H is the component of magnetic moment in the direction of the field and H_o is the strength of the external magnetic field measured in gauss. For a nuclei with spin quantum number I and magnetic quantum number m is given by:

$$E = -\frac{m\mu}{I}\beta H_o \qquad ...(5)$$

where m has values +I, +(I-1),……, -I, β is a constant known as nuclear magneton ($\beta = 5.049 \times 10^{-24}$ erg/gauss), μ is the magnetic moment of the particle expressed in the units of nuclear magnetons (for proton m is 2.7927 nuclear magneton and for electron it is 1836 nuclear magneton). As for proton I = 1/2 has two values of magnetic quantum number +1/2 and -1/2. These two values represent two spin states of proton and the energies corresponding to these two spin states is given by:

$$m = +\frac{1}{2} \qquad E_1 = -\frac{\frac{1}{2}\mu}{\frac{1}{2}}\beta H_o = -\mu\beta H_o \qquad ...(6)$$

$$m = -\frac{1}{2} \qquad E_1 = -\frac{\frac{1}{2}\mu}{-\frac{1}{2}}\beta H_o = +\mu\beta H_o \qquad ...(7)$$

These two nuclear spin states are the possible orientation of the spin axis with respect to direction of applied magnetic field. The orientation $m = +\frac{1}{2}$ is aligned to the external magnetic field and similarly the orientation $m = -\frac{1}{2}$ is opposed with respect to the external field. The aligned state i.e. $m = +\frac{1}{2}$ has lower energy while the opposed state $m = -\frac{1}{2}$ has higher energy. The energy difference between these two spin states for hydrogen nucleus is given by:

$$\Delta E = 2\mu\beta H_o \qquad ...(8)$$

In general for any nucleus with I spin quantum number the energy difference between spin states is given by the following expression:

$$\Delta\mathrm{E} = \frac{\mu\beta\mathrm{H_o}}{\mathrm{I}} \qquad \ldots(9)$$

For excitation from lower to higher spin states if hν energy is absorbed then $\Delta\mathrm{E} = \mathrm{h}\nu$, so above equation becomes:

$$\mathrm{h}\nu = \frac{\mu\beta\mathrm{H_o}}{\mathrm{I}} \qquad \ldots(10)$$

In general in NMR studies $\mathrm{H_o} = 10^4$ gauss, then the frequency of radiation required for this transition is obtained by putting $\mu\beta = 2.79 \times 5.05 \times 10^{-24}$ magentons, $\mathrm{h} = 6.626 \times 10^{-27}$erg-sec and I = 1/2. On putting these values in equation (10), the values of frequency (ν) is calculated to

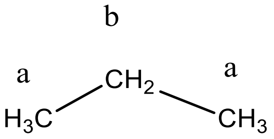
$$\nu = 4 \times 10^7 \mathrm{Hz} \qquad \ldots(11)$$

This frequency value is in the region of radio waves of the electromagnetic spectrum. Thus radio wave radiations are required to cause transitions between spin levels, so that the spin with aligned orientation changes to spin with opposed orientation.

1.6 CHEMICAL AND MAGNETIC EQUIVALENCE:

All the protons having identical chemical (electronic) environment within molecule are chemically equivalent. In a given external magnetic field, protons when same precessional frequency of their nuclear magnetic moment vector are said to be magnetically equivalent, such nuclei absorbs same radiowave frequency and thus gives same signal. In general protons that are chemically equivalent are also magnetically equivalent. This generalisation helps to know the similar type of protons from a signal observed in the NMR spectrum.

Consider the example of propane



There are two types of protons as shown in the structure marked as ‘a’ and ‘b’, the chemical environment of six protons of ‘a’ type are equivalent, similarly the chemical environment of two ‘b’ type protons are equivalent. In the NMR spectrum of propane we get two peaks, in the ratio of 3:1 for these protons. The two signal confirms that propane has two different protons magnetically, which corresponds to chemically distinct type ‘a’ and ‘b’ protons. The ratio 3:1 gives empirical numbers between chemically (magnetically) distinct protons. In propane it confirms 6:2 ratio (from empirical 3:1) for six ‘a’ and two ‘b’ type protons.

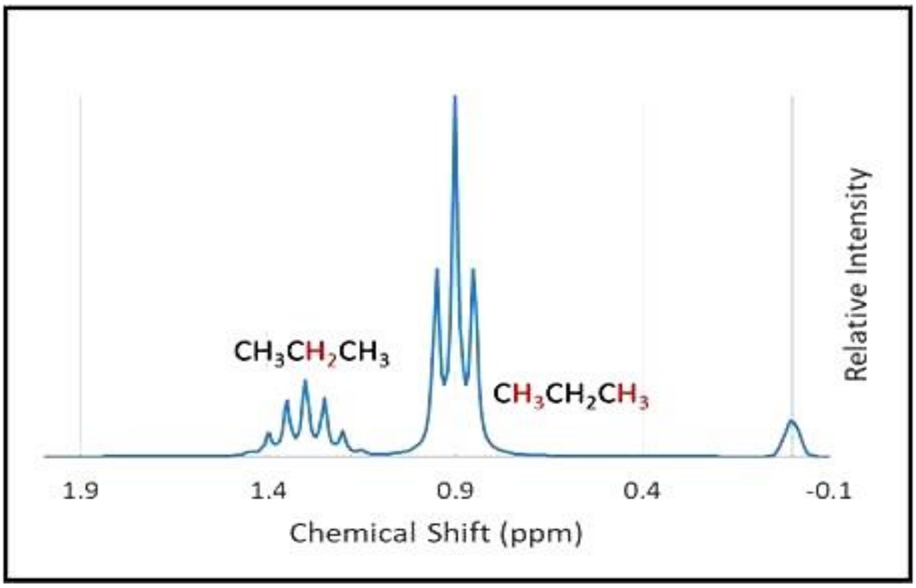
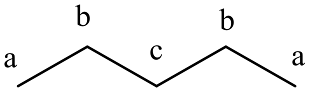


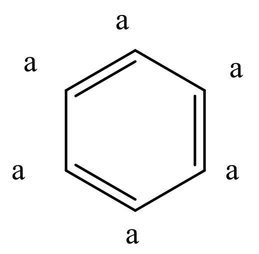
Figure 1.3: NMR spectrum of propane

1.7 NUMBER OF SIGNALS:

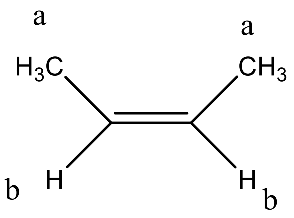
The number of signal for a given compound give information about a number of different set of chemically (that is magnetically) equivalent protons. In the following examples, the number of signals (peaks) has been determined.



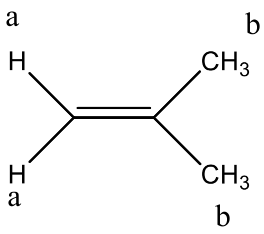
Three signals



Single signal



Two signals



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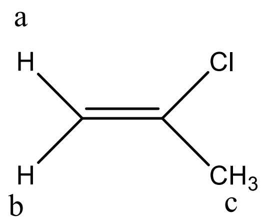
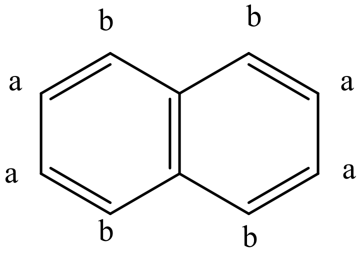
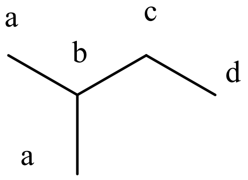


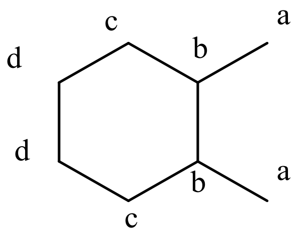
Three signals



Two signals



Four signals



Four signals

1.8 CHEMICAL SHIFT:

The different types of protons will have different electronic environment and they absorb radio waves at different frequency in presence of applied magnetic field. It is the electronic environment which determines where the proton shows absorption in the spectrum. When a proton is placed in a magnetic field then surrounding electrons circulate and thus produce a secondary (induced) magnetic field. The electrons move about the proton generates a magnetic field in such a way that it opposes the external field, then the proton is said to be shielded.

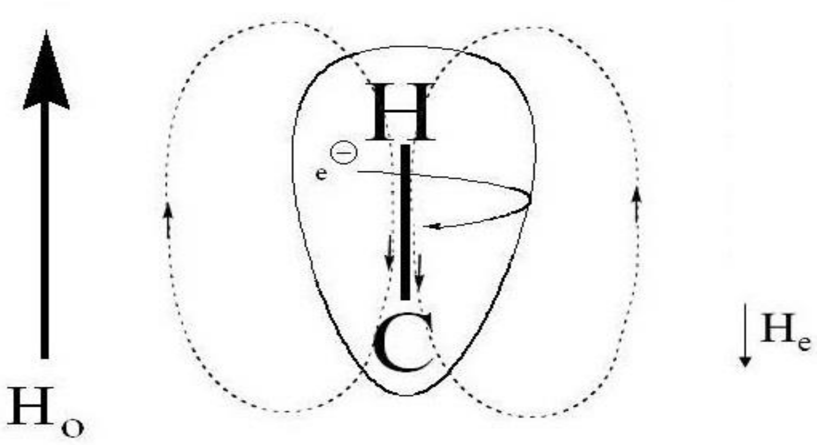


Fig 1.4: Shielding of proton

The circulation of electrons, if the induced magnetic field produced opposes the external field, then this circulation of electrons is called local diamagnetic current and the shielding caused is called diamagnetic shielding or diamagnetic anisotropy. The higher the electron density around the nucleus, greater is the diamagnetic shielding. Thus the nucleus precess at lower frequency, also the radiofrequency radiation absorbed will be of lower frequency.

If the induced field (H_i) reinforces the applied field, then the proton experience greater strength and proton is said to be deshielded. We will consider the different cases of shielding and deshielding in next unit.

The protons in a molecule are in slightly different chemical environment thus the difference in radiofrequency is very small, that can be measured in hertz. It is important to remember that the applied magnetic field has the strength corresponding 60 MHz (in case of proton absorption). For example- The difference in resonance frequency of protons in chloromethane and fluoromethane is only 72Hz, when the field applied is 1.41T (and the radiofrequency used is 60MHz). Thus there is a large difference between radiofrequencies of the instrument and difference in signal for various protons.

In NMR a reference compound is placed in the solution of substance and the resonance frequency of each proton in the sample is measure relative to the resonance frequency of the protons of reference substance. The standard reference substance used is Tetramethylsilane, $(CH_3)_4Si$ (TMS). This compound was chosen because the protons of its methyl group are more shielded and give a signal at one end of the range. The shift from TMS for a given proton depends upon the strength of applied magnetic field. The following are the advantages of TMS as reference in NMR-

- Due to low electronegativity of silicon, the shielding of equivalent proton in TMS is greater than most of the organic compounds, and therefore the signal of protons of TMS is well highly upfield shifted and generally well separated from signal proton attached with carbon in NMR spectrum.
- TMS is inert and it does not react with most of the organic compounds.
- It is volatile and easily miscible with most of the organic compounds.

The separation between a signal of a specific proton (of the compound under study) and the signal of proton of TMS is known as ***chemical shift***, which is a measure of variation in electronic environment of the concerned proton. The chemical shift is defined by:

$$\delta = \frac{\text{Shift in Hz}}{\text{Spectrometer frequency in MHz}} \times 10^6 \text{ppm}$$

This parameter is independent of applied field strength and is obtained in ppm. The chemical shift in δ units express the amount by which a proton resonance is shifted from TMS signal. The chemical shift also expressed in terms τ (tau), which is related with δ as

$$\tau = 10 - \delta$$

The chemical shift values helps to establish the electronic environment of the proton, and thereby helps in ascertaining the possible groups surround the specific proton. The increase in electron density around the proton, enhances the induced secondary field which opposes the applied field. Thus greater external field will be needed to cause the proton absorption. The concept of shielding is represented in terms of shielding parameter (α), the field H felt by the proton is given by

$$\mathrm{H} = (1 - \alpha)\mathrm{H}_0$$

where, H_0 is the applied magnetic field. Thus the net magnetic field felt by proton is different from applied field. Also greater the value of α, greater is the applied field needed by proton to undergo resonance with radiowave.

$$\upsilon = \frac{\gamma \mathrm{H}}{2\pi}$$

$$\upsilon = \frac{\gamma \mathrm{H}_0(1 - \alpha)}{2\pi}$$

In terms of chemical shift, shielding means increase in τ value or decrease in δ value. The presence of electronegative atom or group results in decrease in electron density around the proton that is deshielding takes place and absorption is shifted downfield. This means decrease in τ value takes place.

Chemical Shift Range

The chemical shift value for magnetically active nuclei depends upon the extent of effect of secondary electronic field. That is why the chemical shift (δ) range for proton is about 0 to 12 ppm, while in case C-13, it is about 0-220 ppm. The reason of greater chemical shift in C-13, is the circulating electron are more close to carbon, than hydrogen atom in hydrocarbon chain. In proton-NMR (PMR), the different protons in functional groups shows peaks in specific chemical shift δ values, depending upon the number and closeness of electronegative atom(s). The possible chemical shift range of some common functional groups is shown in figure 1.5

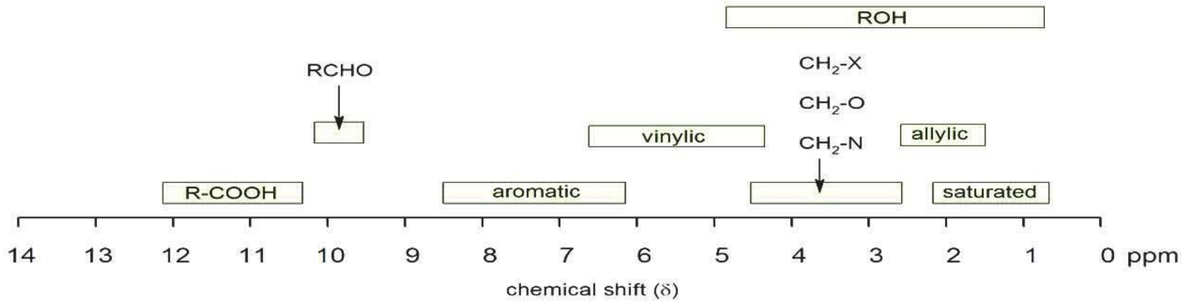


Figure 1.5: Chemical shift values of some functional groups

Field Strength and Position of Signal

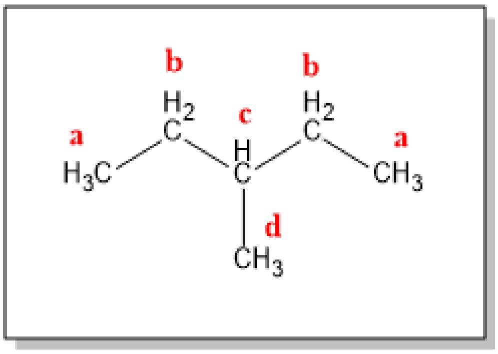
In external magnetic field of 1.41 T the resonance of a proton is approximately 60 MHz, but in the magnetic field of 2.35 T the resonance appears at approximately 100MHz. The ratio of resonance frequencies is the same as the ratio of the two field strength.

$$\frac{100MHz}{60MHz} = \frac{2.35T}{1.41T} = \frac{5}{3}$$

Thus for a given proton a shift from TMS is 5/3 times larger in 100MHz range, than in 60MHz range. Thus the peaks (signals) of non-magnetically equivalent protons are more separated in higher external field strength. The resolution of the instrument is therefore dependent on the applied magnetic field strength, higher the field strength, greater is the separation between the signals, better is the resolution of spectrum.

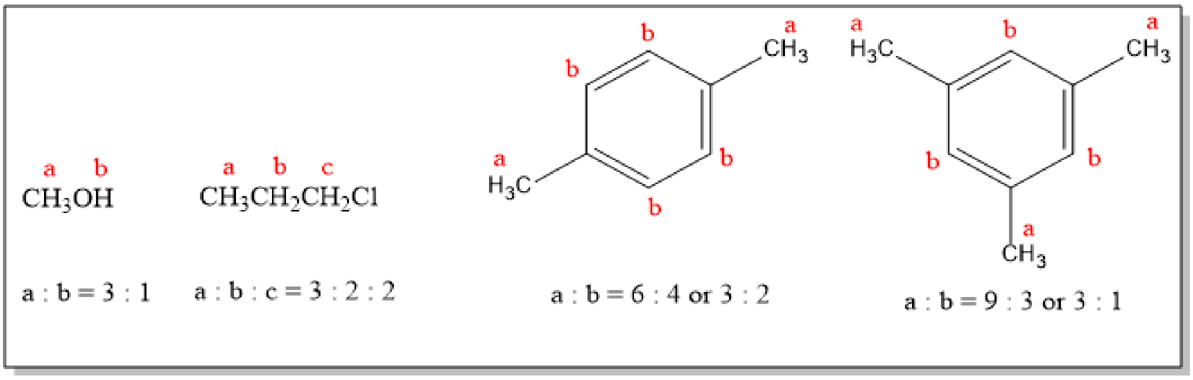
1.9 INTENSITY AND PEAK AREA-INTEGRATION:

In NMR signal intensity is not characterized as strong, medium and weak as in other types of spectroscopic methods (for example carbonyl group give strong band means intense bands in IR spectrum). The relative intensities of different peaks in NMR spectrum for a given sample are directly proportional to the number of same type of chemically (or magnetically) equivalent protons. In order to understand this let us consider an example of simple compound and its NMR spectrum. Consider the case of 3-methylpentane, which has 4 signals in NMR spectrum as shown below:



In NMR spectrum of 3-methylpentane four signals are obtained (represented as a, b, c and d), where signal ‘a’ is due to six protons of two CH_3 groups; signal ‘b’ is due to four protons of two CH_2 groups; signal ‘c’ is due to one proton of CH group and signal ‘d’ is due to three protons of CH_3 group. Due to different chemical environment these protons has different chemical shift and position in NMR spectrum. The intensity of each signal is proportional to the numbers of chemically equivalent protons of each type. Thus the intensity ratios for each signal for 3-methylpentane (a : b : c : d) is 6 : 4 : 1 : 3.

When you are asked to get structural information about a compound from its NMR spectrum, it is important to attain information about number of each type of chemically equivalent protons from their signal intensities. This information can be obtained by **integration** method, in which area under a signal (band) is measured, which is proportional to the number of particular type of equivalent protons causing that signal. This procedure was previously preformed manually by using NMR spectrum on graph paper and then calculating areas of respective signals. But now computer system is used to determine the area of a signal and it directly provides the information regarding number of protons of each type in a given signal. Some other examples with respective signal intensities are shown below.



1.10 FOURIER TRANSFORM TECHNIQUE AND FT-NMR:

The sensitivity and efficiency of NMR signal can be greatly increased by exciting all possible protons in a sample simultaneously rather than successively as in scanning method.

In this method the sample is irradiated with a pulse of radiation of all possible frequencies in the specified range, which is followed by time to frequency conversion i.e. Fourier transformation. The wide range of frequencies can be obtained by using a pulse of radio wave frequencies. This pulse is equivalent to band of frequencies entered around the oscillator frequency, with band width depending upon the duration of the pulse. The protons in sample start emitting signals at their characteristic Larmor frequencies as they relax back to their ground states. This emission following the excitation pulse is known as **free induction decay (FID)**. The FID of a single resonant frequency takes the form of an exponentially decaying sine wave. The Fourier transform is performed by the computer system and the spectrum thus obtained is termed as FT-NMR spectrum. In FT-NMR the sample is irradiated with all radiofrequencies in the required range i.e. covering all proton frequencies at once, at constant applied field. All protons absorb at their individual frequencies. The interactions of all the frequencies give an interferogram that is converted to the spectrum by Fourier transform.

Traditionally in NMR the sample is irradiated with a constant frequency while the magnetic field is varied, this method is known as magnetic scanning NMR. In other type, the spectrometers can operated by applying a constant magnetic field while radiowave frequency is varied, this method is termed as RF scanning NMR or continuous wave NMR (CW-NMR). In FT-NMR, spectra are presented in time domain, while in CW-NMR the spectra are presented in frequency domain. In time domain the intensity of signal is function of time and in frequency domain, the intensity of radiation is function of frequency. The characteristic features of frequency domain and time domain spectra are further explained in unit 2. The FT-NMR has three important advantages over NMR, which are as follows:

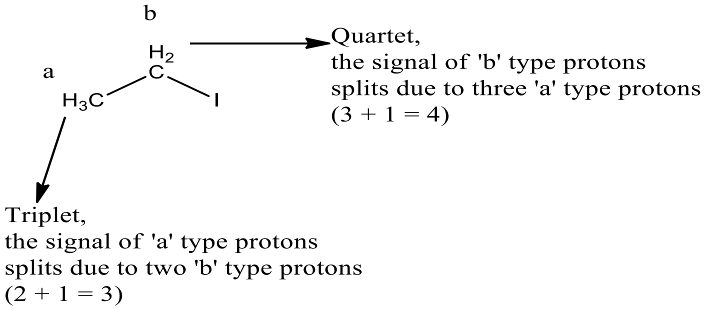
a) In CW-NMR, a very broad range of frequencies are to be scanned which is a time consuming process. In order to record spectrum in time domain (FT-NMR), all frequencies are irradiated in a very short time. Thus CW-NMR spectrum takes minutes to scan while in FT-NMR scanning takes only some seconds. Thus FT-NMR is much faster and less time consuming than CW-NMR.

b) As excess population is required to attain NMR signal and therefore sensitivity of instrument is crucial factor to get good spectrum. FT-NMR helps to increase the intensity of signal by passing successive pulses so that the signal to noise (S/N) ratio improves.

c) FT-NMR provides good spectra with very small samples. The successive pulses are irradiated on the sample and therefore large sample size is not necessary to attain spectrum, even samples in milligrams can give good spectra.

1.11 SPIN–SPIN COUPLING, SPLITTING OF A SIGNAL AND COUPLING CONSTANT:

The spin–spin splitting is explained by ‘n + 1’ rule, each type of proton are affected by number of equivalent proton ‘n’ on the atom adjacent to the carbon atom with which hydrogen under observation is attached. Thus its resonance peak split into ‘n + 1’ components. In order to understand the spin-spin coupling, consider the following examples:

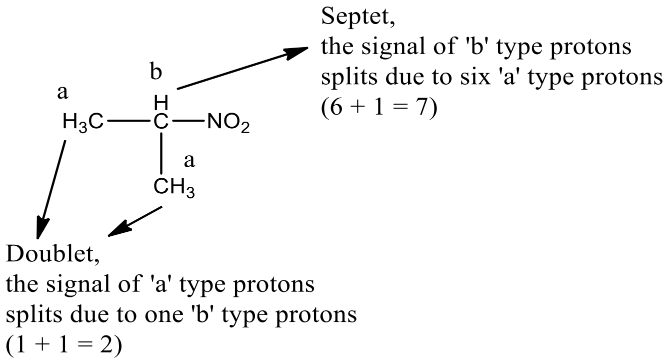


Quartet,
the signal of 'b' type protons
splits due to three 'a' type protons
(3 + 1 = 4)

Triplet,
the signal of 'a' type protons
splits due to two 'b' type protons
(2 + 1 = 3)

Figure 1.6: Multiplets in Iodoethane.

In the above example the set of protons ‘a’ have equivalent electronic atmosphere, so gives one signal. But due to interaction of neighbouring nuclear spins of two ‘b’ type protons, the one signal of ‘a’ type protons splits. The splitting is according ‘n+1’, where n is the number of neighbouring protons. So in this case the one signal splits into triplet, as n=2 (two ‘b’ type protons), so ‘n+1’ gives 2+1 = 3. Similarly the ‘quartet’ for ‘b’ type protons can be explained.



Septet,
the signal of 'b' type protons
splits due to six 'a' type protons
(6 + 1 = 7)

Doublet,
the signal of 'a' type protons
splits due to one 'b' type protons
(1 + 1 = 2)

Figure 1.7: Multiplets in 2-nitropropane.

Spin – Spin Coupling

The spin–spin splitting arises because protons on adjacent carbon atom can sense the spin direction of each other. The chemical shift of proton is influenced by the direction of spin of neighbouring protons. This interaction is magnetic in nature and is termed as spin – spin coupling.

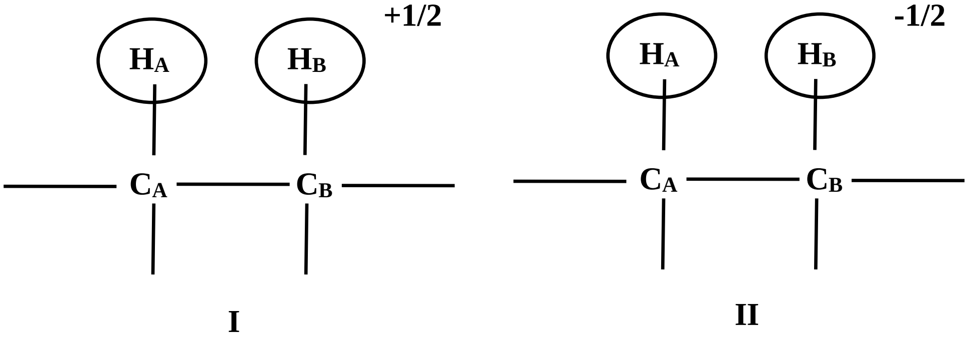


Figure 1.8: Spin-spin coupling

Consider two cases I & II, the hydrogen on the carbon A (presented as C_A) can sense the spin direction of the hydrogen on carbon B (presented as C_B). In some molecules H_B has spin +1/2 (I) and in some molecules H_B has spin -1/2 (II). The chemical shift of proton A is influenced by the direction of spin of H_B. Proton A is said to be coupled with proton B and its magnetic environment is affected by spin of proton B. Thus proton A absorb at a slightly different chemical shift in case I and II. As there is a equal probability of case I and II, the two absorptions have nearly equal intensity. The resonance of proton A is said to be split by proton B and the phenomenon is known as spin-spin coupling.

Coupling Constant 'J'

The spacing between the adjacent peaks in the multiplet is known as coupling constant J. It is basically the distance between the peaks centre of two adjacent peaks in a multiplet. The coupling constant is a direct measure of the spin coupling of the protons. The spacing between the peaks of a multiplet are all equal, which is represented by J, and expressed in terms of cycles per seconds, cps or Hertz (Hz). The values of J, in general found to be in the range of 1 to 20 Hz. For the interaction of most aliphatic protons in acyclic system the J value are always close to 7.5 Hz. The two H atoms on adjacent C atoms can be described as three bond interaction (^3J), and observed coupling for such a system is approximately 6-8 Hz.

The value of J, that is the separation between adjacent peaks is equivalent only if the ratio of chemical shift difference between the peaks ($\Delta\upsilon$) and the coupling constant J, is greater than 7. This condition $\Delta\upsilon/J>7$ is for first order splitting. In first order splitting the interacting protons have sufficient difference in the chemical shift, so that spin-spin coupling can takes place.

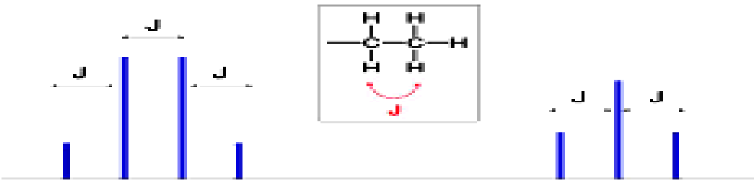


Figure 1.9: Coupling constant

The coupling constant is a measure of how strongly a nucleus is affected by the spin state of its neighbouring nucleus. The coupling constant is a constant and its value is independent of the external field. The value of J remains same (means the separation of adjacent peaks) in multiplet if the applied magnetic field is changed. The spacing of the lines of triplet or any multiplet do not expand when the spectrum is determined at higher field. From the values of coupling constant J, two singlets and one doublet can be distinguished. If the separation between the lines does not change then the signal is a doublet. If the separation between the lines changes with variation in external magnetic field then the signals are two singlets.

The departure from symmetrical nature of the multiplet results when the absorption positions (chemical shift) of interacting group are close enough. If a signal split due to influence of two different sets of protons, then the peak separation in multiplet observed will not be equidistant that is J does not remain constant. Thus in case a particular proton is under the influence of two different types of proton then the peak separation in the multiplet is unsymmetrical.

1.12 FIRST AND SECOND ORDER SPECTRA :

In NMR the **first order spectra** is the spectra which show undistorted multiplets due to spin-spin coupling, with multiplicity according to ‘n+1’ rule and intensities according to Pascal’s triangle. The essential conditions to attain first order spectra are:

a) As already discussed the condition to attain first order spectra is the ratio of difference in chemical shift ($\Delta\upsilon$) and the coupling constant J should be greater than 7 ($\Delta\upsilon/J > 7$).

b) Another important condition is the value of the coupling constant J of all protons in neighbouring groups with the proton (under study) must be identical.

When the above two conditions are followed then the NMR spectra is said to be first order spectra. Thus ‘n+1’ rule can be used to know the number of neighbouring protons to a specific proton.

The coupling constant J is independent of the applied field strength. But value of chemical shift depends on the applied field strength. As the essential condition to get first order spectra is $\Delta\upsilon/J > 7$, so on increasing applied field strength the value of $\Delta\upsilon$ can be increased and therefore the possibility to get first order spectra increases. Consider the example of two signals 30 and 36 Hz in a NMR spectrum measured on 60 MHz spectrometer, and then corresponding chemical shifts are 0.5 and 0.6 respectively. At 300 MHz the absorption frequencies corresponding to theses chemical shifts are 150 and 180 Hz respectively. Thus these two signals are separated by 6 Hz at 60 MHz but at 300 MHz these signals are separated by 30 Hz. Thus these signals appear as first order multiplets at higher applied field strength due to increased $\Delta\upsilon/J$ ratio.

The non-first order spectra are also known as **second order spectra**. When the ratio $\Delta\upsilon/J$ is lesser than 7 then the spectra becomes more complicated and it is known as second order spectra. In case of second order spectra, splitting pattern does not follow ‘n+1’ rule and intensities of the peaks in multiplets are not approximately given by Pascal’s triangle. The conditions to attain second order spectra are as follows:

a) The ratio of difference in chemical shift ($\Delta\upsilon$) and the coupling constant J should be lesser than 7 ($\Delta\upsilon/J < 7$).

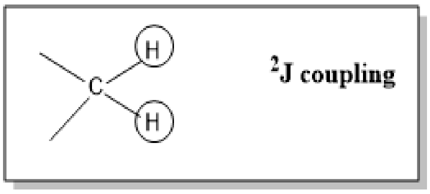
b) Also the value of the coupling constant J of protons in neighbouring groups with the proton (under study) is different.

1.13 *CLASSIFICATION OF SPIN-SPIN COUPLING :*

Depending on the number of bonds between the nuclei undergoing spin-spin interactions, the spin- spin coupling can be classified as:

a) ^{2}**J**-Two bond coupling, in this case the interacting nuclei are separated by two bonds.

This type of coupling is also classified as Geminal Coupling. In case of saturated compounds the value of J depends upon the value of angle between the geminal bonds. This dependence is shown in figure 1.10.



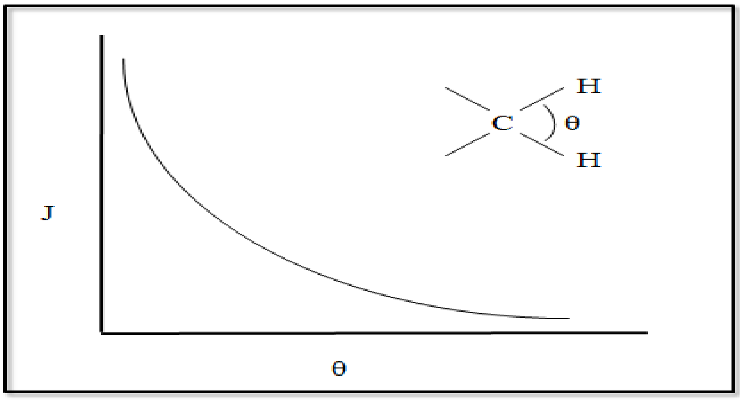
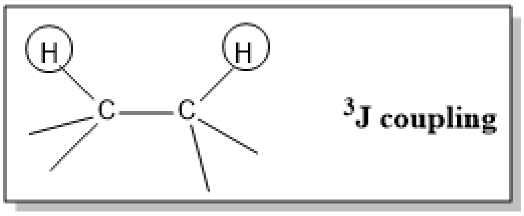


Figure 1.10: Variation of Coupling Constant (J) with Bond Angle (θ)

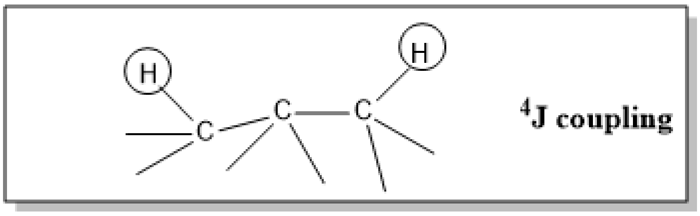
The geminal protons are separated by two bonds and if these protons are in different environments then the coupling is generally strong. The geminal coupling is also affected by the presence of electronegative atom. J_{Gem} increases with increase in electronegativity of the atom with which geminal protons are attached.

b) ^{3}**J**-Three bond coupling, where the interacting nuclei are separated by three bonds, as shown below:



This type of coupling is also known as vicinal coupling. For vicinal protons the value of J_{Vic} varies with the dihedral angle. When dihedral angle is 0° or 180° then the value of J_{Vic} are greater as compared to the value of J_{Vic} for dihedral angle 90°, in general the J_{Vic} is slightly negative when dihedral angle is 90°. In alkenes, in general trans-protons shows greater vicinal coupling than cis-protons so $J_{trans} > J_{cis}$.

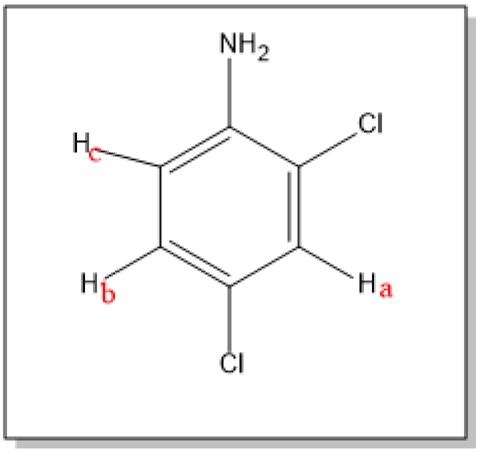
c) **^{4}J**-Four bond coupling, which is categorised under Long Range Coupling. In this case the interacting nuclei are separated by four bonds as shown below:



1.14 LONG RANGE COUPLING:

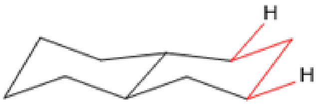
When the bonds between two interacting nuclei are four or more then the spin-spin coupling interactions are said to be long range coupling. Thus proton-proton coupling beyond three bonds is long range coupling. Some important examples of long range coupling are as follows:

a) Aromatic systems: The long range coupling between protons which are separated by more than three bonds can occur when pi-bonded atoms are present between. Due to greater separation between interacting nuclei in such systems the values of coupling constants are small and mostly between the range of 0 – 3 Hz. The signal splitting may give first order spectra if the interacting nuclei have well separated absorption frequencies.

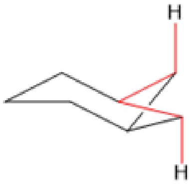


Consider the example of 2,4-dichloroaniline in which the proton ‘a’ at 3-position interacts with the proton ‘b’ at 5-position by spin-spin coupling so that the signal of ‘a’ proton shows doublet in NMR spectrum. As you can easily visualise that the number of bonds between Ha and Hb is four, so this is an example of long range coupling.

b) Saturated Systems: When the four sigma bonds between two interacting protons have a ‘W-arrangement’ then such systems may show long range spin-spin coupling. Some important examples are rigid cyclohexane system, bicyclo-[3, 1, 1] heptane system, etc.



Rigid Cyclohexane system



Bicyclo-[3, 1, 1]-heptane system

But in most cases coupling between ‘W-arrangement’ protons is not strong and either weak resulting in broadening of peak or not observed.

1.15 SIMPLIFICATION OF SPECTRA:

In complex NMR spectra of a compound, it is difficult to perform complete analysis due to overlap of signals. Thus useful information is unattainable in such circumstances. The intense, broad and completely unresolved signal for methylene groups which appear between δ1-2 is such an example. Also when coupling constant is comparable with the chemical shift difference of coupled protons, then spectra becomes complex and it is difficult to attain any useful information under such condition. There are some methods which decrease the complexity of NMR spectra and thereby helps in attaining useful information. The two important methods of simplification of NMR spectra are use of shift reagents and decoupling experiments. Let us consider each of these methods in detail:

Rigid ring system

a) Use of shift reagents: One of the most useful technique for spreading out ^{1}H NMR spectra is use of shift reagents. Shift reagents help is decreasing complexity of NMR spectra without making any change in strength of applied magnetic field.

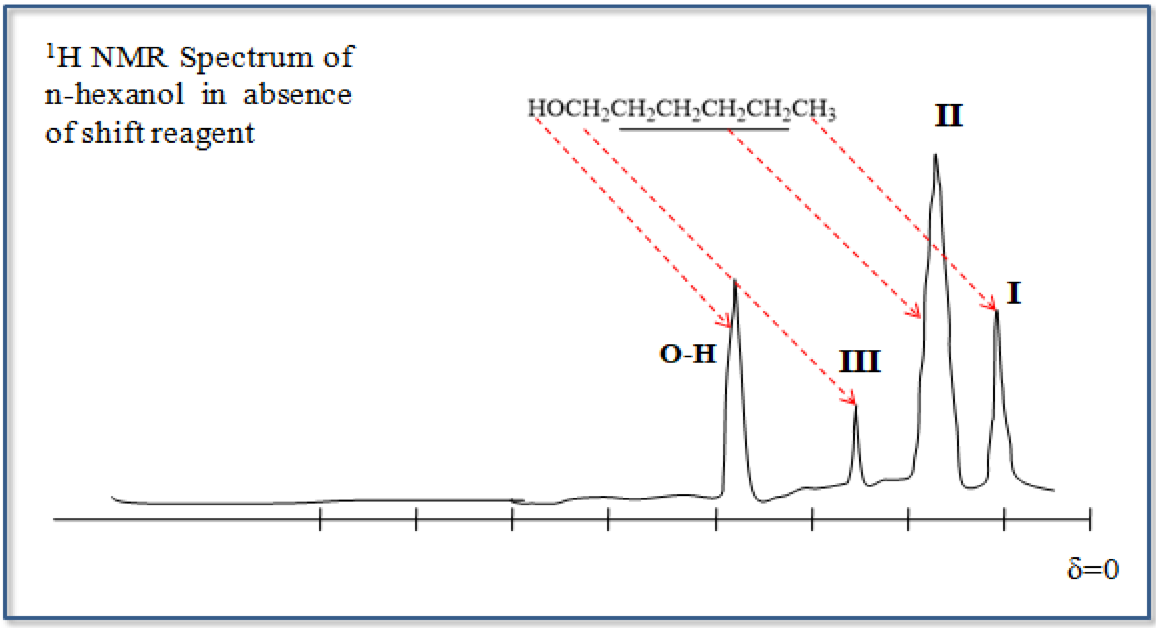


Figure 1.11: NMR spectrum of n-hexanol

Consider the example of ^{1}H NMR spectrum of n-hexanol in which a high field triplet is distorted due to adjacent CH_2 group (I). The low field broad (non-first order) multiplet is due to the CH_2 group adjacent to OH group (II). The protons of other CH_2 groups are observed as a broad signal between δ1.2-1.8 (III), as shown in figure 1.11.

When the same spectrum is recorded after using a shift reagent such as europium (III) complex $Eu(DMP)_3$, then the spectrum spreads out over a wider range of frequencies and it becomes simplified almost to the level of first order spectra (figure 1.12).

The shift reagents of this type has a lanthanide ion that increases its coordination number by bonding interaction with lone pair electrons of groups like NH_2, OH, C=O, O, CN and COOR. This lanthanide ion is paramagnetic in nature therefore significantly affects induced field of protons of the sample, leading to mark variation in chemical shifts of the protons of sample. That is why such reagents are known as shift reagents.

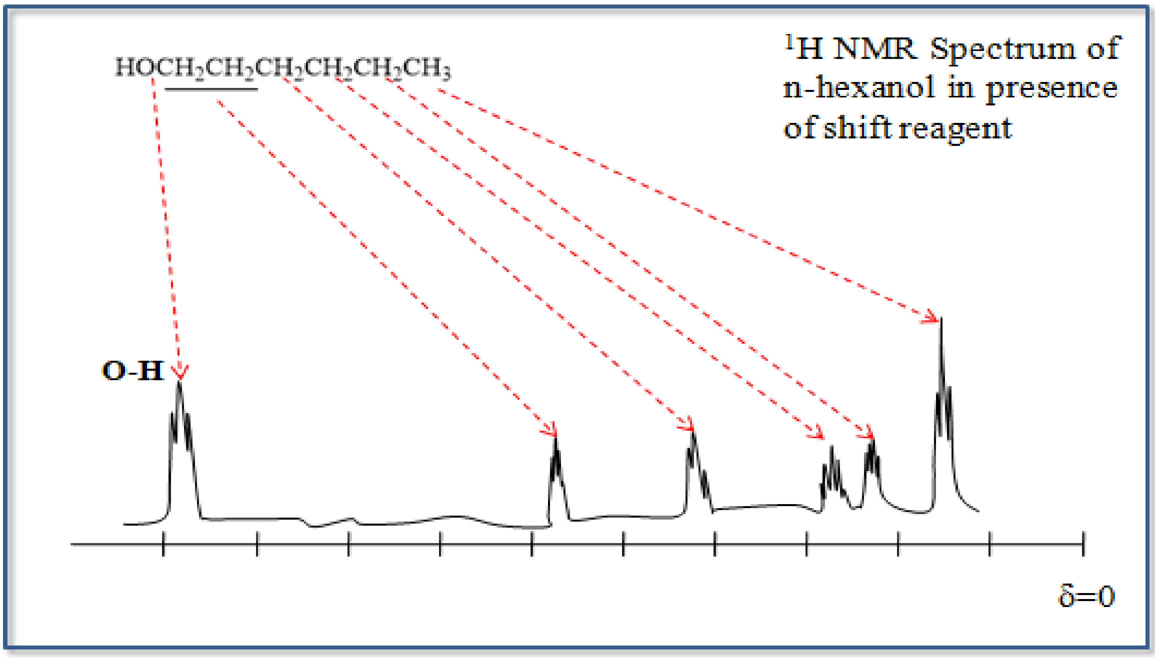


Figure 1.12: NMR spectrum of n-hexanol with shift reagent

The paramagnetic europium (III) ion complexes with n-hexanol, to induce significant shift of protons signal. Thus separate signal is now observed for each methylene group and the shift is function of distance of the group from the coordinating OH group, with greater downfield shift for groups close to OH group.

In general europium complexes results in downfield shifts, while the praseodymium complexes produce upfield shifts. Other lanthanides such as ytterbium, erbium etc also shifts the signals but may cause broadening of peaks and complicates the analysis of multiplets.

b) Spin Decoupling: The complex NMR spectrum results from coupling between neighbouring nuclei, which splits the signals into multiplets and sometimes this complex overlapping multiplets hinders the analysis of NMR spectrum. Spin decoupling is one the method which helps in simplification of NMR spectrum. In spin decoupling experiment, in addition to basic NMR spectrometer, a second strong tunable RF source is required to irradiate a particular proton with the frequency such proton absorbs. This results in rapid transitions of this proton between its nuclear spin states. Now the effect of this proton is averaged out and therefore does not affect the induced magnetic field on neighbouring proton. Thus the spin-spin coupling vanishes resulting in disappearance of corresponding multiplets.

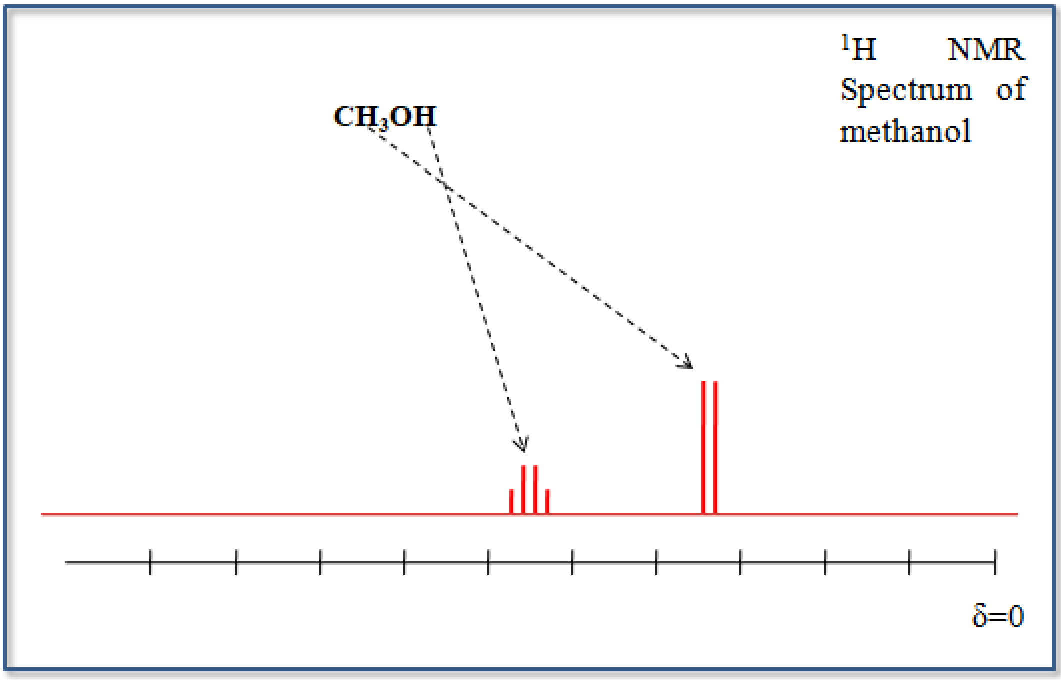


Figure 1.13: NMR spectrum of methanol

Consider the example of methanol, in which two signals are obtained in NMR spectrum due to protons of CH_3 group and OH group. The signal of three protons of methyl group (I) splits into doublet, it is due to spin-spin coupling with neighbouring proton of OH group. Similarly the signal of OH proton splits into quartet due to three protons of CH_3 group (as per ‘n+1’ rule), as presented in figure 1.13.

Now if the sample is irradiated with the radiation of frequency that can cause nuclear spin transitions of three protons of methyl group (using second tunable RF source), then these protons undergoes rapid excitation and relaxation (between nuclear spin states). Thus the magnetic effect of these protons on the proton of OH group cancel out and the quartet signal of OH proton is reduced to singlet. Similarly the decoupled experiment results eliminate the spin-spin coupling between protons of methyl group with that of OH group and therefore the doublet of methyl group reduce to singlet. The decoupled spectra of methanol is presented in figure 1.14

In this way the spin-spin decoupling helps in simplification of complex NMR spectrum. This method is very useful when the multiplets of two different groups of protons overlap. By decoupling experiments the multiplets of different sets of protons reduces to singlet and a simplified spectra of compound is attained.

The decoupling experiment is successful if the chemical shift positions for the coupling multiplets are no closer than 1 ppm. Also decoupling of second order spectra reduces to first order spectra when coupling multiplets are spaced more than 1 ppm.

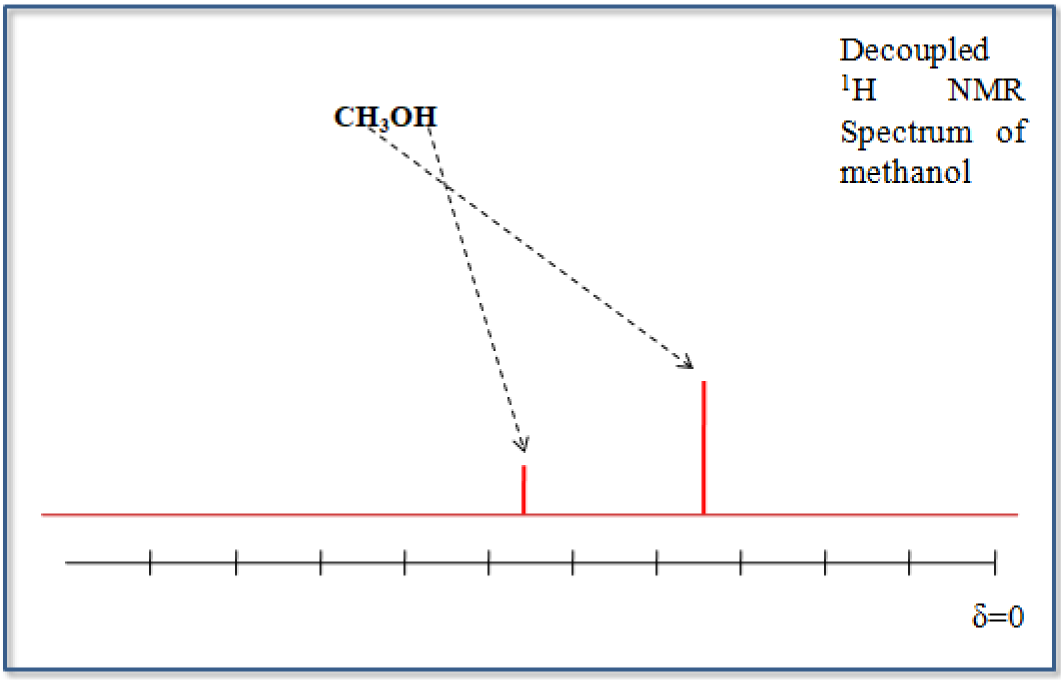
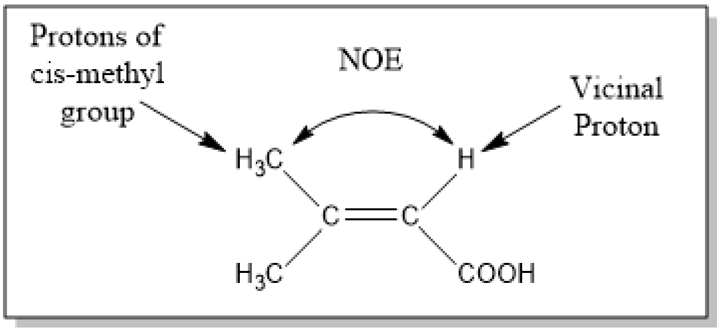


Figure 1.14: Decoupled-NMR spectrum of methanol

1.16 NUCLEAR OVERHAUSER EFFECT (NOE) AND STEREOCHEMISTRY:

The nucleus in excited spin state may undergo spin relaxation, in which transfer of its spin energy to and adjacent nucleus takes place. The efficiency of this energy transfer is directly dependent to the distance between the two nuclei between which energy transfer taking place. This NOE takes place through space and therefore it is different from spin-spin coupling interactions. But this NOE effect is only noticeable over short distances (2 to 4 Å). In simple experiments, normal NMR spectrum of the sample is recorded and intensity is measured. In NOE, sample is also irradiated with radiation of specific frequency (as in decoupling experiments) that results in rapid transition of nuclei (say ‘a’ nuclei) between its nuclear spin states. This nuclei ‘a’ transfers its energy to closest nuclei (say ‘b’ nuclei) through space and this result in enhanced intensity of signal of this nuclei ‘b’.



NOE method is useful in determining the stereochemical relationships between groups in molecules. Consider the example of 2-methylbut-enoic acid, in which due to NOE the intensity of signal of cis-methyl group increases (about 17 %) in comparison to trans-methyl group. This is due to energy transfer via space from vicinal proton to the protons of cis-methyl groups.

1.17 SUMMARY:

In this unit, you learnt about nuclear magnetic resonance, the important analytical method used structure elucidation of compounds. The main concepts you learnt in this unit are:

- The fundamental property of nucleus i.e. nuclear spin quantum number (I), due to which nuclei acquires different spin states. In presence of external magnetic field these spin states are non-degenerate and by absorption of radiowaves nuclear spin state transitions takes place.
- In presence of external magnetic field, nuclear magnetic moment vector starts precessing, known as Larmor precession and the frequency of precession is known as Larmor frequency.
- It is from fundamental NMR equation it can be concluded that the frequency of radiowave must matches with the precessional frequency, so that radiowaves get absorb to give nuclear spin transitions and NMR signal.
- In general chemically equivalent protons are magnetically equivalent, thus gives same signal in NMR spectrum.

- Isotopic ratio/natural abundance is important and isotopes with I > 0 and high isotopic ratio gives good signal in NMR spectrum.
- The difference in energy of nuclear spin states is of the order of energy that of radiowaves and therefore radiowaves are required to cause nuclear spin states transitions.
- The electronic environment of proton produces an induce magnetic field, thus the actual field experienced by proton is different from the applied field. This is measured in terms of chemical shift and used to characterise different protons which are chemically different in a compound.
- Tetramethylsilane is used as reference compound in NMR.
- Intensity of signal is related to number of chemically equivalent protons and it is peak area obtained by method of integration.
- In FT-NMR, sample is irradiated by a pulse of radiofrequency range, that causes all protons to absorbs and give signal simultaneously. The spectrum obtained after applying Fourier transformation is in time domain. The FT-NMR method is highly efficient, gives intense peaks and requires small sample size.
- Spin-spin coupling takes place due to magnetic interaction between neighbouring protons, this result in splitting of signal to give multiplets. The separation between successive peaks of multiplets is known as coupling constant J. Spin-spin coupling can be classified as ^2J, ^3J and Long range coupling.
- Depending on the symmetrical nature of multiplets and constancy of J, the NMR spectra can be classified as First and non-first order (second order) NMR spectra.
- The occurrence of multiplets and second order spectra results in complicated spectrum. In order to simplify complex spectra two important methods i.e. use of shift reagents and decoupling experiments are used.
- Nuclear Overhauser Effect is basically transfer of energy from two close protons through space and it is useful in stereochemical studies.

1.18 SUGGESTED READINGS:

- Spectrometric Identification of Organic Compounds 7th edition by R. M. Silverstein, F. X. Webster and D. J. Kiemle, J. Wiley & Sons Publication.
- Elementary Organic Spectroscopy- Principles and Chemical Applications by Y. R. Sharma, S Chand Publication.
- Introduction of Spectroscopy 5th edition by D. L. Pavia, G. M. Lampman, G. S. Kriz and J. R. Vyvyan, Cengage Publication.
- Spectroscopy of Organic Compounds by P. S. Kalsi, New Age International Publication.

1.19 TERMINAL QUESTIONS:

1) Write short notes on the following:
a) Nuclear spin
b) Isotopic ratio
c) Flipping
d) First order spectra
e) Shift reagent

2) Discus the principle of NMR spectroscopy?

3) What is chemical shift in NMR spectroscopy?

4) Why NMR signal splits into multiplet? How does multiplicity of signal depends on surrounding protons?

5) What methods are used for simplification of complicated NMR spectra?

6) What is Nuclear Overhauser Effect? How does it is useful in stereochemical analysis?

7) Predict the number of signals in the following:
a) Propan-2-ol
b) Ethylacetate
c) 1-Chlorocyclobutane
d) Napthalene

8) Predict the signals with their multiplicity in the following compounds:

a) n-butane and iso-butane

b) Propanone and Propanal

c) o-Xylene, m-Xylene and p-Xylene

d) 1-Chlorobut-1-ene and 1-Chlorobut-2-ene

Unit 2: ^{1}H NMR Spectroscopy-II

2.1 OBJECTIVE :

You have learnt basic concepts related to ^{1}H-NMR in previous unit. In order to understand this subject in-depth you need to understand some more details related to nuclear spin states, relaxation processes, factors affecting chemical shift, etc. In this unit you will learn about:

- Nuclear spin quantum number I and nuclear spin states.
- Definition of Larmor precession and frequency.
- Condition of radiowave absorption in NMR spectroscopy.
- Concept of relaxation and spin-spin, spin-lattice relaxation in NMR.
- Selection rule in NMR spectroscopy.
- Origin of chemical shift
- Reference compounds used in ^{1}H-NMR.
- Shielding and Deshielding effects.
- Factors such as inductive effect, anisotropic effect and hydrogen bonding which affects chemical shifts of ^{1}H nuclei.
- Paramagnetic induced field due to diamagnetic ring current in aromatic compounds and NMR definition of aromaticity.
- NMR signals for proton in hydrocarbons, alkenes, alkynes, aromatic compounds, alcohols, etc.
- Spin systems and AX, AB and AMX types of spin-spin coupling interactions.
- Spin-decoupling method and its advantages.
- Chemical exchange and spin-decoupling.
- Pulsed FT-NMR spectroscopy and its advantages.
- Time domain and frequency domain spectra.
- NMR spectroscopy involving Multinuclei.

2.2 INTRODUCTION :

In NMR transition between nuclear spin states takes place and these spin states of a nucleus depends on nuclear spin quantum number I. The values of I depends on the atomic number Z and mass number A of the nucleus. Thus spin states of a nucleus is dependent of the number and nature of nucleons in it. The nuclei with I > 1/2 have a nuclear magnetic moment μ and in presence of external magnetic field μ vector undergoes a precessional motion, known as Larmor precession. When frequency of radiowave is equivalent to the Larmor frequency then only absorption of radiowave takes place and corresponding signal is attained. The signal of NMR is obtained only if the ground spin state has excess population and that is why relaxation of nuclei to ground state is important. The spin-spin and spin-lattice relaxations results population excess in ground state, thus enhancing signal intensity. The nuclear spin transition follows $\Delta m = \pm 1$ selection rule and accordingly signals are obtained in NMR spectrum.

The separation between a signal of a specific proton of sample and the signal of proton of TMS is known as chemical shift, which is a measure of variation in electronic environment of the concerned proton. This parameter is independent of applied field strength and is obtained in ppm. The chemical shift is a measure of the amount by which a proton resonance is shifted from TMS signal. TMS is important reference frequently used in NMR signal measurements, but other reference compounds such as TSP, acetone, etc can also be used in ^{1}H-NMR. The chemical shift values helps to establish the electronic environment of the proton, and thereby helps in ascertaining the possible groups surround the specific proton. The chemical shift of a proton depends on various factors such as inductive effect, anisotropic effect, etc which causes shielding or deshielding effects thereby causing shift in signal position. The signal position of ^{1}H nuclei in different types of groups (such as CHO, COOH, C=C-H, etc) depends on these factors and this information is used in structural elucidation of organic compounds.

The extent of spin-spin interaction further affects the NMR spectrum of a compound. The coupling constant and symmetry of multiplet depends on the spin system i.e. AX, AMX and AB systems. Depending on spin system, the multiplet may be first order or non-first order. In general, spin-spin coupling interactions result in complexity in NMR spectrum. The spin-decoupling methods, such as use of shift reagents, decoupling experiments and chemical exchange can be used to reduce complexity in NMR spectrum. The pulsed-FT NMR is used to take fast scans and to increase signal intensity. FT-NMR provides the scope of study of NMR signals for less sensitive nuclei like ^{13}C which have low signal intensity.

2.3 NUCLEAR SPIN STATES AND LARMOR PRECESSION:

In NMR spectroscopy, nuclear spin state transitions take place by absorption of radiowave energy in presence of external magnetic field. In this section we will discuss about nuclear spin quantum number, nuclear magnetic moment and nuclear spin states. In later part of this section we will discuss the precessional motion of nuclear magnetic moment vector and Larmor precession.

We know that atom consists of nucleons (i.e. neutrons and protons) inside the nucleus and electrons in extra nuclear part of atom. The mass number (A) of an atom is sum of neutrons and protons in its nucleus and atomic number (Z) is the number of protons in nucleus of an atom. The **nuclear spin quantum number (I)** is a fundamental property of nucleus and it can be determined on the basis of values of mass number (A) and atomic number (Z). In table 2.1, the relation between mass number (A), atomic number (Z) with nuclear spin quantum number (I) is presented.

Table 2.1: Nuclear Spin Quantum Number (I) of some nuclei

Mass Number A	Atomic Number Z	Nuclear Spin Quantum Number I
Odd	Odd/Even	1/2, 3/2, 5/2,…
Even	Even	0
Even	Odd	1, 2, 3,….

Thus it can be easily concluded that

- When both mass number (A) and atomic number (Z) are even then nuclear spin quantum number I is zero (I = 0).
- When both mass number (A) and atomic number (Z) are either or both are odd then nuclear spin quantum number I is positive (I > 0).

The nuclei with positive nuclear spin quantum number (I > 0) are magnetically active nuclei, this means such nuclei have **nuclear magnetic moment (μ)**. Thus the atomic nucleus that posses either odd mass (A) or odd atomic number (Z), or both, has a quantized spin angular momentum and a nuclear magnetic moment.

The two important properties can now be introduced in order to explain NMR in detail. First is ‘nuclear spin states’, which is directly related to nuclear spin quantum number (I) and second is ‘precessional motion of nuclear magnetic moment vector and Larmor frequency’, which is related to nuclear magnetic moment of the nucleus. Let us first consider the case of nuclear spin states.

The nuclei with I = 0 does not influenced by external magnetic field and therefore such nuclei are said to be magnetically inactive nuclei. As already discussed, the nuclei with I > 0 are affected by external magnetic field and therefore known as magnetically active nuclei (and these nuclei are also known as NMR active nuclei). Some important nuclei and their nuclear spin quantum number I values are presented in table 2.2.

Table 2.2: Nuclear spin quantum number of some isotopes

Isotope	Nuclear Spin Quantum Number (I)
${}^{1}_{1}\mathrm{H}$	$\frac{1}{2}$
${}^{2}_{1}\mathrm{H}$	1
${}^{1}_{1}\mathrm{H}$	$\frac{1}{2}$
${}^{12}_{6}\mathrm{C}$	0
${}^{13}_{6}\mathrm{C}$	$\frac{1}{2}$
${}^{14}_{7}\mathrm{N}$	1
${}^{16}_{8}\mathrm{O}$	0
${}^{19}_{9}\mathrm{F}$	$\frac{1}{2}$
${}^{31}_{15}\mathrm{P}$	$\frac{1}{2}$
${}^{35}_{17}\mathrm{Cl}$	$\frac{3}{2}$

The number of allowed spin states is determined by nuclear spin quantum number I. For nucleus with I > 0, there are 2I + 1 allowed nuclear spin states with integral differences ranging from –I to +I. For example for hydrogen I = + 1/2. Thus the allowed spin states = $= 2 \times \frac{1}{2} + 1 = 2$. So, the states are -1/2 & +1/2. Similarly for chlorine I = 3/2, thus it has four allowed spin states -3/2, -1/2, +1/2, +3/2. The nuclear spin states of some nuclei are presented in table 2.3.

Table 2.3: Nuclear spin states of some nuclei

Element	**I**	**Number of Spin States (2I + 1)**	**Nuclear Spin States**
^{1}H	1/2	2	+1/2, -1/2
^{2}H	1	3	+1, 0, -1
^{13}C	1/2	2	+1/2, -1/2
^{14}N	1	3	+1, 0, -1
^{17}O	5/2	6	+5/2, +3/2, +1/2, -1/2, -3/2, -5/2
^{19}F	1/2	2	+1/2, -1/2
^{31}P	1/2	2	+1/2, -1/2
^{35}Cl	3/2	4	+3/2, +1/2, -1/2, -3/2

In absence of applied magnetic field, all the spin states of the given nucleus are of equivalent energy (degenerate) and in a given sample all the spin states should be almost equally populated with same number of atoms having each of the allowed spins. The spin states are not degenerate in presence of an applied magnetic field. It is because nucleus is a charged particle and any moving charge generates a magnetic field of its own. Thus the nucleus has a magnetic moment (μ) generated by its charge and spin. For example, the hydrogen has two spin states + 1/2, - 1/2, the possible orientation in external magnetic field is presented in figure 2.1.

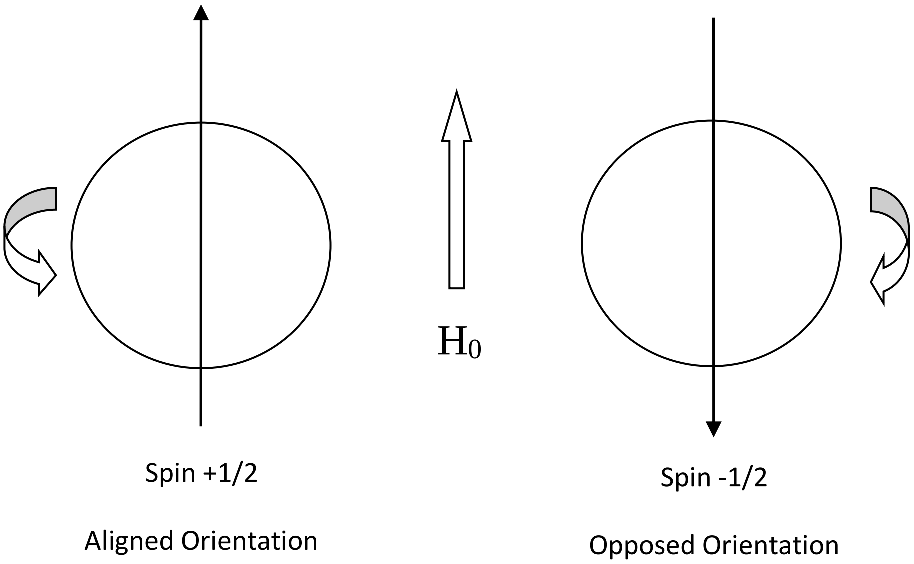


Figure 2.1: Possible nuclear spin states for hydrogen

The nuclear magnetic moment μ in two cases of hydrogen are pointed in opposite directions. In an applied magnetic field, all protons have their magnetic moments either aligned or opposed to external field. The spin state + 1/2 is of lower energy (aligned), while – 1/2 spin state has higher energy (opposed). The aligned orientation is more stable (lesser in energy) as compared to opposed orientation (greater in energy). Thus in an external magnetic field the degenerate spin state split up into two states of unequally energy (figure 2.2).

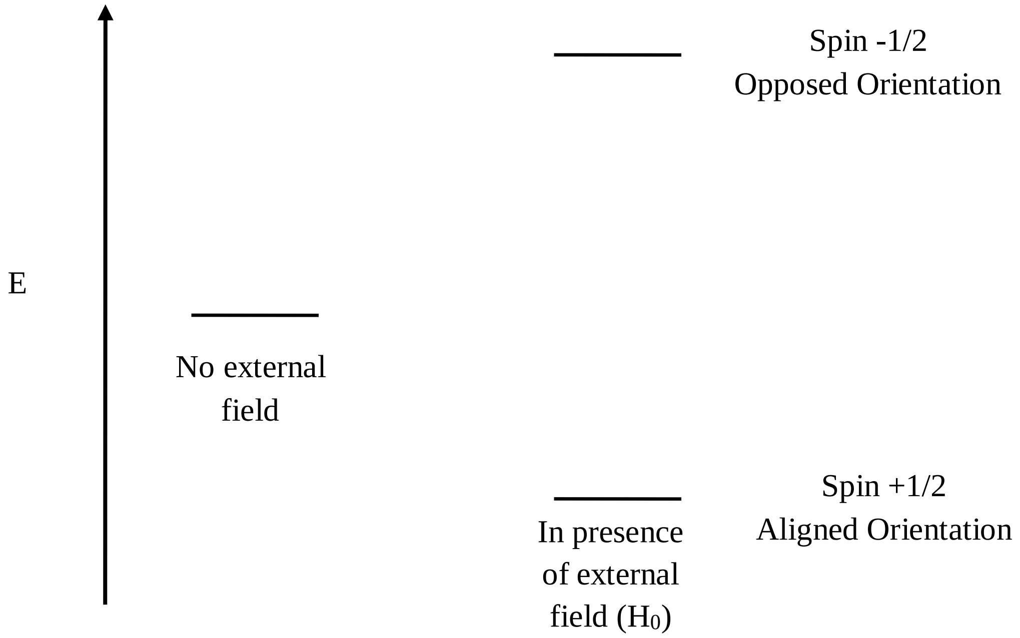


Figure 2.2: Splitting of nuclear spin states in hydrogen

Similarly in case of Cl the four energy states splits in external magnetic field as shown in figure 2.3.

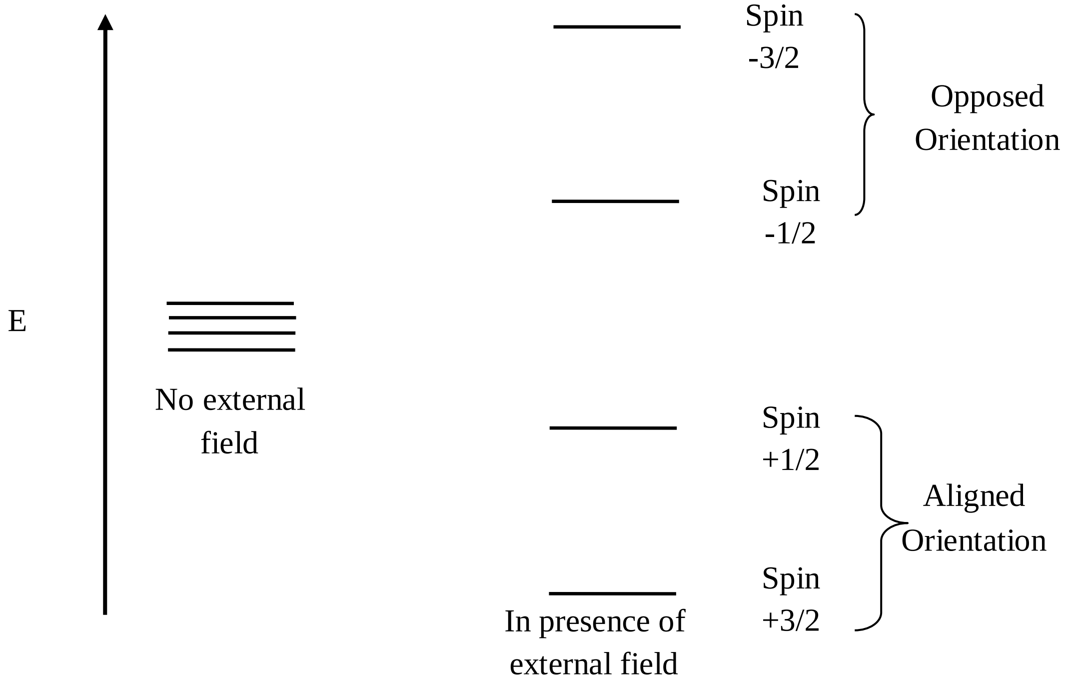


Figure 2.3: Splitting of nuclear spin states in chlorine

It is due to the nuclear spin quantum number I, different nuclei attains distinct nuclear spin states in presence of external magnetic field. Now we will discuss the other important aspect of precessional motion of nuclear magnetic moment vector which is related to nuclear magnetic moment of the nucleus.

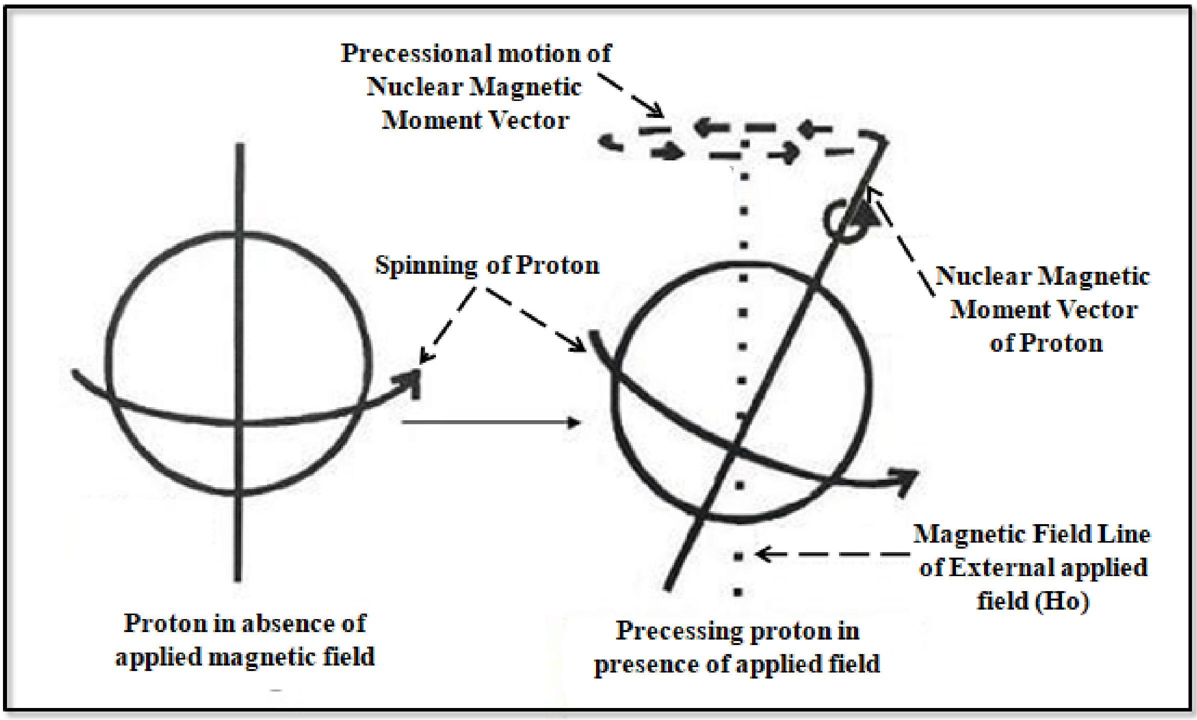


Figure 2.4: Precessional motion of Nuclear Magnetic Moment of Proton

In unit 1 (section 1.3) we already introduced the precessional motion and condition of resonance. The precessional motion of nuclear magnetic moment vector associated with the nuclei is conceptually similar to the precessional motion of a tilted classical gyroscope in an external gravitational field. In presence of applied magnetic field the magnetic moment vector of nuclei starts precessing about the direction of external magnetic field at certain frequency. This precessional motion is known as Larmor precession.

The frequency of precession i.e. precessional frequency is also known as Larmor frequency of the nuclei. It is defined as number of revolution made by the magnetic moment vector of the nucleus about the external field H_o.

Consider a nucleus for which I = 1/2, so it has two spin states represented as m = +1/2 and -1/2 (see section 2.5). From quantum mechanics, the energy of these two spin states is given by:

$$E_\alpha = +\frac{1}{2}v \qquad \dots(12)$$

$$E_\beta = -\frac{1}{2}\nu \qquad \dots(13)$$

where ν is Larmor frequency of nuclear magnetic moment vector of the nucleus. In general the energy is given by:

$$E_m = m\nu \qquad \dots(14)$$

The Larmor frequency depends on the gyromagnetic ratio γ, chemical shift δ and strength of the applied magnetic field. Now the most important point is this precessional frequency or Larmor frequency depends upon the magnetic field experienced by the proton. In a NMR experiment, different protons in a compound experienced different magnetic field depending upon the electronic surrounding. As the surrounding electrons, by virtue of their motion in orbitals (in presence of external magnetic field) produce a induce magnetic field that may be diamagnetic or paramagnetic (i.e. shielding and deshielding). This induced magnetic field may increases or decreases the actual magnetic field on protons. In this way the magnetic field experienced by protons in a compound is varied and depends on electronic surrounding. That is why the precession and Larmor frequency of chemically equivalent protons is same and similarly the Larmor frequency of non-equivalent protons is different.

In order to absorb radiowaves, the frequency of radiowave must matches with the precessional (or Larmor) frequency (ν). In other words, the frequency of radiowave (ν) must be equal to the precessional frequency for absorption of radiowave by a proton i.e. to attain NMR signal.

So from the above discussion it can be concluded that the nuclear spin quantum number (I) and nuclear magnetic moment forms the basis of NMR spectroscopy. The various nuclear spin states arise due to spin quantum number and the transition between these states results in NMR signal. The concept of NMR signal is related to resonance between radiowaves and precessing nuclear magnetic moment vector. When this resonance takes place radiowaves are absorbed and NMR signal in attained.

2.4 SPIN-SPIN AND SPIN-LATTICE RELAXATION :

In NMR, nuclei continuously absorb radiowave radiations and get excited to higher energy nuclear spin state. This results in decrease in number of nuclei in lower energy nuclear spin state. Consequently the intensity of the absorption signal also decreases and with time under certain circumstances may vanish completely. This actually happens when the condition of saturation is attained, in which the population of nuclei in both lower and higher energy nuclear spin states becomes equal. Thus the revert back of various nuclei from higher energy to lower energy spin states is necessary, so that the lower energy spin state has excess of population, which again absorbs radiation is excess and give a signal with considerable intensity. These various types of transitions from higher energy nuclear spin state to lower energy spin state is termed as relaxation processes. There are two kinds of relaxation processes: spin-spin relaxation and spin-lattice relaxation.

a) Spin-Spin Relaxation: In this kind of relaxation process, two precessing nuclei mutually exchange their energy due to interactions between nuclear magnetic moment. As each precessing nucleus is associated with a nuclear magnetic moment vector, it has a component rotating in a (X-Y) plane perpendicular to the direction of external magnetic field (Z-axis).

In case two nuclei are extremely close to each other, then this small rotating magnetic field is sufficient to induce a transition in the neighbouring nucleus. This mutual exchange of magnetic energy shortens the lifetime of an individual nucleus in higher energy spin state, as after loss of energy nucleus revert back to lower energy spin state. The time required for this relaxation is denoted by T_1 relaxation time and its value is generally very small for crystalline solids or viscous liquids, about 10^{-4} seconds. This result in broadening of peaks/signals for such samples and therefore such samples should be not used in high resolution studies.

b) Spin-Lattice Relaxation: In a NMR experiment, with respect to nuclei, lattice is surrounding molecules of sample or solvent. All molecules undergo translational, rotational and vibrational motion and have magnetic properties, giving rise to small magnetic field in the lattice. This magnetic field of lattice may induce nuclear spin transition from higher energy spin state to lower energy spin state. Thus the nucleus in higher energy spin state loses its magnetic energy to lattice, which may increase the translational and vibrational energy of lattice molecules. In this way, the nucleus reverts back to ground spin state or excess population in excited state increases. The time required for this type of relaxation is referred as T_2 and it is measure of the average lifetime of the nuclei in the higher energy spin state.

The relaxation time T_2 depends on the Gyrometric ratio of the absorbing nuclei and it is strongly affected by the mobility of the lattice. As the mobility in crystalline solids and viscous liquids is low thus T_2 is large. When mobility increases T_2 becomes shorter but at very high mobilities may results in broadening of signal.

The spin-lattice relaxation time is greatly affected by the presence of element with an unpaired electron, which causes strong fluctuations in magnetic field. This effect is also observed by nuclei of $I > 1/2$.

2.5 SELECTION RULES AND RELATIVE INTENSITIES OF LINES:

In unit 1 you already learnt about nuclear spin and nuclear spin quantum number I. On the basis of value I, nucleus has distinct spin states, which is given by 2I + 1. For example for proton I = 1/2, so the total spin states should be 2. According to quantum mechanics, the two spin states are described by another quantum number 'm', such that the values of m represents spin states with values –I to +I. So for proton (I = 1/2), there are only two values of m i.e. -1/2 and +1/2. Conventionally these spin states are represented as 'α' for m = +1/2 and 'β' for m = -1/2 respectively. Also you already know that α state is lower energy spin state. The energy of a spin state depends on m (from equation (14)), which is a sort of quantum number as its value represents spin states of a nucleus. As you know that the selection rule i.e. the conditions or rules which governs that which transitions can takes place and related to quantum numbers which are characteristics of each energy state.

In NMR the selection rule therefore depends on m and as per the selection rule 'only transitions in which m changes by unity (up or down) are allowed transitions'. Mathematically this is expressed as:

$$\Delta \mathrm{m} = \pm 1$$

Thus according to selection rule, the transition from m = +1/2 to m = -1/2 is allowed transition. As the transitions that takes place according to selection rule are allowed transitions and the intensity of the signal corresponding to allowed transitions is high. In NMR the signals that follow selection rule i.e. $\Delta \mathrm{m} = \pm 1$ are only observed in the spectrum.

Thus the transitions that are not according to selection rule are strictly forbidden in NMR spectroscopy.

Thus the signals obtained in NMR spectrum are only according to selection rule. But the relative intensity of signals depends on various factors. The factors that affect relative intensity of signals in NMR spectrum are as follows:

- Relative number of chemically equivalent nuclei.
- Relaxation processes and excess population in ground state.

The relative intensities of signals directly depend on the number of chemically equivalent protons (see section 1.9). The effect of relaxation processes and excess population on intensity of NMR signal is already discussed in previous section. The relative intensity of signals is independent of applied magnetic field. In FT-NMR, the radiofrequency pulse is irradiated on the sample that is useful in attaining intense signals of various chemically equivalent protons (see section 1.10).

2.6 CHEMICAL SHIFT IN NMR :

In unit 1 you learnt about the definition of chemical shift, in this section you learn about the theory and concepts related to chemical shift in NMR spectroscopy. The chemical shift arises due to different electronic environment of protons (section 1.8). When a sample containing nuclei with I > 0 is placed in external magnetic field, the circulation of electrons takes place due to which a diamagnetic induced magnetic field shield the nuclei. Thus the field at the nucleus is not equal to the applied magnetic field. The chemical shift is defined as the nuclear shielding with respect to a reference divided by the applied magnetic field.

$$\delta = \frac{(\mathrm{B_{Reference}} - \mathrm{B_{Sample}})}{\mathrm{B_{Reference}}} \times 10^6 \text{ ppm} \qquad \dots(15)$$

where $\mathrm{B_{Reference}}$ is the magnetic field experienced by the reference nuclei $\mathrm{B_{Sample}}$ is the magnetic field experienced by the sample nuclei. Chemical shift can also be defined as:

$$\delta = \frac{(\nu_{\mathrm{Reference}} - \nu_{\mathrm{Sample}})}{\text{Operating Frequency (in MHz)}} \times 10^6 \text{ ppm} \qquad \dots(16)$$

The important characteristics of chemical shift are as follows:

- It depends on the nucleus and its environment, thus chemical shift is a property of the nucleus and surrounding electronic framework. Thus the nuclei with equivalent electronic surrounding are magnetically equivalent and give absorption at same radiofrequency.
- The chemical shift can be measured by using external reference, which is a compound separately introduced in sample tube. Also internal reference can be used in which reference compound is added to the solution containing sample under study and sometimes solvent peak itself used as reference.
- It is measured relative to same nuclei in reference. Thus a suitable reference is required for determination of chemical shift. From equation (15), it can be easily concluded that chemical shift is simply the measure of applied field experienced by a nuclei in the sample with respect to the applied field on the nuclei in reference.
- The applied magnetic field is the primary factor that influences absorption frequency ν_{Sample} without affecting the value of chemical shift for specific nuclei. For example, in ^{1}H-NMR spectrum at 60 MHz if a signal is observed with a separation of 60 Hz with respect to reference ($\nu_{\text{Reference}} - \nu_{\text{Sample}} = 60\,\text{Hz}$), then chemical shift calculated using equation (16) is 1 ppm. Now same two peaks when observed in a 100 MHz spectrometer would be separated by 100 Hz apart and chemical shift is same as 1 ppm. Thus the separation between two signals (in Hz) increases with increase in operating frequency (or applied magnetic field) but the value of chemical shift remains same. This can be utilised in simplification of NMR spectrum by increasing separation between signals, as increase in operating frequency the separation between signals increases.
- Chemical shift can only be determined when operating frequency of the instrument is recorded along with absorption frequency of nuclei in sample and in reference. But chemical shift does not depend on the value of operating frequency of the instrument. As described above, the chemical shift is same 1 ppm, even if the operating frequency of the instrument is increased from 60 MHz to 100 MHz.

2.7 REFERENCE COMPOUNDS USED IN NMR:

In ^{1}H-NMR the most commonly used reference is Tetramethylsilane (TMS), $Si(CH_3)_4$. In TMS, due to low electronegativity of silicon the electron density on CH_3 groups of TMS increases, such that the protons experienced low magnetic field i.e. such protons are highly shielded. Due to shielding of protons, their signal is highly upfield shifted. In δ-scale, the TMS scale has assigned the value of $\delta = 0$. The NMR spectrum is measured from right to left in frequency units. The other scale use in chemical shift measurement is τ-scale, which is related to δ-scale as '$\tau = 10 - \delta$' in which signal for TMS protons is at $\tau = 10$.

TMS is insoluble in water and therefore it is not recommended reference compound in aqueous solvent system. The reference compound used under such condition is 3-methylsilylpropionate-2,2,3,3-D_4 $Me_3SiCD_2CD_2CO_2Na$, commonly known as TSP, in which the signal of protons of CH_3 group is taken as reference. The chemical shift for these protons is assigned the value of $\delta = 0$. The other compounds that may be used as reference in aqueous solutions are presented in table 2.4, along with their chemical shift δ_H in ^{1}H-NMR

Table 2.4: Reference compounds and their chemical shift δ_H in aqueous solutions

Reference Compound	Chemical Shift (δ_H) in Aqueous Solution
TSP	0.0
Acetone	2.216
Dioxan	3.743
DMSO	2.710
t-butyl alcohol	1.231

2.8 SHIELDING AND DESHIELDING EFFECTS:

When external magnetic field is applied to a sample, the electrons present in various molecular orbitals and non-bonding orbitals in molecule starts circulating. This circulation of electrons is such that a secondary induced field is produced with direction opposing the applied magnetic field (figure 2.5).

This induced magnetic field due to electrons opposes the applied external field passing through the orbital region. The magnitude of this induced field is very small in comparison to that of applied field. The neighbouring proton may experience this induced field along with the external magnetic field. Thus the net field experienced by the proton is different from that of applied magnetic field.

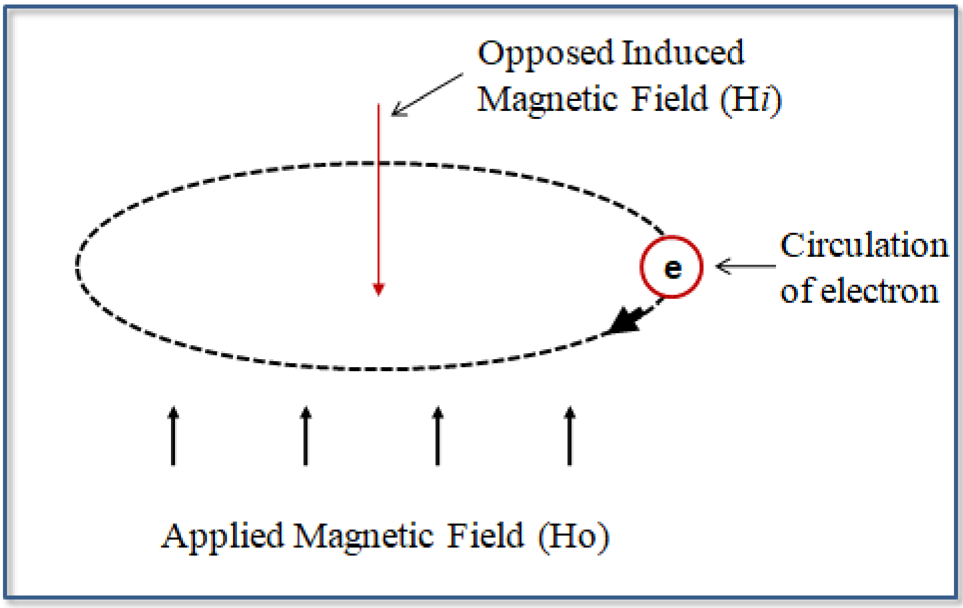


Figure 2.5: Induced magnetic field due to circulation of electron

The fundamental NMR equation which correlates electromagnetic radiation frequency 'υ' with the magnetic field on the proton in spin state, as $\gamma H = 2\pi\upsilon$. The two conditions that concluded from this equation are as follow:

- Firstly if the field experienced by the proton is lesser than the applied field then such proton is said to be shielded and this phenomenon is known as **shielding effect**. This is the case when the proton lies in a region where the induced field due to circulating electrons opposes the applied field (figure 2.6).

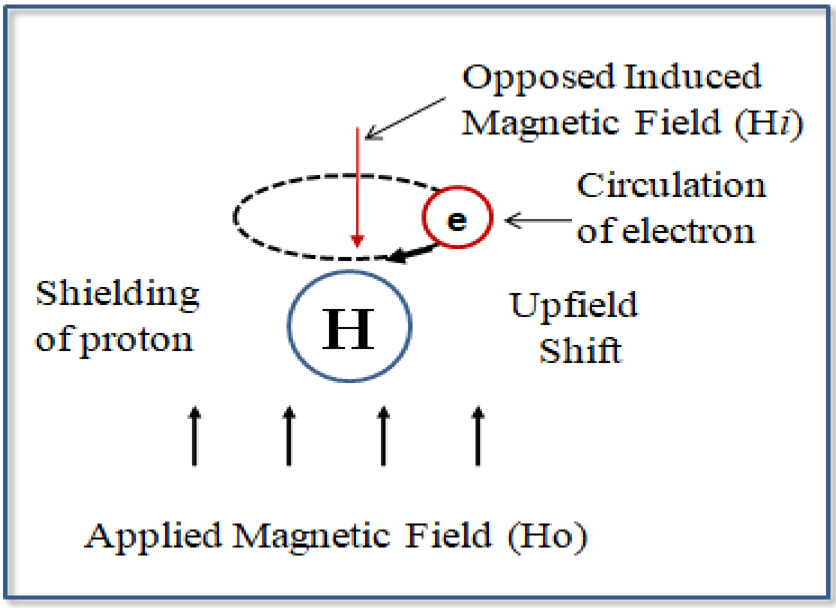


Figure 2.6: Shielding of Proton

Now consider if this opposing magnetic field is not present then for resonance the field should be greater i.e. the applied magnetic field (H < Ho). Thus if frequency of radiowave that causing nuclear spin transition at applied magnetic field Ho is υ, then in presence of opposing magnetic field, the actual magnetic field on the proton should be increases so that same frequency of radiowave i.e. υ is absorb by proton. This increase in actual magnetic field to cause same radiofrequency absorption is termed as **upfield shift** of signal.

Thus it can be concluded that whenever shielding takes place, signal is upfield shifted. As already discussed that the signal of TMS ($\delta = 0$) is highly upfield shifted. So under the condition of shielding, the signal gets close to signal of TMS (figure 2.6).

- Now consider the second case, if the field experienced by the proton is more than the applied field then such proton is said to be deshielded and this phenomenon is known as **deshielding effect**. This happens when the proton lies in a region where the induced field due to circulating electrons get aligned with the applied field (figure 2.7). As discussed in previous case, the actual magnetic field on the proton should be decreases to cause same frequency of radiowave (υ) absorb by proton to cause flipping. This decrease in actual magnetic field to cause same radiofrequency absorption is termed as

downfield shift of signal. Thus signal is downfield shifted when deshielding of proton takes place and the signal get far away from the signal of proton.

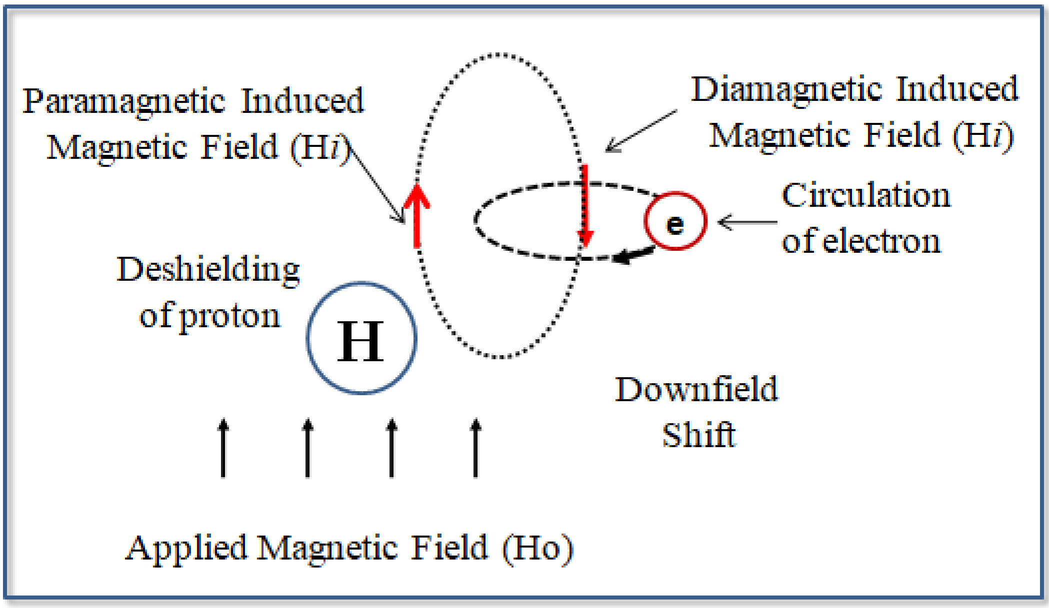


Figure 2.7: Deshielding of Proton

It is important to note that the increase or decrease of electron density around proton also increases or decreases the shielding or deshielding effect respectively. Thus if an electronegative atom is introduced near a proton, the electron density around the proton decreases. This result in decrease in shielding effect and i.e. deshielding takes place and downfield shift takes place.

Also the induced magnetic field shows directional effect (anisotropic property), due to continuous nature of magnetic field lines. Thus circulation of electrons may cause opposing (diamagnetic) induced field in a region then it is also causing aligned (paramagnetic) induced field in some other region, with respect to applied field (figure 2.7). This concept is further explained in case of alkene and alkyne in next section.

2.9 FACTOR AFFECTING CHEMICAL SHIFT:

Inductive Effect

The inductive effect is a permanent effect. It causes polarisation of carbon chain or ring, due to variation in electronegativity of bonding atoms. The presence of electron-releasing group (group having + I effect) increases electron density around the neighbouring protons. Similarly the electron-withdrawing group (group with – I effect) decreases the electron density around the proton. A proton is said to be deshielded if it is attached to electronegative atom or group. So –I effect causes deshielding means δ value increases (or τ value decreases). In the same way + I effect causes shielding, so δ value increases (or τ value increases). The effect of shielding and deshielding is shown in figure 2.8.

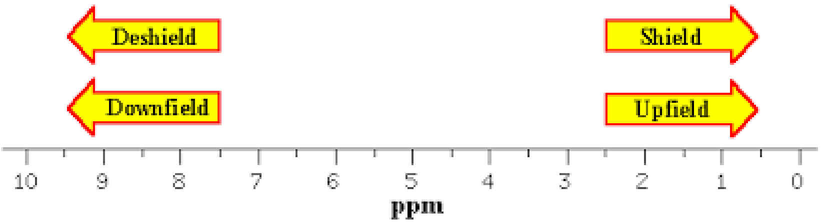


Figure 2.8: Downfield and upfield shifting

The effect of presence of electronegative atom can be explained with the example of substituted methane. The values of chemical shift for halogen substituted methane are shown in figure 2.9. The electronegativity of halogen varies as F > Cl > Br > I, so deshielding occurs and there is a downfield shift (increase in δ values) as electronegativity (-I effect) increases from I to F.

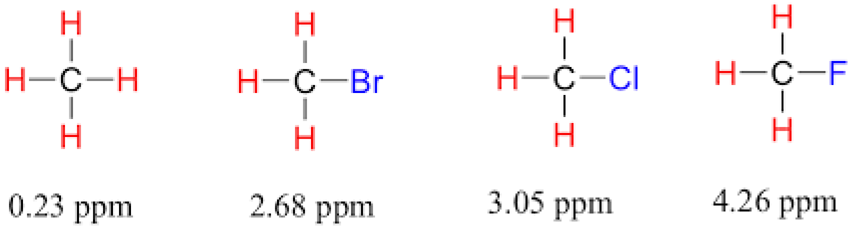


Figure 2.9: Chemical shifts for halogen substituted methane.

The inductive effect varies with the distance from the electronegative atom (or group). Thus with distance the deshielding effect and thereby downfield shift decreases. This can be explained by considering the example of 1-chlorobutane. The various chemical shift values form different types of protons in 1-chlorobutane are shown below in figure 2.10.

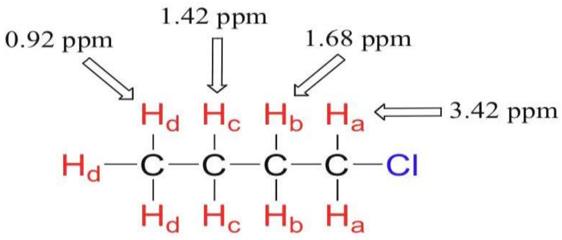


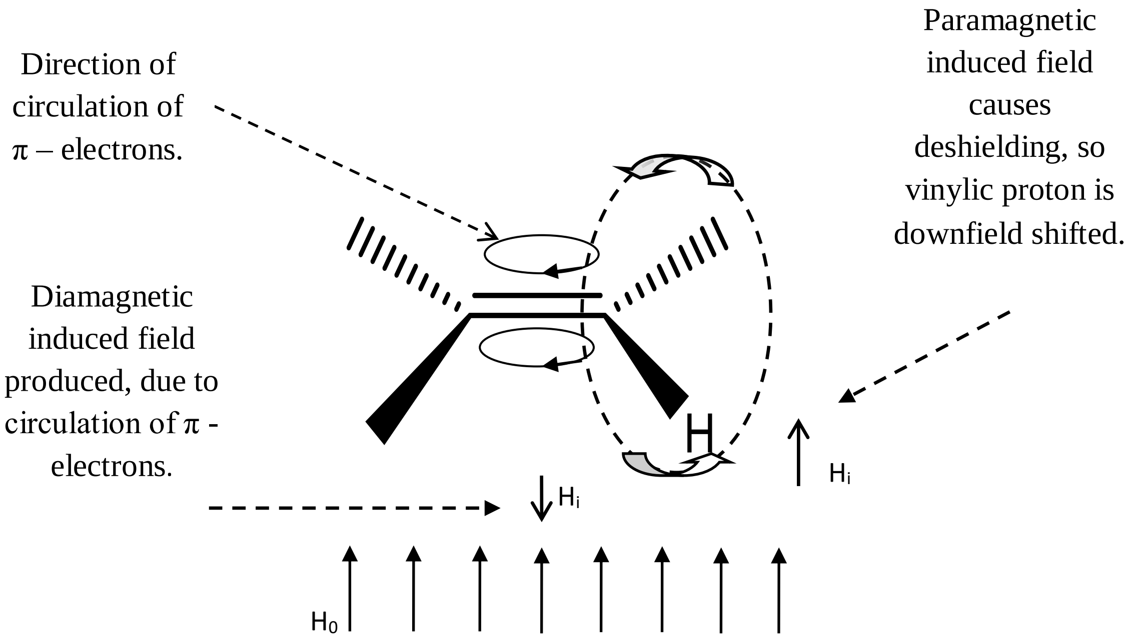
Figure 2.10: Chemical shift in Chlorobutane

Anisotropic Effect

The value of chemical shift can be explained by understanding the circulation of electrons under the influence of applied field. The absorption depends upon the manner in which π-electrons circulate. The π-electrons are loosely attracted and therefore free to circulate. This circulation is such that the magnetic field lines opposed the passage of external magnetic field lines form within the molecules. Thus diamagnetic field is generated within the molecule. As the magnetic field lines are continuous, there are regions of paramagnetic fields around the molecule. Therefore circulation π electrons generates diamagnetic shielding along with paramagnetic deshielding in the specific part of the molecule.

Case I: Ethene

The ethylene molecule is so oriented that the plane of the double bond is at right angle to the applied field. The circulation of π-electrons takes place in a plane along the axis of the bond. The molecule is oriented to the field as shown in figure 2.11.



Direction of circulation of π – electrons.

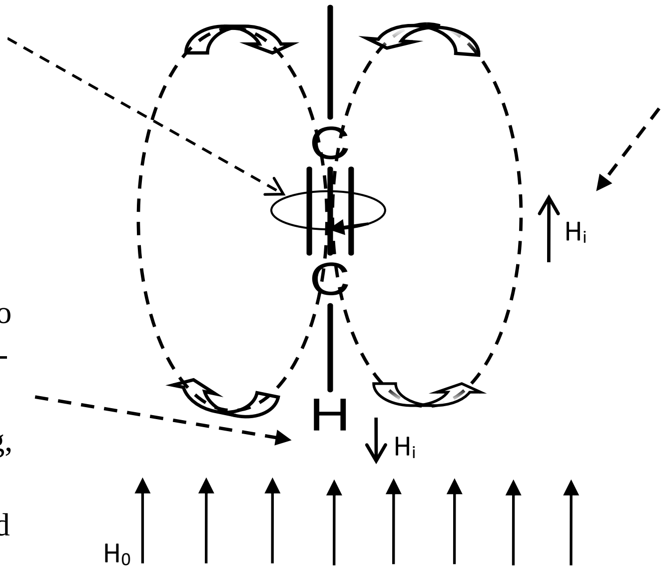
Paramagnetic induced field causes deshielding, so vinylic proton is downfield shifted.

Diamagnetic induced field produced, due to circulation of π - electrons.

Figure 2.11: Anisotropic effect in alkene and its effect on vinylic proton

The circulation π-electrons take place such that the induced field is diamagnetic above or below the alkene molecule. But there is a paramagnetic field on the vinylic protons, due to which deshielding occurs. The vinylic protons are downfield shifted because of deshielding, as compared to corresponding protons in alkanes.

Case II: Alkynes



Direction of circulation of π–electrons.

Paramagnetic induced field, due circulation of π-electrons.

Diamagnetic induced field produced, due to circulation of π-electrons. It causes shielding, so acetylenic proton is upfield shifted.

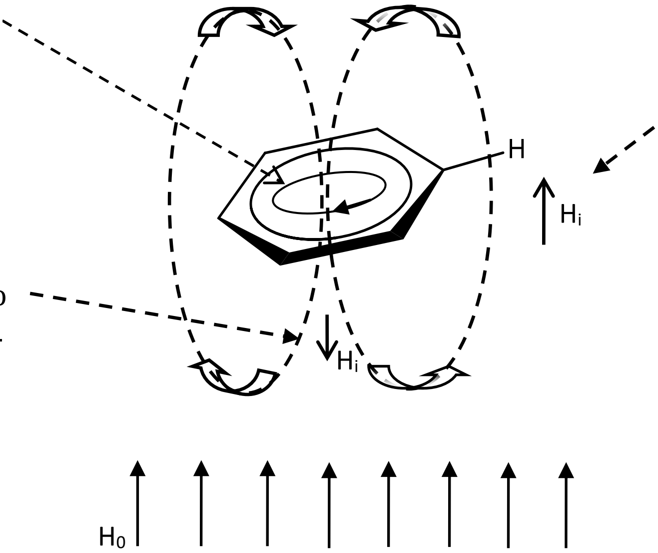
Fig. 2.12: Anisotropic effect in alkyne and its effect of acetylenic-proton

The circulation of electrons in the triple bonds takes place in cylindrical manner as shown in the figure 2.12. This mode of circulation results in the diamagnetic field along the C≡C bond axis. That is why the alkyne molecules get oriented parallel to the external magnetic field lines as shown in the figure 2.12.

The generated diamagnetic field at the acetylenic-proton decrease the field strength at it. Thus the field experienced by is lesser, than that required for resonance to occur. This means acetylenic proton is shielded and thereby upfield shifted.

Case III: Benzene

The case of benzene is unique as it has highly delocalised electron density. The electrons undergo circulation parallel to the plane of ring, to produce diamagnetic ring current, with the benzene ring. The ring current produces diamagnetic field (opposed to external field) within the ring and paramagnetic (aligned) field outside the ring (in the region of protons). It is this paramagnetic deshielding of aromatic protons which is responsible for downfield shifting. The protons outer to the benzene ring system are downfield shifted are observed at higher chemical shift (δ) values as compared to vinylic proton. The vinylic proton is generally observed in the chemical shift range of 5 ppm to 6 ppm, while for aromatic proton the range is 6 ppm to 9 ppm. The figure 2.13 presents the anisotropy in benzene ring.



Direction of circulation of electrons.

Diamagnetic induced field produced, due to circulation of π-electrons.

Paramagnetic induced field, due circulation of π-electrons, which cause deshielding of proton of benzene, that causes downfield shift.

Figure 2.13: Anisotropic effect in benzene and its effect on proton

The conclusion of the above discussion gives '**modern (NMR) definition of aromaticity**'. According to this definition 'aromatic compound is a compound in which the proton (outside the ring) is downfield shifted, due to paramagnetic induced field and inner protons (if present) are upfield shifted due to diamagnetic induced field'. Such a compound is said to have diamagnetic ring current and it is known as diatropic and diatropic compounds are aromatic compounds.

Hydrogen Bonding

Hydrogen bonding shifts the absorption downfield that is deshielding takes place. It is because of presence of electronegative atom around the proton, which results in decrease in electron density around proton and thus absorption shifted downfield. The downfield shift depends upon the strength of H-bonding. In case of carboxylic acid, the chemical shift is about 10 -12 ppm as COOH proton is highly deshielded. This large downfield shift is due to resonance effect, electronegativity of oxygen and hydrogen bonding.

Also intermolecular and intramolecular H-bonding can be distinguished by NMR. The proton involved in intramolecular H-Bonding does not have any shift in absorption due to change in concentration. The absorption due to intermolecular H-bonding is concentration and temperature dependent.

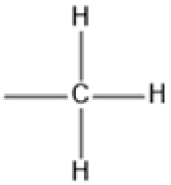
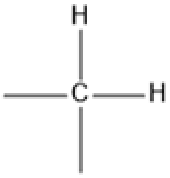
2.10 CHARACTERISTIC NMR SPECTRUM OF ORGANIC COMPOUNDS:

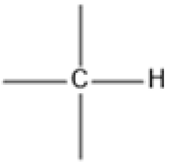
a) Hydrocarbons: In hydrocarbons the proton are bonded with only carbon atoms. The chemical shift variation in hydrocarbons is affected by the following factors:

- Hybridisation of carbon with which hydrogen is bonded.
- Diamagnetic or paramagnetic field effect of π-electrons.

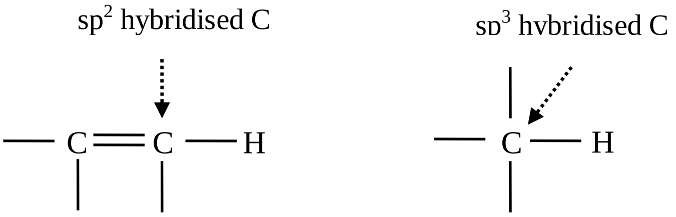
Alkanes have C-C bond due to which anisotropic effect is not important in this case. The chemical shift range for sp^3-hybridised carbon atom is about δ 0-2 ppm. In general methyl group gives signal close to δ 1 ppm. The methylene group shows signal close to δ 1.2-1.4 ppm and CH group has chemical shift higher than that of CH_2 group.

Table 2.5: Chemical shift in alkanes

Alkyl group	Chemical shift δ in ppm
	1 ppm
	1.2–1.4 ppm

	> 1.5 ppm

Alkenes have both sp^3 and sp^2 hybridised carbon atoms, the proton associated with these carbon atoms shows signals with distinct chemical shifts. The chemical shifts associated with sp^3 carbon are already discussed in case of alkanes. The proton bonded with sp^2 carbon atom is affected by paramagnetic induced magnetic field and hybridisation. The sp^2 hybridised carbon is relatively more electronegative as compared to sp^3 hybridised carbon. This result in slight decrease in electron density on proton attached with sp^2 hybridised carbon, causing a downfield shift (see section 2.9).



Due to greater s-character sp^2 hybridised C is more electronegative and there sp^{2}C-H proton is downfield shifted.

Figure 2.14: s-character and electronegativity of carbon

Due to paramagnetic field on sp^{2}C-H proton deshielding and therefore downfield shift of its signal takes place. The proton attached with sp^2 hybridised carbon (alkenes) shows signal in the range of δ 4.5-6 ppm.

In alkynes the proton is bonded with sp hybridised carbon atom. The proton in this case also affected by hybridisation and diamagnetic induced field. As sp hybridised carbon is more electronegative, it causes decrease in electron density on the attached proton, resulting in deshielding effect. In presence of external magnetic field the π-electrons of C≡C circulate in such a way to produce diamagnetic induced magnetic field around the acetylenic proton. This causes shielding and upfield shift.

These two effect are opposing with deshielding effect is slightly dominating, so there is net downfield shift of C≡C-H proton. The deshielding effect results in downfield shift, in the range of δ 2-3 ppm. Also due to shielding of C≡C-H proton chemical shift is lesser as compared to signal of proton in alkenes.

In benzene and other aromatic compounds, a diamagnetic ring current develops in presence of external magnetic field. Due to this diamagnetic ring current there is a diamagnetic induced field inside the ring but outside at proton there is paramagnetic field. Due to this strong paramagnetic deshielding the proton bonded with aromatic ring is highly deshielded, the chemical shift range is δ 6-9 ppm.

b) Carbonyl compounds: In carbonyl compounds there are two factors which determine the chemical shift range are as follows:

- Paramagnetic induced field of π-electrons on aldehydic proton.
- Presence of electronegative oxygen atom.

The paramagnetic effect of π-electrons in carbonyl compounds is same as in case of alkene. The proton bonded with carbonyl carbon is deshielded due to paramagnetic induced field in surrounding region. This results in deshielding effect and downfield shift in the signal of aldehydic proton.

The observed chemical shift in aldehyde is δ 8-10 ppm, which is much greater as compared to chemical shift in alkenes (δ 3-6 ppm). It is because of presence of electronegative oxygen atom present in carbonyl group. Due to high electronegativity of oxygen, there is decrease in electron density in the neighbourhood of aldehydic proton (due to –I effect of oxygen).

c) Alcohols and Phenols: The OH group in alcohols and phenols is a polar group. Also there is a strong –I effect of electronegative oxygen atom. The important factors affecting chemical shift in alcohols and phenol are:

- Inductive effect of oxygen.
- Hydrogen bonding due to OH group.

The proton of OH group is bonded with electronegative oxygen atom. Due to which the electron density on the proton decreases. This causes the deshielding effect resulting in downfield shift. Due to polarity of O-H group it can formed hydrogen bond with protic solvent or with other O-H group. The hydrogen bond causes deshielding effect and further downfield shift the signal of proton of OH group. The chemical shift range in alcohols is δ 3-5 ppm. The chemical shift is quite dependent on the concentration in case of intermolecular hydrogen bonding.

The chemical shift in phenols is further downfield shifted which is due to delocalisation of lone pair of oxygen in the benzene ring due to which oxygen acquired partial positive charge and become more electronegative.

Also the circulation of π-electrons of benzene ring in external field generates paramagnetic induced field on OH group. Due to which the proton of OH group is deshielded and further downfield shifted. That is why the chemical shift range in phenols is δ 5-11 ppm, which is much larger than the chemical shift in alcohols.

d) Carboxylic acid: In carboxylic acid the proton is bonded with C=O group. The important factors which effect chemical shift in COOH are:

- Electronegativity of oxygen atom of OH group.
- Paramagnetic deshielding of C=O group.
- Resonance in COOH group.
- Hydrogen bonding.

The electronegative oxygen atom decreases the electron density on the proton of COOH group. This results in deshielding and downfield shift similar to that in alcohols. The C=O group of COOH group causes paramagnetic deshielding due to its circulating electrons. This also results in downfield shift as in the case of carbonyl compounds. The carboxylic group shows resonance due to which the oxygen with which hydrogen in bonded acquired partial positive charge. Due to this the electronegativity of oxygen further increases causing greater downfield shift.

e) Amines: The important factors which effects the chemical shift values in amines are:

- Inductive effect of nitrogen.

- Hydrogen bonding of NH_2 group.

The electronegative nitrogen atom causes decrease in electron density on bonded proton, causing deshielding and downfield shift. But the electronegativity of nitrogen is lesser than oxygen, the downfield shift is also lesser in case of amines. Also NH_2 group forms hydrogen bond with itself and protic solvents but the extent of hydrogen bonding is much lesser as compared to that in alcohols, and that is why the chemical shift range in amines in δ 2-5 ppm.

2.11 SPIN SYSTEM AND SPIN-SPIN COUPLING IN AX, AMX AND AB PROTON NMR:

The spin-spin coupling arises because protons on adjacent carbon atoms can experience the spin orientation of each other. The chemical shift of proton is influenced by the direction of spin of neighbouring protons. This interaction takes place through the electronic environment and this interaction is magnetic in nature. The origin of spin-spin coupling, splitting of signal and their relative intensities is already discussed in section 1.11.

The origin of spin-spin splitting is characteristic of the spin system involved in spin-spin coupling. The spin system constitutes nuclei along with their relative positions and surrounding electronic environment. Thus a spin system has spin-spin coupling interactions between two or more nuclei. The nuclei is a spin system affects each other magnetically via spin-spin coupling interactions, but do not affect any other nuclei outside the spin system.

Notations used for different nuclei in spin system:

(1) The group of nuclei (protons) for which chemical shift difference is comparable to the spin-spin constant J, are designated as A, B, C, etc in order of increasing shielding.

(2) The group of nuclei (protons) having much difference in their chemical shift as compared to A or B are designated as X, Y, Z, etc.

(3) The number of protons of each type is indicated by a subscript i.e. 2, 3, etc. For example the ethyl group in ethyl acetate is designated as A_2X_3 spin system.

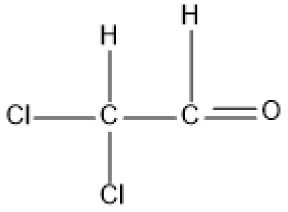
(4) To notify chemically equivalent but magnetically non-equivalent proton, prime (’) symbol is used (example AA’).

Classification of Spin System

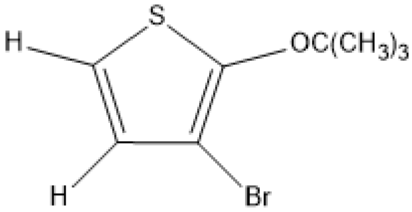
Depending on the number of nuclei and their type involved in spin-spin coupling interactions, the spin systems can be classified as A_2, AX, AB, A_3, AX_2 or AX_2, AMX, ABC and ABX type systems. Now we will discuss some important types of spin systems:

AX spin system: This spin system involves interaction between nuclei (protons) which have well separated chemical shift. In this case the proton absorbing at lower field should be represented by ‘A’ and proton absorbing at higher magnetic field is represented by ‘X’. In this spin system, the molecule has two types of protons having different chemical shift, for example molecule of dichloroacetaldehyde.

This is condition to attain first order spectra (see section 1.12). In this case the multiplets are symmetrical and the value of coupling constant J and chemical shift δ can be directly determined from the spectrum in case of AX coupling.


Dichloroacetaldehyde

AB spin system: In such a system the two types of protons have almost similar chemical shift difference Δδ and coupling constant J. The splitting pattern of AB system does not follow rules of first order splitting. This case is already discussed in non-first order i.e. second order spectra (section 1.12). Thus the pattern of splitting in AB spin system is distorted and unsymmetrical as in the case of second order splitting, is 3-bromo-2-tert.butoxy-thiophene (δ_{AB} = 0.17 ppm and J = 6 Hz at 100 MHz instrument operating frequency). The chemical shift should be at the centre of the doublets, also when the A and B has same chemical shift then the outside lines of multiplets disappear resulting in formation of the singlet. The chemical shift coupling constant and intensities of signal can be calculated from the following expression:


3-bromo-2-tert.butoxy-thiophene

a) Coupling constant (J_{AB}): On numbering the peaks from 1 to 4 in order of increase in shielding, the coupling constant is given by:

$$J_{AB} = \delta_1 - \delta_2 = \delta_3 - \delta_4$$

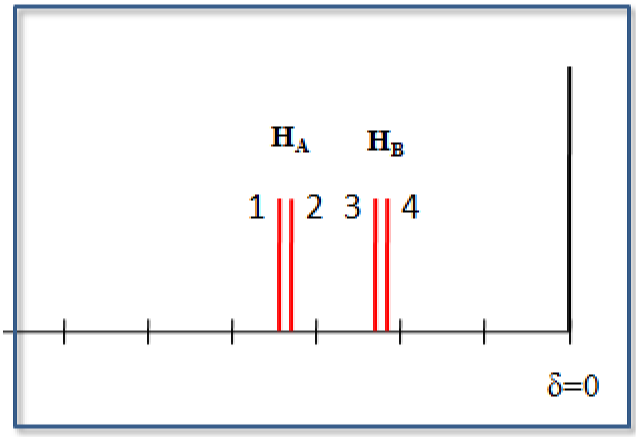


Figure 2.15: Splitting in AB spin system

b) Chemical shift (δ_{AB}): It can be determined from the following expression:

$$\delta_{AB} = \sqrt{(\delta_1 - \delta_4)(\delta_2 - \delta_3)}$$

c) The relative intensities of signals can be obtained from the following ratio:

$$\frac{I_3}{I_4} = \frac{I_2}{I_1} = \frac{(\delta_1 - \delta_4)}{(\delta_2 - \delta_3)}$$

AMX spin system: In AMX spin system, the three protons are non-equivalent magnetically (and chemically) with the condition $\Delta\delta/J > 7$. Thus this system follows the condition of first order spectra. These protons get coupled equally, resulting in three chemical shift and three coupling constants i.e. J_{AM}, J_{AX} and J_{MX}. The spectrum of AMX spin system consists of twelve peaks of almost equal intensity arranged in three parts of quartet from which chemical shifts and coupling constant can be easily determined.

The origin of twelve peaks can be explained by considering the splitting of signal of X proton into doublet by its coupling with M proton, which again spilt into pair of doublets by coupling with A proton. Similarly the signal of M and A proton splits into pair of doublets respectively resulting in total twelve peaks.

2.12 SPIN-DECOUPLING METHOD:

It involves irradiation of a nuclei (or proton) with an intense radiofrequency signal at its resonance frequency to eliminate coupling to the neighbouring proton. A signal of a particular proton or a group of equivalent proton splits into multiplet due to interaction with neighbouring proton having different environment.

Consider a compound in which H_A and H_B are in different environment resulting in two doublets corresponding to each proton in the NMR spectrum.

Now if appropriate radiofrequency is irradiate on proton H_B then this proton undergoes rapid and continuous transition between lower and upper spin states. This results in much decrease in time of stay in a particular state to eliminate its coupling with other proton H_A. Thus the coupling between H_A and H_B vanishes and this phenomenon is known as spin-decoupling. Due to absence of coupling, the signal of H_A appears as singlet in the spectrum. This spin decoupling helps in simplification of NMR spectrum when the spectrum is complicated due to presence of too many close multiplets. This simplification of NMR spectrum by using spin-decoupling method is already explained in section 1.15.

2.13 CHEMICAL EXCHANGE :

When in a molecule a proton transit between two magnetic environments at a rate which is faster in comparison with the nuclear spin transition, then resonance observed for that proton is due to averaged effective field in the two environments, resulting in observance of single peak (without any multiplet). In case of alcohols the chemical exchange of proton can be presented as:

$$\mathrm{R-O-H^a + H-O-H^b \rightleftharpoons R-O-H^b + H-O-H^c}$$

In the above chemical process, there is an exchange of protons between alcohol and water molecule. As you see the $\mathrm{H^a}$ shuttles between alcohol and water molecule, this process is known as chemical exchange and if this exchange rate is faster than the rate of nuclear spin transition then this H^a proton experience averaged magnetic field and gives single signal in NMR spectrum.

The OH proton in water has different chemical shift as compared to proton of alcohol say ethanol (EtOH). But in NMR spectrum, only one signal is observed for the proton of EtOH in water. It is because the proton of OH of ethanol shuttles between EtOH molecule and surrounding water molecules. As this exchange of proton between EtOH molecule and surrounding water molecules is much faster than the nuclear spin transition of proton, the magnetic field experienced by proton is average of the magnetic field in ethanol molecule and in water molecule. This average magnetic influence limits the spin-spin coupling of proton of OH group of ethanol with neighbouring protons of CH_2 group. The elimination of spin-spin coupling results in one signal for OH proton of EtOH. Thus it can be concluded that spin-decoupling takes place when chemical exchange takes place. The necessary conditions for this kind of spin-decoupling due to chemical exchange are:

- Sample should have nuclei bonded with polar bond, which can be ionised under suitable conditions.
- Sample should be dissolved in a polar solvent having same nuclei, with which chemical exchange is possible.
- Signal should be recorded at moderate to high temperature.

When signal is observed at low temperature and if the solvent is less polar, than rate of chemical exchange is less and resulting decoupling does not takes place. Chemical (proton) exchange also takes place in some other compounds in which hydrogen is attached with nitrogen, sulphur and oxygen, i.e. carboxylic acids, amides, amines, thiols, etc also shows proton exchange. Thus in these compounds also the effect of spin-spin couple of proton undergoing chemical exchange does not takes place and single signal is observed in NMR spectrum.

2.14 PULSED FT-NMR- TIME AND FREQUENCY DOMAIN SPECTRA :

In FT-NMR the sample is subjected to a high powered short duration radiofrequency pulse. This pulse of radiation contains a broad band of radiofrequency and causes all the magnetically active nuclei to resonate simultaneously at their Larmor frequencies. Thereafter the sample radiates a signal known as free induction decay (FID), which is modulated by all the frequencies of the nuclei excited by the pulse the signal detected as nuclei returns to the equilibrium and intensity as the function of time is recorded, digitized and presented by the help of the computer.

The FID of a signal resonance frequency takes the form of an exponentially decaying sine wave of frequency given by $\nu = \nu_L - \nu_C$, where ν_L is Larmor frequency and ν_C is carrier frequency. The plot obtained is known as interferogram, the information gain from interferogram can be converted into a useful form by a computer program using Fourier transformation.

The use of powerful short burst of energy, known as pulse, simultaneously excites all magnetically active nuclei in the molecule. When the pulse is discontinued, the excited nuclei begin to lose their excitation energy and return to lower energy spin state. This relaxation of nuclei is accompanied by simultaneous emission of electromagnetic radiation. As a molecule contains many types of chemically equivalent nuclei, different frequencies of radiowave radiation are emitted simultaneously. This emission of radiations of different radiofrequency is known as free-induction decay (FID). The intensity of FID decays with time as all the nuclei loses their excitation energy. The FID is presented as a plot between time and intensity and thus the FID is also known as time-domain signal and spectra are known as time-domain spectra. The FID constitutes the combination of all emitted frequencies and therefore it is very complicated. For the simplification FID, a mathematical method known as Fourier transform (FT) is used using computer system. This mathematical method separates each of the individual components of FID and converts them to frequencies. Thus this complex mathematical procedure i.e. Fourier transforms is used to break the FID into its separate sine and cosine wave components by the use of computer system. In this way the frequency-domain spectra of a compound can be attained, which is the standard format for a spectrum, as is obtained by CW-NMR instrument.

The FT-NMR instrument thus have computer as a component, which not only process the data by Fourier transform but also it is used to control all settings of the spectrometer. The most important advantage of FT-NMR over CW-NMR is related to more sensitivity on FT-NMR instrument so that weak signals are also measured with high intensity. Also pulsed FT analysis is much faster and a measurement of FID can be performed in few seconds. Thus this experiment can be repeated many times to get an average FID signal. Another advantage of FT method is when the sample size is small, FID is weak in intensity and high level of noise is associated with it. As you know that noise random electronic signals that fluctuations of the baseline in the signal. As noise is random and its intensity does not increases when repeated FID signal is attained, but the intensity of FID enhances with each repetition. Thus using this procedure the signal to noise ratio increases i.e. the FT-NMR has appreciably good spectrum as compared to CW-NMR spectrum for sample of small size. Pulsed FT-NMR is also suitable for the examination of nuclei that are not very abundant in nature, nuclei that are not strongly magnetic or dilute samples.

2.15 MULTINUCLEI NMR, RELATIVE ABUNDANCES AND INTENSITIES :

There are many nuclei other than ^{1}H which have nuclear magnetic moments and sensitive enough for practical use and give ^{1}H like nuclear magnetic resonance. These are ^{19}F (83 % sensitive), ^{203}Tl and ^{205}Tl (13.5 %), ^{31}P (6.63 %), ^{129}Xe (0.55 %), ^{115}Sn and ^{119}Sn (0.45 %), ^{195}Pt (0.34 %), ^{123}Te and ^{125}Te (0.22 %), ^{207}Pb (0.19 %), ^{77}Se (0.062 %), ^{29}Si (0.037 %) and ^{13}C (0.018 %). In organic chemistry, beside ^{1}H-NMR the nuclei like ^{19}F, ^{13}C and^{14}N NMR are most useful for the purpose of structural analysis. In inorganic chemistry the nuclei with are important for the purpose of NMR studies are ^{31}P, ^{11}B, ^{27}Al, ^{119}Sr, ^{119}Hg, etc.

The magnetically active isotopes of fluorine, carbon and phosphorus i.e. ^{19}F, ^{13}C and ^{31}P are used extensively. The magnetic resonance condition of these isotopes is almost same as that of proton. These isotopes have nuclear spin quantum number $I = 1/2$, and therefore the signal is obtained by nuclear spin transition between lower energy spin state to higher energy spin state as in the case of proton. The isotopic abundance of ^{19}F and ^{31}P is very high as these are the only isotopes of their particular elements. Thus the intensity of signal of these isotopes is quite high and their signal is easily recognizable. But in case of ^{13}C the isotopic abundance is much lower (as compared to ^{12}C) and this limits the use of ^{13}C-NMR as a technique in analytical chemistry. With the advent of Fourier transform method, it is possible to attain good intensity signals, even if the original signal is weak. This helped in using ^{13}C signals observable and therefore using FT-based instrument it is possible to use ^{13}C-NMR is structural analysis in organic compounds (details are in unit 3). The important features of ^{19}F, ^{31}P and ^{13}C based NMR methods are as follows:

a) ^{19}F-NMR: Fluorine (F) has atomic number 19 and spin quantum number I = 1/2 (same as that of H). The resonance frequency of fluorine in similar field is only slightly lesser than proton, that is why the ^{19}F-NMR is carried out with similar methods as that for ^{1}H-NMR. The chemical shift range in ^{19}F-NMR is about δ 0 to 300 ppm (compared to about δ 0-12 ppm). The signals due to ^{19}F are greatly affected by solvent effect, due to high electronegativity of fluorine. The splitting pattern in ^{19}F-NMR is same as that in case of ^{1}H-NMR.

b) ^{31}P-NMR: Phosphorus (P) has I=1/2, which is same as that for ^{1}H and ^{19}F. But the sensitivity of ^{31}P is lower than that of ^{1}H and ^{19}F. The chemical shift range for ^{31}P is much larger i.e. δ 0-300 ppm in comparison to ^{1}H and ^{19}F. The splitting pattern in ^{31}P-NMR is also similar as that in case of ^{1}H-NMR.

c) ^{13}C-NMR: The principle governing ^{13}C-NMR is same as that for proton, but the scale of observed chemical shift and coupling is greater in case of ^{13}C (δ 0-220 ppm). In ^{13}C-NMR study, the chemical shifts can be measured in ppm using TMS as reference, but it is the signal of carbon atoms of methyl groups of TMS is used as reference instead of proton signal as in the case of ^{1}H-NMR. The important features and concepts related to ^{13}C-NMR spectroscopy are explained in unit 3.

2.16 SUMMARY:

- The nuclear spin quantum number I depends on atomic number Z and mass number A of the nucleus.
- The various nuclear spin states arise due to spin quantum number and the transition between these states results in NMR signal.
- The concept of NMR signal is related to resonance between radiowaves and precessing nuclear magnetic moment vector.
- Spin-spin and spin-lattice relaxation processes are used to obtain excess population in ground state, which helps in attaining intense NMR signal.
- The selection rule $\Delta m = \pm 1$ is followed in NMR spectroscopy.
- TMS proton signal is used as reference in ^{1}H-NMR.
- Inductive effect, anisotropic effects, etc causes shielding or deshielding thereby affecting signal position of ^{1}H nuclei.

- In alkene ^1H signal is deshielded and downfield shifted, in alkyne it is shielded and upfield shifted.
- In aromatic compound, ^1H signal is highly deshielded by paramagnetic induced field of circulating pi-electrons and therefore downfield shifted.
- The splitting of signal, symmetrical nature of peaks and value of coupling constant depends on the type of spin system i.e A_2, AX, AB, AMX, etc.
- In order to simplify proton-NMR spectrum, spin-decoupling methods such as shift reagents, decoupling experiments and chemical exchange can be used.
- FT-NMR method is used to attain spectrum efficiently and with intense signals.
- The magnetically active isotopes of fluorine, carbon and phosphorus i.e. ^{19}F, ^{13}C and ^{31}P can also be studied in NMR.
- ^{19}F, ^{13}C and ^{31}P nuclei have $I = 1/2$ same as for ^1H. Due to high abundance ^{19}F and ^{31}P shows intense signals, but ^{13}C is less abundant and less sensitive for NMR studies.

2.17 SUGGESTED READINGS:

- Spectrometric Identification of Organic Compounds 7th edition by R. M. Silverstein, F. X. Webster and D. J. Kiemle, J. Wiley & Sons Publication.
- Elementary Organic Spectroscopy- Principles and Chemical Applications by Y. R. Sharma, S Chand Publication.
- Introduction of Spectroscopy 5th edition by D. L. Pavia, G. M. Lampman, G. S. Kriz and J. R. Vyvyan, Cengage Publication.
- Spectroscopy of Organic Compounds by P. S. Kalsi, New Age International Publication.
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2.18 TERMINAL QUESTIONS:

1. Define the following terms:

a) Nuclear spin state

b) Larmor frequency

c) Spin-spin relaxation

d) Spin system

2. What is the selection rule in NMR spectroscopy?

3. Why reference compound is required in NMR signal measurement? What are the different reference compounds used in NMR spectroscopy?

4. Discuss the factors affecting chemical shift in NMR spectroscopy?

5. What are the important features of NMR signal of proton in alkene and aromatic compound?

6. Explain the spin-spin coupling in AX, AB and AMX spin systems?

7. Explain chemical exchange method used to attain.

Unit 3: Carbon -13 NMR Spectroscopy

3.1 OBJECTIVE OF THE UNIT:

In this unit you will learn about the following aspects of ^{13}C-NMR:

- Common features of ^{13}C-NMR and ^{1}H-NMR methods.
- Contrasting features of ^{13}C-NMR and ^{1}H-NMR methods.
- Chemical shift and spin-spin coupling in ^{13}C-NMR.
- Problem related to observance of ^{13}C-NMR signal: low abundance and low sensitivity.
- Chemical and Magnetic equivalence.
- Number of signals and their respective multiplicity.
- Chemical shift range of ^{13}C in various groups.
- Factors such as electronegativity, anisotropy, etc which affects chemical shift.
- Chemical shift for carbon of aliphatic, alkene and alkyne compounds.
- Chemical shift for carbon of aromatic and heteroaromatic compounds.
- Chemical shift for carbonyl carbon.
- Characteristic features of proton coupled ^{13}C- spectra.
- Characteristic and advantages of proton decoupled ^{13}C- spectra.
- ^{13}C-^{1}H Spin-spin coupling and related splitting to characterise nature of alkyl group.
- Homonuclear and heteronuclear coupling constant J.
- Multiplicity of signal for homonuclear and heteronuclear spin-spin coupling.
- Dependence of coupling constant J on hybridisation of carbon.
- Merits and demerits of proton coupled ^{13}C spectra.
- Advantages of proton decoupled ^{13}C spectra over proton coupled ^{13}C spectra.

3.2 INTRODUCTION:

In unit 1 and 2 you learnt about ^{1}H-NMR spectroscopic method in great details. In this unit you will study nuclear magnetic resonance of another important nucleus i.e. ^{13}C. This isotope of carbon has nuclear spin quantum number $I = 1/2$, which is as that of ^{1}H. That is why ^{13}C has two spin states and in presence of external magnetic field these spin states have difference in energy, as in the case of ^{1}H. On absorption of radiowaves of specific energy, nuclear spin transitions takes place, resulting in signal for ^{13}C present in the sample.

The low abundance of ^{13}C isotope (1.1 %) restricts the possibility of occurrence of two or more ^{13}C atoms in a molecule. Thus the spectrum attained in ^{13}C-NMR is collectively due to whole sample and not due a single molecule. The important factor that hinders the quality of spectrum is related to small gyromagnetic constant, causing low sensitivity of ^{13}C nuclei. This affects the signal intensity and limits the use of ^{13}C-nuclei in NMR related studies.

The concept of chemical and magnetic equivalence can be extended to ^{13}C and carbon atoms with same electronic environment gives a signal. These carbons atoms are said to chemically equivalent carbons and in general chemically equivalent carbon atoms are also magnetically equivalent. The chemical shift range in ^{13}C-NMR is quite large (δ 0 – 220 ppm) as compared to that is ^{1}H-NMR (δ 0 – 12 ppm). This large range is very useful is structural elucidation of organic molecule, as the signals due to different groups are well separated and least overlap.

The signal in ^{13}C-NMR is affected by factors such as electronegativity of surrounding nuclei, change in electronegativity due to variation in hybridisation on carbon atom and anisotropic effects. The effect of these factors is in general same as that in case of ^{1}H-NMR (see section 2.7), but the magnitude of effect is varied thereby the variation in chemical shift is greater in case of ^{13}C-NMR as compared to ^{1}H-NMR. The effect of hydrogen bonding significantly affects the ^{1}H-NMR signal, while ^{13}C-signal is not much affected by hydrogen bonding. The chemical shift of carbon in alkane is affected by inductive effect and using these values and multiplicity of signal it is possible to predict the nature of substitution on carbon. In case of alkene, alkynes and aromatic compounds, the anisotropic effects and hybridisation of carbon that causes significant downfield shift. The substitution of polar groups on aromatic ring further shifts the value of signal due to variation in electron density on carbon atoms due to delocalisation. The ^{13}C-signals for carbon in heteroaromatic compounds show much variation in chemical shift due to interplay of resonance along with electronegativity of heteroatom. Similarly in carbonyl compounds also, the paramagnetic effect of circulating π-electrons of C=O group and presence of electronegative oxygen atom causes large downfield shift in the signals of carbonyl carbon. Also this downfield shift is affected by presence of groups such as OH, NH_2, etc. These groups may alter the electron density on the carbonyl carbon and causes shift in its signal position.

^{13}C-nucleus interacts with ^{1}H nuclei attached with it in organic molecule, resulting in characteristic splitting pattern. This splitting information is very useful in identification of CH_3, CH_2, CH types of groups in the molecule under study. The spectrum in which this multiplicity of signals is presented is known as proton-coupled spectrum. This splitting is very useful in the study of simple molecules, but for large molecules this splitting complicates the spectrum. Due to this reason the ^{13}C-NMR spectra are generally recorded with proton-decoupled conditions and such a spectrum is termed as proton-decoupled spectrum.

3.3 GENERAL CONSIDERATIONS:

In previous two units, ^{1}H-NMR has been discussed in great details. As already discussed nuclei with $I > 0$ can interact with external magnetic field and have non-degenerate spin states. In organic compounds carbon is predominant element but its most abundant isotope i.e. ^{12}C is not magnetically active as for ^{12}C nuclear spin quantum number is zero ($I = 0$). Thus ^{12}C isotope is of no use in NMR related studies. But the less abundant isotope of carbon i.e. carbon-13 (^{13}C) has $I = 1/2$ like proton and it can interact with external magnetic field to attain two spin orientations and with absorption of radio frequencies corresponding ^{13}C-NMR signal is attained. The principle of ^{13}C-NMR is similar to that of Proton-NMR (PMR), but ^{13}C-NMR is different from ^{1}H-NMR in the following respect:

a) The natural abundance of ^{13}C is only 1.1% as compared to that of ^{12}C. Thus due to low abundance the possibility of occurrence of ^{13}C in a molecule of organic sample is very low. The spectrum obtained in ^{13}C is therefore the collection of signals obtained from many molecules, as no single molecule can provides the complete ^{13}C spectra.

   Also it is very unlikely that a molecule contains more than one ^{13}C atom and the possibility of having two ^{13}C atoms bonding together is almost negligible. This limits the possibility of ^{13}C-^{13}C spin-spin coupling in ^{13}C-NMR and therefore ^{13}C-^{13}C signal splitting is a not case in ^{13}C-NMR.

b) The sensitivity of ^{13}C is much lesser (only 1.6 %) to that of ^{1}H. This is attributed to lower value of gyromagnetic ratio of ^{13}C (1.4043) as compared to that of ^{1}H (5.5854). The gyromagnetic ratio of ^{13}C is smaller as compared to that of hydrogen, thus the ^{13}C nuclei has lower resonance frequency as compared to proton. Due to which the excess spin population decreases and therefore there is a decrease in sensitivity of ^{13}C signal detection.

c) The range of chemical shifts in ^{13}C-NMR is much larger when compared with the case of ^{1}H. The chemical shift range in ^{13}C-NMR is about δ 0-220 ppm while for ^{1}H-NMR it is about 0-12 ppm.

d) In organic compounds, carbon is directly attached with hydrogen atom(s) and therefore it is possible that ^{13}C-^{1}H spin-spin coupling results in splitting of ^{13}C signal. In this way the different types of alkyl groups (such as CH_3, CH_2, etc) can be distinguished.

In ^{1}H-NMR spectroscopy provides the following information about the structure of organic compound:

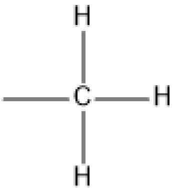
- The number of signal in ^{1}H-NMR spectrum gives the number of chemically equivalent protons in the compound.
- The chemical shift (δ) values provide information about the chemical or electronic environment of these protons. From this information it possible to know the nature of groups present in the compound.
- The relative area under the peak is related to the number of proton which are chemically equivalent and giving that peak. The quantitative information is useful in structure elucidation of the compound.
- The splitting pattern in peaks gives information about the neighbourhood of the proton(s) responsible for that peak. This information is very useful in structure elucidation studies.

In comparison to ^{1}H-NMR spectroscopy, the ^{13}C-NMR or CMR spectra provides following information about a compound:

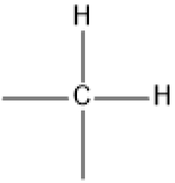
- The number of signal in ^{13}C-NMR gives information about chemically equivalent carbons in the compound.
- The chemical shifts (δ) in ^{13}C-NMR provide information about the electronic environment of different carbon atoms in the compound. The range of chemical shift in case of ^{13}C-NMR is wide i.e. δ 0-220 ppm relative to TMS, while for ^{1}H-NMR this

range is δ 0-14 ppm. Thus in ^{13}C-NMR the separation of peaks is greater and therefore there is fewer overlap of peaks in contrast to ^{1}H-NMR.

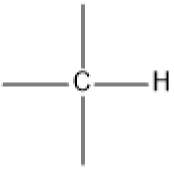
- Due to low isotopic abundance (1.1 %) of ^{13}C, the ^{13}C-^{13}C coupling is negligible and not observed.
- But ^{13}C nuclei can interacts with attached proton, ^{13}C-H spin-spin coupling takes place and signal of ^{13}C can splits depending on number of protons attached with ^{13}C. The rule of spin-spin interaction is same as in case of ^{1}H-NMR i.e. the signal splits according to 'n + 1' rule, where n is number of H atoms bonded to carbon atom. In this way, CH_3 (n = 3) gives quartet, CH_2 (n = 2) gives triplet, CH (n = 1) gives doublet and carbon not attached with any hydrogen (n = 0) gives singlet. Thus multiplicity of carbon signal in ^{13}C-NMR provides useful information about nature of substitution on different carbon atoms in a compound.



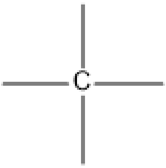
Number of H nuclei = 3
Multiplicity of signal n + 1 = 3 + 1 = 4
Thus signal of C in CH_3 is **quartet**



Number of H nuclei = 2
Multiplicity of signal n + 1 = 2 + 1 = 3
Thus signal of C in CH_2 is **triplet**



Number of H nuclei = 1
Multiplicity of signal n + 1 = 1 + 1 = 2
Thus signal of C in CH is **doublet**



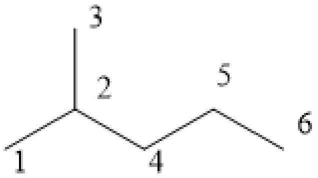
Number of H nuclei = 0
Multiplicity of signal n + 1 = 0 + 1 = 1
Thus signal in tetrasubstituted C is **singlet**

- In ^{13}C-NMR, the areas under the peaks are not necessarily proportional to the number of carbon atoms giving that signal.

- In comparison to proton, the gyromagnetic ratio for ^{13}C is about one-fourth of the proton, thus the resonance frequency is also about one-fourth of that of proton. Thus a spectrometer operating with 14092 Gauss magnet required 15.1 MHz transmitter for ^{13}C compared to 60 MHz for protons. Also a spectrometer with a 23486 Gauss magnet requires 100 MHz transmitter for protons, but only 25.2 MHz transmitter for ^{13}C.

3.4 NUMBER OF SIGNALS IN ^{13}C-NMR :

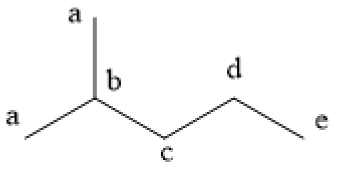
You already learnt about the chemical and magnetic equivalence in section 1.6. It was described for ^{1}H nuclei, but this concept can be extended to ^{13}C nuclei present in the sample. ^{13}C nuclei having identical chemical (electronic) environment within molecule are chemically equivalent. In a given applied magnetic field, ^{13}C nuclei with same precessional frequency of their nuclear magnetic moment vector are said to be magnetically equivalent, such nuclei absorbs same radiowave frequency and thus gives same signal. Thus it can be concluded that ^{13}C nuclei that are chemically equivalent are also magnetically equivalent. In this way we can determine the number of signals in ^{13}C-NMR spectrum of a molecule.



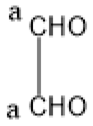
Consider the example of 2-methylpentane, the different carbon atoms are presented by number, in this compound there are five signals in ^{13}C-NMR spectrum. These signals are:

- Signal a: It is due to two ^{13}C-nuclei at ‘1’ and ‘3’ positions. These ^{13}C-nuclei have same chemical environment as these are bonded with similar groups. So these ^{13}C-nuclei give a signal or we can say that this signal is due to two carbon atoms. Now due to spin-spin coupling with bonded proton, this ^{13}C signal is obtain as quartet for these two carbon atoms.
- Signal b: Due to one ^{13}C nucleus at ‘2’ position, which is doublet as only one proton is present on this carbon atom.

- Signal c: It is also due to one ^{13}C nucleus at ‘4’ position and it is doublet as only one proton is present on this carbon atom.
- Signal d: This signal is due to one ^{13}C nucleus at ‘5’ position, it is also doublet as only one proton is present on this carbon atom.
- Signal e: Similarly due to one ^{13}C nucleus at ‘6’ position, which is doublet as only one proton is present on this carbon atom.

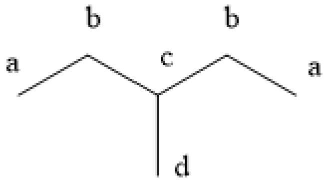


In this way the signals and their respective multiplicity can be determined. Some examples and number of signals in their ^{13}C-NMR are illustrated below:



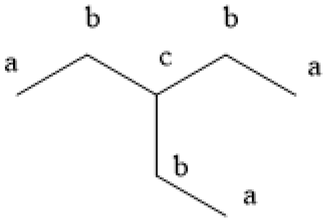
One Signal

a: 2C, doublet



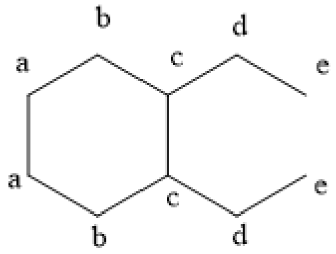
Four Signals

a: 2C, quartet
b: 2C, triplet
c: 1C, doublet
d: 1C, quartet



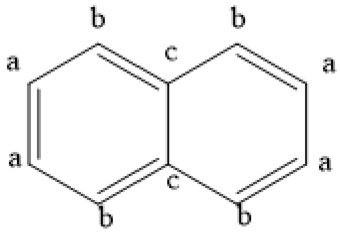
Three Signals

a: 3C, quartet
b: 3C, triplet
c: 1C, doublet



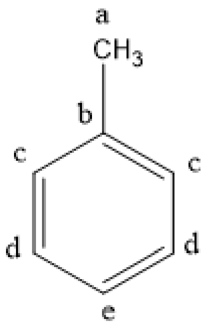
Five Signals

a: 2C, triplet
b: 2C, triplet
c: 2C, doublet
d: 2C, triplet
e: 2C, quartet



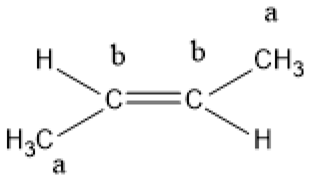
Three Signals

a: 4C, doublet
b: 4C, doublet
c: 2C, singlet



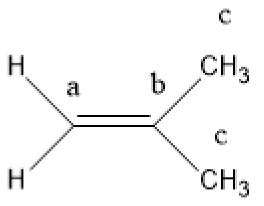
Five Signals

a: 3C, quartet
b: 1C, singlet
c: 2C, doublet
d: 2C, doublet
e: 1C, doublet



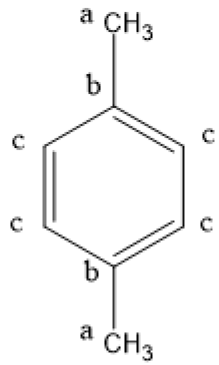
Two Signals

a: 2C, quartet
b: 2C, doublet



Three Signals

Signal a: 1C, triplet
Signal b: 1C, singlet
Signal c: 2C, quartet



Three Signals

Signal a: 2C, quartet
Signal b: 2C, singlet
Signal c: 4C, doublet

3.5 CHEMICAL SHIFT IN ^{13}C-NMR:

In order to determine the chemical shift in ^{13}C-NMR, Tetramethylsilane (TMS) is used as reference compound as in ^{1}H-NMR. But in ^{13}C-NMR it is the signal of methyl group of TMS is used as reference. That is why the signal of TMS in ^{13}C-NMR is quartet, due to C-H coupling in methyl group. The chemical shift in case of ^{13}C-NMR appears in the range of δ 0-220 ppm, which is much larger as compared to range in ^{1}H-NMR (δ 0-12 ppm). This broad range of chemical shift in case of ^{13}C-NMR is due of greater effect of induced magnetic field due to circulating electrons on carbon atom. Due to this large range of chemical shift every non-equivalent carbon in organic compound give rise to a peak with a different chemical shift and almost no overlap of signals occur. This is in contrast with the chemical shift range in ^{1}H-NMR, where due to small range the signals overlap. The chemical shift of carbon atom with characteristic electronic environment is presented in table 1.

Table 1: Chemical shift values for different types of carbon.

Types of Carbon	Chemical Shift Range (in ppm)
CH_3	8 – 30

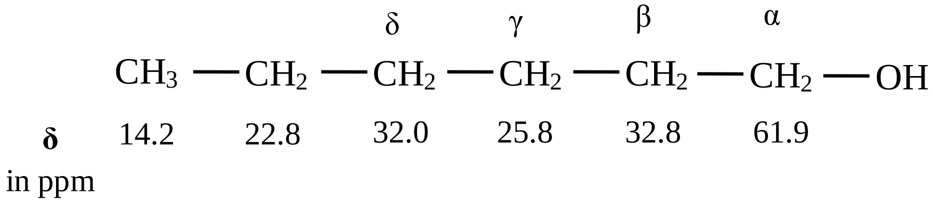
CH_2	15 – 55
CH	20 – 60
C-I	0 – 40
C-Br	25 – 635
C-N	30 – 65
C-Cl	35 – 80
C-O	40 – 80
C≡C	65 – 90
C=C	100 – 150
C≡N	110 - 140
Benzene	110 – 175
Carboxylic Acid and Ester	155 - 185
Amides	155 – 185
Aldehyde and Ketones	185 – 220

The carbon bonded with hydrogen in aliphatic compounds has chemical shift values upto δ 60 ppm. In such compounds the chemical shift is largely affected by +I (inductive effect) of surrounding alkyl groups. But when carbon is bonded with an electronegative element such as oxygen, nitrogen, etc then the chemical shift lie upto δ 90 ppm. In such compounds the –I effect causes deshielding effect and thereby shifting signals to such downfield values. Carbon shows large downfield shift (upto δ 150 ppm) when it is bonded with heteroatom by as π-bond. In such compounds the anisotropic effects along with –I effect of heteroatom causes such a large downfield shift. The carbonyl carbon is strongly affected by anisotropic effects and –I effect of oxygen and therefore the signals for carbonyl carbon have much large chemical shift upto δ 220 ppm. The chemical shift region in ^{13}C-NMR is divided into four sections:

- Saturated carbon atom with chemical shift δ 8-60 ppm.
- C-X (where X is electronegative atom) have chemical shift in the range of δ 40-80 ppm.
- Alkene and aromatic ring have chemical shift in the range of δ 110-175 ppm.
- Carbonyl carbon have chemical shift in the range of δ 155-220 ppm.

3.6 FACTORS AFFECTING CHEMICAL SHIFT IN ^{13}C NMR:

1. The ^{13}C-NMR is affected by similar factors as in the hydrogen NMR. The important factors are: electronegativity, hybridisation and anisotropy affects ^{13}C chemical shift in the same way as in hydrogen chemical shift, but ^{13}C chemical shifts are about 20 times larger. Electronegativity produces the same deshielding effect in ^{13}C as in PMR, and the electronegative atom produce a large downfield shift. It is because the electronegative atom is directly attached to ^{13}C atom and the effect occurs through a single bond, C-X. In case of PMR the shift occurs through two bonds (X-C-H). In PMR, the effect of electronegative on chemical shift decreases with distance, but it is always in same direction (deshielding and downfield). In ^{13}C NMR an electronegative atom causes a downfield shift in α & β carbon, but there is a small upfield shift for the γ carbon. For example in hexanol, α & β carbons are upfield shifted and γ carbon is upfield shifted as shown in figure 3.1.



$$\underset{14.2}{CH_3}-\underset{22.8}{CH_2}-\underset{32.0}{\overset{\delta}{CH_2}}-\underset{25.8}{\overset{\gamma}{CH_2}}-\underset{32.8}{\overset{\beta}{CH_2}}-\underset{61.9}{\overset{\alpha}{CH_2}}-OH$$

Figure 3.1: Chemical shift for signals in hexanol

2. Similar to ^{1}H chemical shift, changes in hybridisation also produce larger shifts for ^{13}C that is directly involved. In ^{13}C NMR the carbon of carbonyl group has largest chemical shift due to sp^{2} hybridisation and presence of electronegative oxygen directly bounded with carbonyl carbon. figure 3.2.

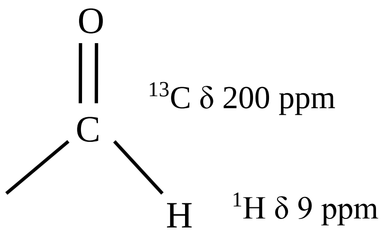


Figure 3.2: Chemical shift for aldehyde group

3. Anisotropy is responsible for large chemical shifts of the carbon in aromatic shifts of the carbons in aromatic ring and alkene. Thus the range of chemical shift is larger for carbon atom than for the hydrogen atom. The factors affecting carbon shift operate either through one bond or directly on carbon, are greater than those for hydrogen which operates through more than one bond. Thus the ^{13}C chemical shift range becomes larger (0-220 ppm) as compared to hydrogen chemical shift (0-12 ppm). figure 3.3.

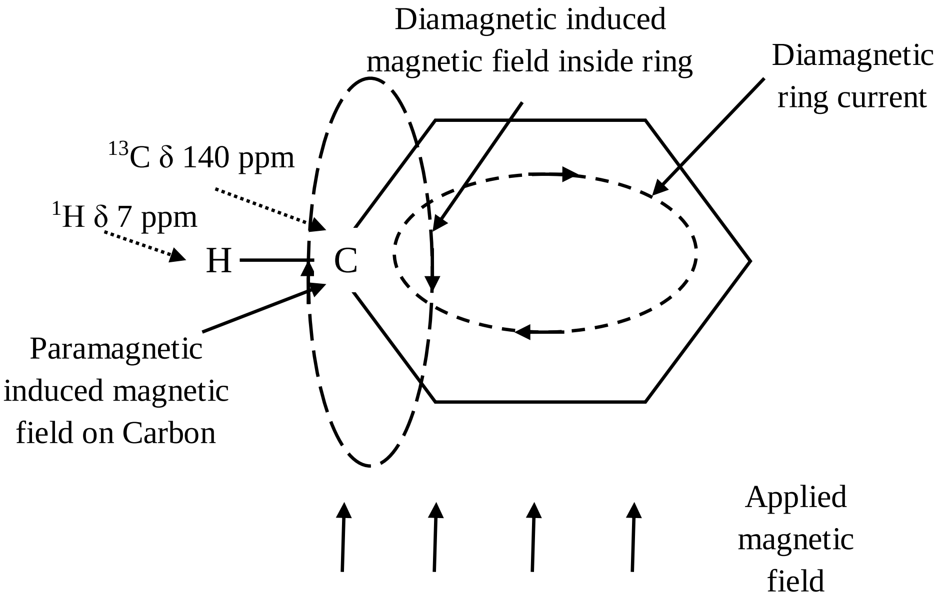


Figure 3.3: Anisotropic effect on carbon of benzene ring

Thus paramagnetic effect arises due to circulation of electrons in aromatic system or in alkenes (in presence of applied magnetic field) has stronger effect on carbon atoms as compared to hydrogen atom.

4. The hydrogen bonding causes a definite chemical shift in case of proton-NMR, due to polar X-H bond in alcohols, amines, etc. But the ^{13}C signal is in general remains unaffected by presence of hydrogen bonding.

3.7 CHEMICAL SHIFT FOR ALIPHATIC, ALKENE, ALKYNE, AROMATIC, HETERO AROMATIC AND CARBONYL CARBON :

a) Aliphatic ^{13}C chemical shift: The chemical shift due to aliphatic carbon i.e. CH_3, CH_2, CH and tetrasubstituted carbon lies in the range of δ 0 – 60 ppm. The chemical shift of carbon of CH_4 is exceptionally δ -2.6 ppm, i.e. carbon of CH_4 resonates δ 2.6 ppm upfield from the reference compound (TMS). In ethane the chemical shift of two equivalent CH_3 carbons is δ 5.7 ppm, thus on substitution of methyl group in place of hydrogen of methane results in downfield shift. Similar effect is also observed when more alkyl groups are substituted in place of hydrogen. For example when one more hydrogen is replaced by methyl group in ethane, then the central carbon has chemical shift value of δ 16 ppm. This downfield shift of a carbon signal due to increase in substitution on it is known as 'α-effect'. The magnitude of this shift adds ~9.1 ppm to the chemical shift of attached carbon. This is a significant difference in ^{1}H and ^{13}C NMR spectra, as simple alkyl substitution has much greater effect on ^{13}C shifts than it does on proton shifts. (figure 3.4)

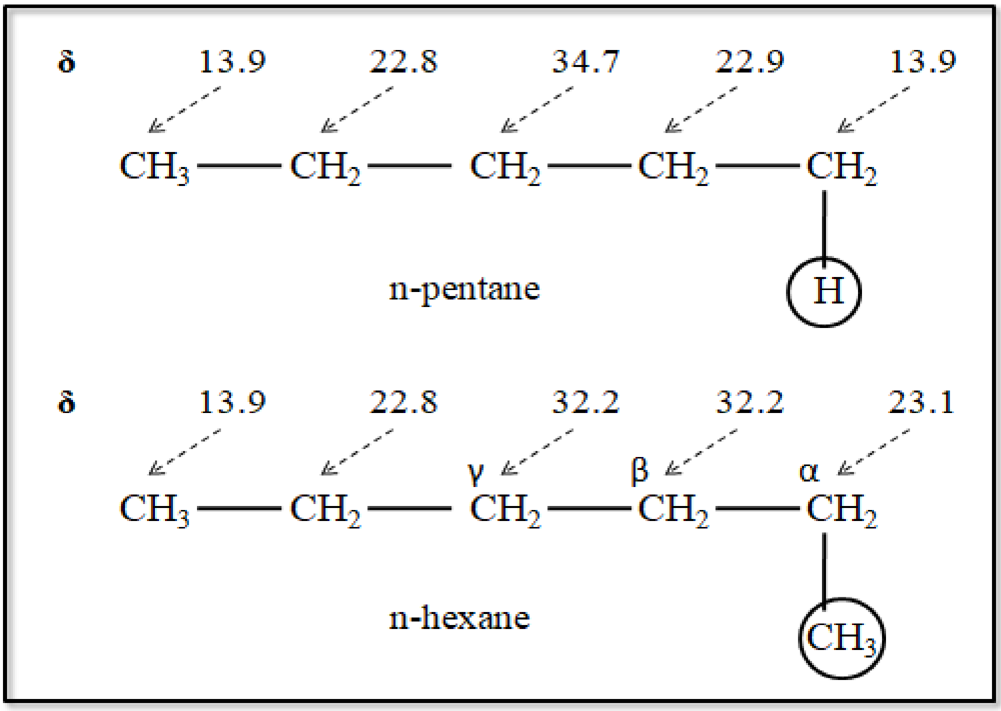


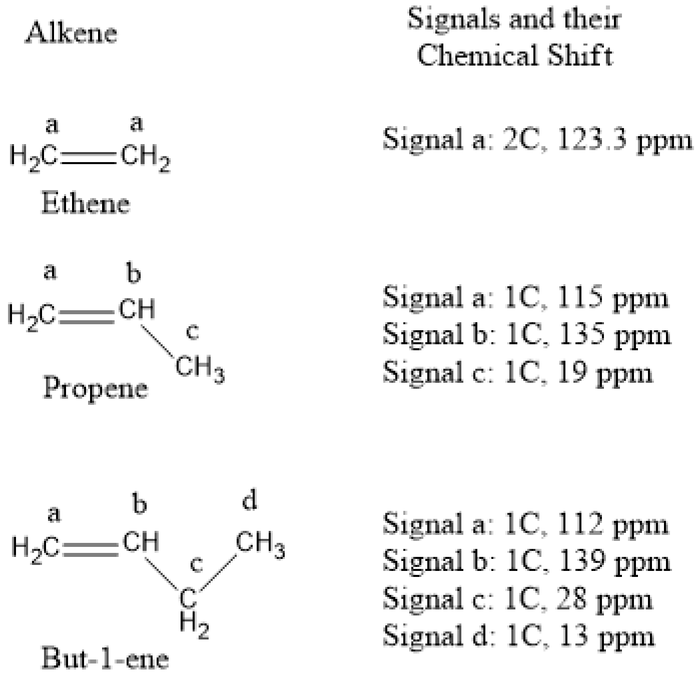
Figure 3.4: Shifts in signals from to n-pentane to n-hexane

Also there occurs a 'β-effect' in aliphatic system in which alkyl group substitution at β-position results in shift in δ by 9.5 ppm. In same way there is a 'γ-shift', which leads to an upfield shift with a typical value of δ -2.5 ppm. These effects can be explained by consider the chemical shift values in pentane and hexane as shown in figure 4. Consider replacement of H in pentane by CH_3 group so that formation of hexane takes place. When carbon to carbon chemical shift are compared, it can be easily concluded that methyl group at α-carbon causes downfield shift of about 9.0 ppm at the α-carbon in hexane (δ 23.1) compared to the carbon bonded with the H in pentane (δ 13.9). This shift in chemical shift at α-carbon is known as α-shift and it is downfield shift with magnitude of +9.1 ppm. Similarly for β-carbon in hexane, the methyl group results in downfield shift from δ 22.8 to δ 32.2, with difference of about δ 9.4 ppm. But at γ-carbon, there occurs a upfield shift of about δ 2.5 ppm as signal of γ-carbon shift from δ 34.7 to δ 32.5 on moving from pentane to hexane.

Thus it can be concluded that a substituent in α and β-carbon position generally deshields the carbon but at γ-carbon position shielding takes place.

b) **Alkene and alkyne ^{13}C chemical shift:** The hybridisation of carbon atom also affects the chemical shift in more prominent way. The sp^3 hybridised carbon absorbs at high field (δ 0 – 60 ppm) but the sp^2 hybridised carbon in alkenes give signals at low field (δ 110 – 200 ppm), similarly the sp hybridised carbon in alkynes give signals at low field (δ 65 – 90 ppm). The chemical shift range of alkynes is in between to that of alkanes and alkenes. This is an important advantage of ^{13}C-NMR over ^{1}H-NMR, as in ^{1}H-NMR the signal of sp^3, sp^2 and sp hybridized carbons lie in a close range of δ 0 – 6 ppm, and therefore their identification is quite difficult in ^{1}H -NMR. But in ^{13}C-NMR these signals are well separated and therefore can be easily distinguished.

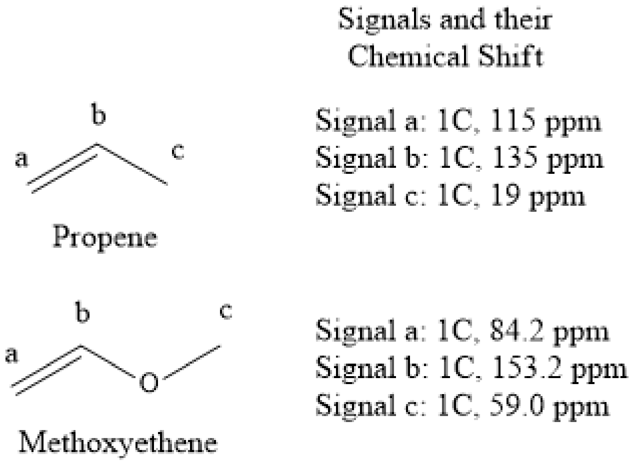
The sp^2-hybridised carbon atoms in alkenes and aromatic compounds appear in the same general region of ^{13}C-NMR, but in ^{1}H-NMR the signal of sp^2-hybridised carbon of alkene and that of aromatic compound appears in distinct regions. This is an important advantage of ^{1}H-NMR over ^{13}C-NMR. The signals of ethene, propene and but-1-ene are presented in (figure 3.5). The signals of sp^2-hybridised carbon atoms are above 100 ppm, for example it is 123.3 ppm for both carbons of ethene, 135 and 115 ppm for carbon C_a and C_b in propene and similarly 112 and 139 ppm for carbon C_a and C_b in but-1-ene. In general the inner sp^2-hybridised carbon atom is downfield shifted as compared to terminal sp^2-hybridised carbon atom, as in the case of propylene. The sp^3-hybridised carbons are upfield shifted with large chemical shift difference as compared with sp^2-hybridised carbon signals. The effect of double bond on sp^3-hybridised carbon atoms is weak, for example methyl group in propene absorbs at δ 19 ppm and in propane it is δ 16 ppm.



Alkene	Signals and their Chemical Shift
$\overset{a}{H_2C}=\overset{a}{CH_2}$ Ethene	Signal a: 2C, 123.3 ppm
$\overset{a}{H_2C}=\overset{b}{CH}-\overset{c}{CH_3}$ Propene	Signal a: 1C, 115 ppm Signal b: 1C, 135 ppm Signal c: 1C, 19 ppm
$\overset{a}{H_2C}=\overset{b}{CH}-\overset{c}{CH_2}-\overset{d}{CH_3}$ But-1-ene	Signal a: 1C, 112 ppm Signal b: 1C, 139 ppm Signal c: 1C, 28 ppm Signal d: 1C, 13 ppm

Figure 3.5: Chemical Shifts in Ethene, Propene and But-1-ene

The ^{13}C-chemical shifts of C=C carbon atoms are very useful to identify polar substituents. It is because there is long range effect of these substituents due to resonance effect in addition to inductive effect. The delocalization of electrons results in shifts in electron density, which affects shielding and deshielding in such molecules. Consider the example of vinyl ether and propene, in which the signals of C=C shows drastic chemical shift in vinyl ether when compared with that of propene. The sp^2-hybridised carbon atoms in propene shows chemical shift of δ 115 and 135 ppm, but in vinyl ether (methoxyethene) same carbon atoms absorbs at δ 84.2 and 153.2 ppm (figure 3.6).



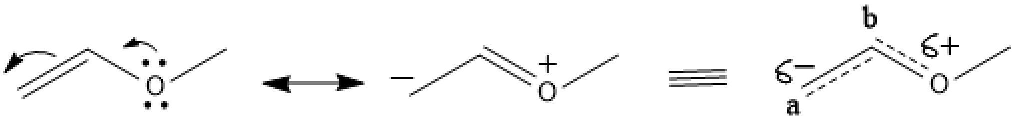
Signals and their Chemical Shift

Signal a: 1C, 115 ppm
Signal b: 1C, 135 ppm
Signal c: 1C, 19 ppm

Signal a: 1C, 84.2 ppm
Signal b: 1C, 153.2 ppm
Signal c: 1C, 59.0 ppm

Figure 3.6: Chemical shifts in Propene and Methoxyethene

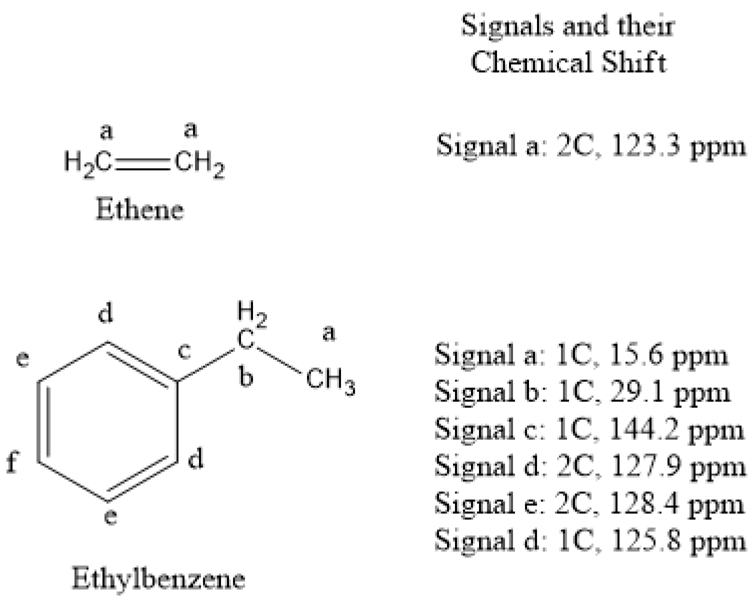
This variation in chemical shift can be explained on the basis of delocalisation of lone pair of oxygen atom in methoxyethene, as shown in figure 3.7. The delocalisation results in increase in electron density on C_a, causing significant upfield shift to δ 84.2 ppm (in comparison to δ 115 ppm in propene). The C_b carbon atom is highly downfield shifted, as it is bonded with electronegative oxygen atom (which acquires partial positive charge due to resonance).



Resonating forms of methoxyethene

Figure 3.7: Delocalisation in Methoxyethene

c) **^{13}C chemical shift in aromatic and heteroaromatic compounds:** The carbons atoms of aromatic ring absorbs in the same region as for sp^2-hybridised carbon atom in alkene. Consider the examples of ethene and ethylbenzene (figure 8), the carbon atoms of benzene ring gives signals with chemical shifts close to that of carbon atoms of ethene. The ethyl substitution on benzene ring results in significant downfield shift (signal c in figure 3.8), which is due to the +I effect of ethyl group.



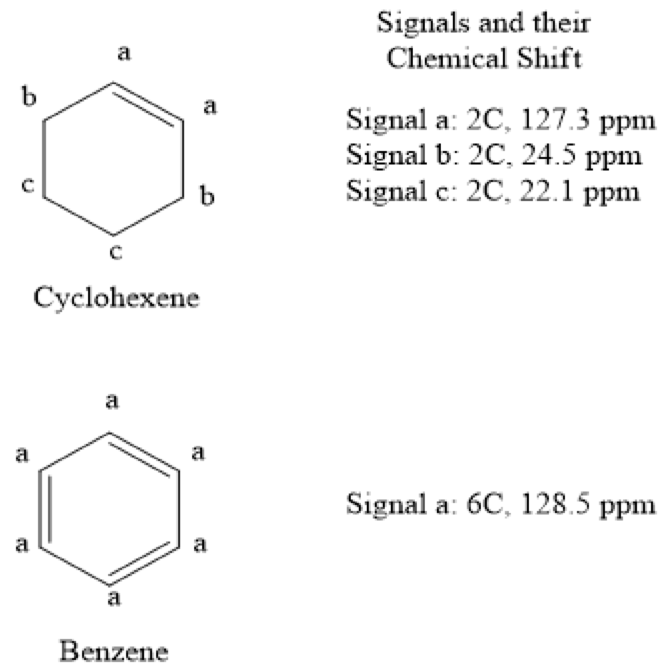
Signals and their Chemical Shift

Signal a: 2C, 123.3 ppm

Signal a: 1C, 15.6 ppm
Signal b: 1C, 29.1 ppm
Signal c: 1C, 144.2 ppm
Signal d: 2C, 127.9 ppm
Signal e: 2C, 128.4 ppm
Signal d: 1C, 125.8 ppm

Figure 3.8: Chemical shifts in Ethene and Ethylbenzene

Consider another example of chemical shift values in cyclohexene and benzene. The benzene carbon atoms absorbs at δ 128.5 ppm, which is close to chemical shift values for sp^2-hybridised carbon atom of cyclohexene δ 127.3 ppm (figure 3.9).



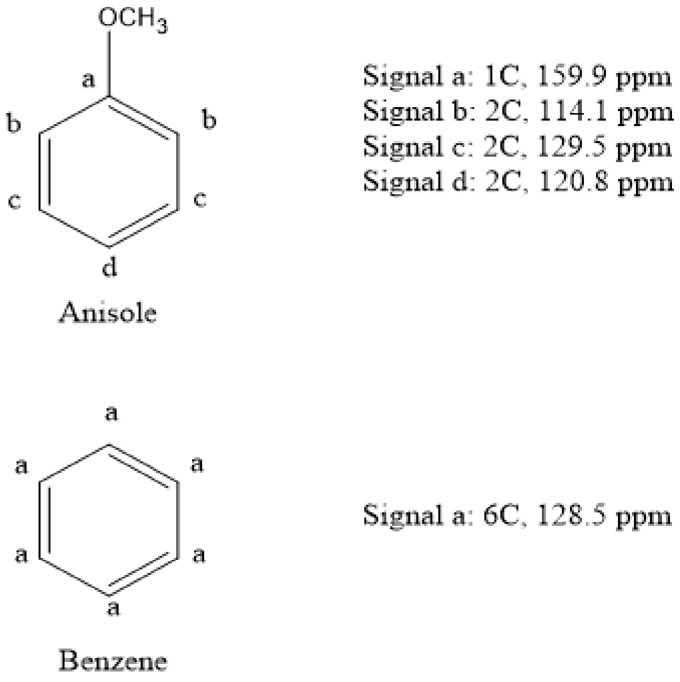
Signals and their Chemical Shift

Signal a: 2C, 127.3 ppm
Signal b: 2C, 24.5 ppm
Signal c: 2C, 22.1 ppm

Signal a: 6C, 128.5 ppm

Figure 3.9: Chemical shifts in Cyclohexene and Benzene

The electron density on benzene ring is affected by presence of a substituent and the ortho, meta and para positions are unequally affected by this variation in electron density. For example consider the case of anisole, in which the -OCH_3 group is substituted on benzene ring and due to delocalisation of lone pair on oxygen to benzene ring affects electron density most particularly on ortho and para positions. It is because the -OCH_3 group is electron releasing group and it increases electron density at ortho and para positions by resonance effect. Thus shielding takes place and the ^{13}C-signal is upfield shifted. In anisole the ortho-carbon absorbs at δ 114.1 ppm and para-carbon gives signal at δ 120.8 ppm, showing marked shift in comparison to the carbon signal in benzene i.e. δ 128.5 ppm (figure 3.10). The carbon at meta position absorbs at 129.2 ppm, which is close to the signal of carbon of benzene (δ 128.5 ppm). This confirms that the meta position remains unaffected by resonance delocalization.



Signal a: 1C, 159.9 ppm
Signal b: 2C, 114.1 ppm
Signal c: 2C, 129.5 ppm
Signal d: 2C, 120.8 ppm

Signal a: 6C, 128.5 ppm

Figure 3.10: ^{13}C-NMR signals in Anisole and Benzene

-The presence of heteroatom in aromatic system affects in same way as if a polar group is substituted to benzene ring. Consider the example of pyridine, in which the nitrogen atom is present in ring and due to its electronegative nature it decreases the electron density on carbon atoms of ring. This is reflected as increased chemical shift for carbon atoms of pyridine ring in comparison to that in benzene ring.

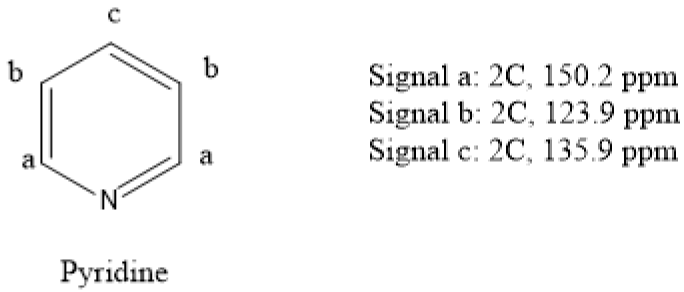


Figure 3.11: ^{13}C-NMR signals in Pyridine

The signal ‘a’ is for carbon atoms that are directly bonded with nitrogen atom (figure 3.11). This signal is highly downfield shifted due to greater –I effect due to electronegative nitrogen atom, observed at δ 150.2 ppm. Also due to delocalisation of π-electrons the carbon bonded with nitrogen acquire partial positive charge in resonance hybrid, resulting in further enhancing downfield shift in signal for these carbon atoms. The –I effect decreases with distance therefore the signal b is only slightly affected by it. Also the resonance effect does not cause any change in electron density for carbons showing b signals. But signal c is comparatively much more downfield shifted, due to resonance effect the carbon atom showing this signal acquire partial positive charge so it is deshielded and downfield shifted. figure 3.12.

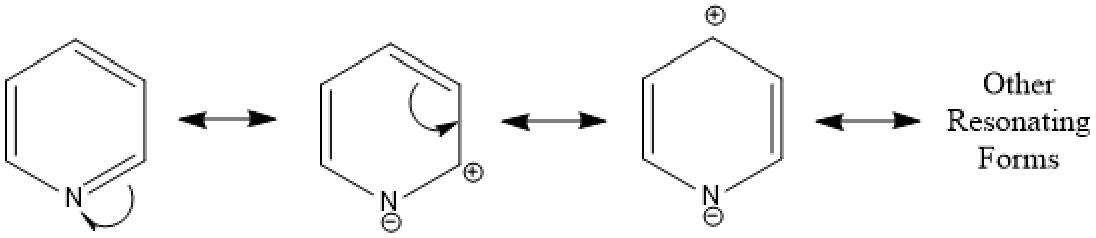


Figure 3.12: Resonance in Pyridine

In same way, consider the example of pyrrole in which there is strong delocalisation of lone pair of nitrogen atom (to attain aromatic sextet and consequent stability). This delocalisation in general increases electron density in the ring and therefore the carbon atoms of pyrrole ring are shielded and upfield shifted in comparison to the ^{13}Csignal in benzene. (figure 3.13)

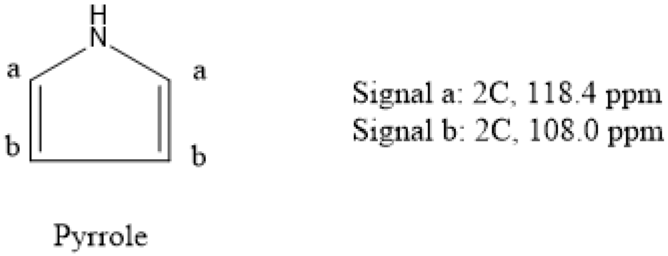


Figure 3.13: ^{13}C-NMR signals in Pyrrole

In same way the signals in other heteroaromatic compounds are shifted (upfield of downfield), depending on the variation electron density due to inductive and resonance effects.

e) **^{13}C chemical shift for carbonyl carbon:** The carbonyl carbon is influenced by electronegativity of oxygen atom with which it is bonded and the paramagnetic induced magnetic field due to circulation of electrons (as you studied in case of alkenes in section). Both these factor causes large downfield shift in the signal of carbonyl carbon. The typical ^{13}C chemical shifts for carbonyl carbon vary from δ 150 to 220 ppm. These chemical shifts value depends on the nature of electron donating or electron releasing nature of the groups bonded with carbonyl carbon. The chemical shift ranges for some compounds containing carbonyl carbon are presented in table 2.

Table 2: ^{13}C chemical shift range in compounds containing carbonyl carbon.

Compound	Chemical shift
Ketone	205-220 ppm
Aldehyde	200-210 ppm
Carboxylic acid	175-185 ppm
Amide	170-180 ppm
Esters	165-175 ppm

3.8 SPIN–SPIN COUPLING AND COUPLING CONSTANT J:

The spin-spin coupling and consequent splitting of signal is already discussed in previous unit (see section 1.11). The spin-spin coupling is measured in terms of coupling constant J and measured by the spacing in Hz between the individual peaks of the multiplet. It is defined as the separation between peaks of the multiplet. Its value is independent of the field strength or operating frequency of NMR spectrometer. The magnitude of J is dependent on the bond angle between the interacting nuclei and can therefore vary with temperature or solvent and the conformation of the compound. Coupling between two nuclei of same type is called homonuclear coupling, while coupling between different types of nuclei is called heteronuclear coupling. The coupling between hydrogen and hydrogen is an example of homonuclear coupling while the coupling between carbon and hydrogen or carbon and fluorine are example of heteronuclear coupling, both gave multiplet according to 'n+1' rule.

The spin-spin coupling can be classified on the basis of the number of bonds present between interacting nuclei and represented as $^{n}J(A\text{-}B)$, where A and B are interacting nuclei and n is the number of bonds between them. Thus spin-spin coupling can be ^{1}J, ^{2}J, ^{3}J, etc as presented in figure 3.14.

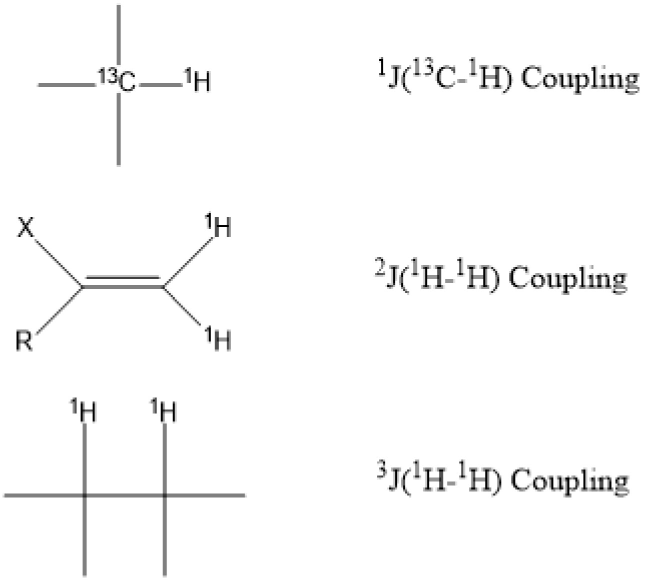


Figure 3.14: Types of Coupling Systems

The magnitude of coupling constant depends to a large extent on the number of bonds between the two atoms that interact. One bond coupling are larger than the two bond coupling, which in term are larger than three bond coupling and so on. Thus the type of coupling can be presented by the symbol having information about the types of atom involved and number of bonds between the interacting atoms. The coupling constant values for $^{1}J(^{13}C\text{-}^{1}H)$ is of the order of 150 Hz, while for $^{3}J(^{1}H\text{-}^{1}H)$ it is much lesser i.e. 8 Hz. The coupling constants are positive for some cases while negative for some other cases. With a negative J, the positions of individual line in a multiplet are reversed that is the downfield and upfield peaks exchanged.

In ^{13}C-NMR, one bond coupling i.e. $^{1}J(^{13}C\text{-}X)$ is most prominent where X can be ^{1}H, ^{19}F, ^{31}P, etc. 1-bond coupling (^{1}J): this type of coupling is exhibited by two different atoms as shown in table 3.

Table 3: ^{13}C-X coupling and corresponding J values

^{13}C-X Coupling	J values
$^{13}C\text{-}^{1}H$	110-270 Hz
$^{13}C\text{-}^{19}F$	-165 to -370 Hz
$^{13}C\text{-}^{31}P$	48-56 Hz
$^{13}P\text{-}^{1}H$	190-700Hz
$^{13}C\text{-}^{2}D$	20-30Hz

Some important points about 1-bond coupling

- The coupling constant depends on the hybridisation in case of $^{1}J(^{13}C\text{-}^{1}H)$. The coupling constant depends on the s character of hybridised orbital of carbon.

  $$^{1}J(^{13}C\text{-}^{1}H) = \left(\frac{1}{n+1}\right)500\text{Hz}$$

  where n is number of p-orbitals undergoing hybridisation, for example for hybridisation of type sp^3 then n is 3.

- The multiplicity depends upon the spin states of the interacting nuclei. The multiplicity of a signal can be calculated as:

  $$\text{Multiplicity} = 2nI + 1$$

  where n is number of neighbouring nuclei of nuclear spin quantum number I. For example if ^{13}C nucleus is bonded with one ^{1}H nucleus, as for ^{1}H nuclear spin quantum number I is 1/2, so

  $$\text{Multiplicity} = 2 \times 1 \times \frac{1}{2} + 1 = 2$$

As multiplicity is obtained as 2, so the signal of ^{13}C splits into doublet. If ^{13}C nucleus is bonded with 'n' ^{1}H nucleus, then

$$\text{Multiplicity} = 2 \times n \times \frac{1}{2} + 1 = n + 1$$

The above expression was frequently used in previous topics to calculate the multiplicity of a signal for ^{1}H and ^{13}C nucleus.

Now consider if ^{13}C nucleus is bonded with one ^{2}D (deuterium) nucleus, for ^{2}D nuclear spin quantum number I is 1, so

$$\text{Multiplicity} = 2 \times 1 \times 1 + 1 = 3$$

Thus multiplicity is 3, so the due to coupling between ^{13}C-^{2}D results in splitting of ^{13}C signal into triplet.

3.9 PROTON COUPLED CARBON–13 SPECTRA :

The probability of finding two ^{13}C atoms in the same molecule is low. The probability of having two ^{13}C atoms adjacent to each other in same molecule is even much lower. Thus homonuclear (C-C) spin-spin splitting are rarely observed.

The spin of protons directly attached to ^{13}C atom interacts with spin of carbon and cause carbon signal to split according to 'n+1' rule (see section 3.3). Thus CH_3 (n = 3) gives quartet, CH_2 (n = 2) gives triplet, CH (n = 1) gives doublet and carbon not attached with any hydrogen (n = 0) gives singlet. In this way multiplicity of carbon signal in ^{13}C-NMR provides useful information about proton substitution on different carbon atoms in a compound.

This is heteronuclear (C-H) coupling involving two different atoms, and it is a case of one bond coupling. The hydrogen atoms are directly attached to C^{13} (one bond coupling), the coupling constants for this interaction are quite large, with J values about 100 – 250 Hz. In case of PMR in general J values are about 1 to 20 Hz. The spectra that show spin – spin splitting or coupling between C^{13} and H^{1} directly attached to it are called proton coupled spectra.

The proton coupled ^{13}C spectra of a compound presents the multiplets for each and every signal. The knowledge of multiplicity of a signal is useful in predicting proton substitution on a carbon. But in general the multiplets complicates the NMR spectrum and it is difficult to attain structural information from such spectra. This problem can be solved by using decoupling methods by which each multiplets shrinks to singlets, thus simplifying the NMR spectrum.

3.10 PROTON DECOUPLED C^{13} SPECTRA:

In the proton decoupled spectra the coupling interaction between carbon and hydrogen is eliminated therefore only singlets are observed in a decoupled C^{13} NMR spectrum. The technique is used to simplify the spectrum and to avoid overlapping multiplets. But it has disadvantage that the information related to hydrogen is lost.

Proton decoupling (section 1.15) is attained by simultaneously irradiating all the protons in the molecule with the broad spectrum of frequencies in the proper range. Most modern NMR spectrometers provide a second tunable radio frequency generator, known as decoupler for this purpose. The irradiation causes the protons to be saturated and they undergo upward and downward transitions among all their possible spin states. These rapid transitions decouple any spin – spin interaction between the hydrogen and C^{13} nuclei being observed. Thus all spin interactions are average to zero by the rapid change. The carbon nucleus experience only one average spin state for the attached hydrogen rather than one or two spin states.

3.11 SUMMARY:

- The nuclear spin quantum number I is zero for ^{12}C, but for ^{13}C it is 1/2. Thus ^{13}C gives NMR signal same as that of ^{1}H nuclei.
- Due to low abundance of ^{13}C, the ^{13}C-NMR spectrum obtained is due to whole sample and not due to individual molecule.
- Low abundance also restricts the possibility of ^{13}C-^{13}C spin coupling.
- Low value of gyromagnetic ratio for ^{13}C results in lesser sensitivity of ^{13}C.
- ^{13}C-^{1}H spin coupling results in splitting of ^{13}C signal due to attached proton, which helps in identification of CH_3, CH_2 and CH groups.
- Chemically equivalent ^{13}C nuclei give a signal in NMR spectrum.
- The chemical shift range in ^{13}C-NMR is much more (δ 0 – 220 ppm) and therefore the signals are well separated with least overlap.

- The signal of ^{13}C is affected by electronegativity, hybridisation and anisotropic effects in the same way as in ^{1}H chemical shift.
- In aliphatic compounds, the ^{13}C signal is affected by α-effect along with β-effect and γ-effect.
- The sp^2 hybridised carbon in alkene and aromatic compounds have same chemical shift range.
- The signal for ^{13}C in alkynes has chemical shift range in between to that of alkenes and alkanes.
- In aromatic compounds, polar groups affects electron density in ring and thereby significant chemical shifts are observed.
- The ^{13}C-signals in heteroaromatic compounds are also affected by electronegativity of heteroatom and resonance effect.
- The carbonyl carbon is highly downfield shifted due to paramagnetic deshielding and high electronegativity of oxygen atom of C=O group.
- The coupling constants of ^{13}C-^{1}H is of the order of 150 Hz while for ^{1}H-^{1}H it is much lesser i.e. 8 Hz.
- The general expression for determination of multiplicity of signal is '2nI + 1', where n is number of nuclei undergoing spin-spin interactions with a nucleus and I is its nuclear spin quantum number.
- The proton coupled ^{13}C spectrum provides details of multiplicity of each signal and it is useful to attain information regarding substitution on ^{13}C. But such spectra are complicated and it is difficult to attain structural information from overlapping multiplets.
- Proton decoupled ^{13}C spectra is more useful as it provides direct information of different chemical equivalent carbon atoms present in the given sample.

3.12 SUGGESTED READINGS:

- Spectrometric Identification of Organic Compounds 7th edition by R. M. Silverstein, F. X. Webster and D. J. Kiemle, J. Wiley & Sons Publication.
- Elementary Organic Spectroscopy- Principles and Chemical Applications by Y. R. Sharma, S Chand Publication.

- Introduction of Spectroscopy 5th edition by D. L. Pavia, G. M. Lampman, G. S. Kriz and J. R. Vyvyan, Cengage Publication.
- Spectroscopy of Organic Compounds by P. S. Kalsi, New Age International Publication.
- Introduction to NMR Spectroscopy by R. J. Abraham, J. Fisher and P. Loftus, Wiley Publication.

3.13 TERMINAL QUESTIONS:

1. Compare the ^{13}C-NMR with ^{1}H-NMR spectroscopy method?

2. What are important features of ^{13}C-NMR spectroscopy?

3. Explain why the ^{13}C-^{13}C spin splitting is not observed in ^{13}C-NMR spectrum?

4. Predict the number of signal and multiplicity of peaks in the following compounds:
   a) 2,5-Dimethylhexane
   b) Cyclohexanol
   c) Benzophenone
   d) p-Xylene
   e) 2,3-Dimethylbuta-1,3-diene

5. Discuss the factors affecting chemical shift in ^{13}C-NMR spectroscopy?

6. Compare the ^{13}C-NMR signals for alkene and aromatic compounds with suitable example?

7. Discuss the factors responsible for downfield shifts for ^{13}C-signal for carbonyl carbon?

8. Explain spin-spin coupling with special reference to ^{13}C-^{1}H coupling? Compare ^{13}C-^{1}H coupling with ^{1}H -^{1}H coupling?

9. What is proton decoupled ^{13}C spectra? What is the advantage of proton decoupled ^{13}C spectra?

Unit 4: 2D NMR Spectroscopy

4.1 OBJECTIVE :

This chapter is dedicated to advanced NMR methods i.e. advanced one-dimensional (1D) and two dimensional (2D) NMR methods. These methods are developed by using physics of RF pulses and delay to attain FID and thereby using Fourier transform to attain signal in frequency domain. These techniques differ in terms of pulse sequence used, delays and presentation of spectrum. On completion of this chapter you learn about following advance NMR methods such as:

- Concept of Net Magnetization
- APT method
- INEPT and DEPT
- Principle of 2D NMR
- HETCOR method
- COSY method
- NOESY method
- INADEQUATE method.

4.2 INTRODUCTION:

In previous units you learnt about the core concepts of NMR method. The basic NMR spectroscopic methods i.e. ^{1}H-NMR and ^{13}C-NMR provides crucial information about structure of compounds under study. But there are certain issues that limit the use of these fundamental spectroscopic methods. The limitations are short chemical shift range in ^{1}H-NMR, low sensitivity in ^{13}C-NMR, also the complex spectrum attained in ^{1}H-NMR and ^{13}C-NMR due to spin-spin coupling. You already learnt about the FT-NMR method and its advantage in increasing sensitivity of ^{13}C-signal. The proton-decoupled ^{13}C-NMR is another method as already in previous unit used to simplify the complex ^{13}C-spectrum.

In order to attain indepth and accurate molecular structure elucidation, methods such as advance one dimensional (1D) and two dimensional (2D) NMR are frequently used. These methods are developed by utilizing physics of pulses and delays to control magnetization transfer and coherence transfer and attaining spectrum in more informative way.

To understand these advance topics of NMR, it is essential to attain an understanding about bulk magnetization vector and change in its position on interaction with RF pulse. This depends on pulse frequency, pulse width RF pulse. The change in position of net magnetization vector affects the excess population on precessing nuclei (which may give signal) in lower energy α-state and higher energy β-state.

The pulse sequence is set of RF frequency pulses with definite frequencies and width, separated by delays results in FID, which is converted to frequency domain spectra. The various methods described in this unit can be differentiated in terms of:

- RF Pulse and delay sequences.
- Presentation of NMR spectrum.
- Nature of J coupling or NOE interactions.
- Interacting nuclei.
- Transfer of net magnetization or coherence.

The APT and DEPT are essentially advanced 1D-NMR methods in which proton-decoupled spectra are obtained with characteristic signal phase presentation. The 2D-NMR methods are based on transfer of magnetization by either J-coupling interactions as in HETCOR or by Nuclear Overhauser Effect (NOE) as in NOEY.

4.3 ORIGIN OF NET MAGNETIZATION AND COHERENCE:

In order to understand advance NMR experiments, you need to understand the theoretical tools used to describe a large population of spins and how such large numbers of spinning nuclei are affected by RF pulses and delays. The first tool is **vector representation or model** that uses a magnetic vector to represent one signal in the NMR spectrum. This vector model is only a method of illustrating the NMR phenomenon in our minds and it is not an accurate description of nuclear spins.

Nucleus is a positively charged sphere that spins on its axis, producing a small magnetic field that is defined in terms of gyromagnetic ratio (γ) which is characteristic of that isotope. Thus gyromagnetic ratio (γ) can be considered as strength of nuclear magnet (ignoring its unit and sign). Gyromagnetic ratio (γ) is dependent on nuclear magnetic moment and the axis (direction) of this nuclear magnetic vector undergoes precessional motion about the direction of applied magnetic field (Ho) under its influence. Consider the angle between the directions of applied magnetic field (Ho) conventionally z-axis and precessing nuclear magnetic moment vector is Θ. The frequency of precession is termed as Larmor frequency and this Larmor frequency is related to applied magnetic field (Ho) and gyromagnetic ratio of the nucleus, as according to fundamental NMR equation (equation (3).

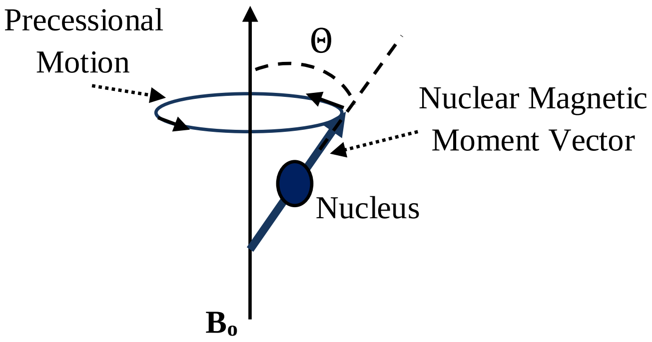


Figure 4.1: Precession of spinning nucleus

Classically all values of Θ are possible, i.e. the nuclear magnetic moment vector can precess between 0° (+z axis) to 180° (-z axis) with respect to direction of applied magnetic field. But for nuclei with nuclear spin quantum number I = 1/2, there will be only two angles corresponding to the two quantum states. The lower energy state is that in which Θ is 45°, represented by 'α-state', and a higher energy state in which Θ is 135°, represented by 'β-state'.(figure 4.2)

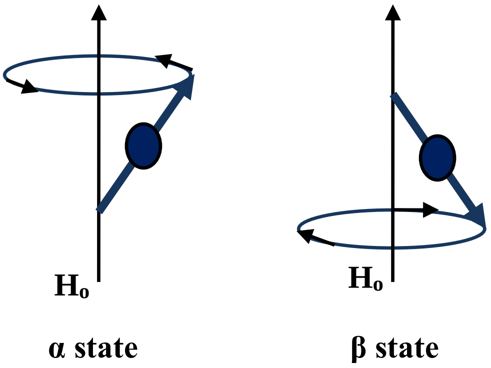


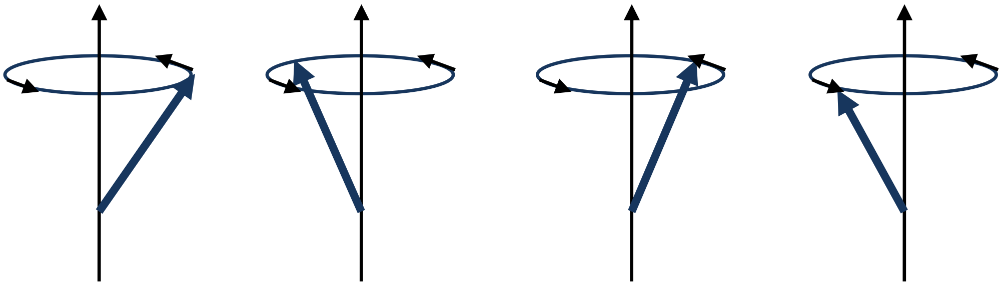
Figure 4.2: α and β nuclear spin states

So the nuclear spin axis sweep out a cone as it precesses around the z-axis. This cone opens upward (towards +z-axis) for spins in α-state and downward (towards –z axis) for spins in β-state. The vector model combines these two fundamental aspects of NMR:

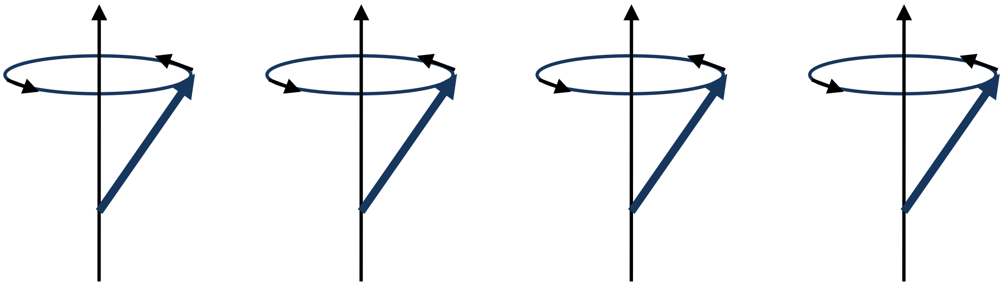
- Precession motion (circular motion in x-y plane), resulting in NMR signal.

- Aligned ‘α-state’ and opposed ‘β-state’ or energy levels.

The nucleus attains its orientation with respect to applied field i.e. α and β nuclear spin orientation. At macroscopic level, all nuclei attains either α and β nuclear spin orientations, and to attain NMR signal there must be some excess population in the aligned α nuclear spin orientation (lower energy state). The orientation of the spin axis of the nucleus can be presented as vector, which gives the direction of nuclear magnetic moment i.e. from south pole to the north pole, with magnitude of gyromagnetic ratio i.e. γ.



Before RF Pulse: No Phase Coherence (random phase)



After RF Pulse: Phase Coherence (same phase, organized precession)

Figure 4.3: Effect of RF pulse and Phase Coherence

Consider large population of identical spins in a sample at equilibrium. The vectors associated with nuclear magnetic moment are rotating with random phase in every possible direction around the cone in both +z axis and –z axis, as illustrated in figure 4.3. For simplicity only some vectors in α-state and β-state are shown, but actually there is random distribution of vectors in every possible direction around the cone in both +z axis and –z axis.

This is technically known as absence of phase coherence in large population of nuclei with identical spins.

Now if the sample is irradiated by a high power pulse of RF for short period of time, then the spins sync in terms of phase i.e. all the spins are pointing in the same direction at any time. Thus the precessional motion of all nuclear magnetic moment vectors is identical and this condition is known as phase coherence in large population of nuclei with identical spins (figure 4.3).

When considered collectively, these individual magnetic vectors add up to give a net magnetic vector which is rotating anticlockwise at Larmor frequency. Thus net magnetization of the sample takes place and a signal in NMR spectrum (FID) can be detected, thereby increasing sensitivity of detection of signal. This concept of phase coherence created by a pulse is basis of Pulsed FT-NMR spectroscopy.

The origin of **net magnetization and coherence** can be correlated with orientation and cancellation of nuclear magnetic moment vectors. These vectors are oriented in these cones such that their south pole of each vector is placed at same point is space. The populations in α- and β-states are nearly equal, with very slightly more spins in the lower energy state i.e. α-state. Also for almost every vector in α-state (upper cone), there is another vector in β-state (lower cone) that is exactly opposite to vector in upper cone. These magnetic vectors exactly cancel out and only some vectors that are in excess remains in lower energy α-state (upper cone). On cancelling the opposing spins, the x and y components of the individual vectors cancels when they are combined to form the vector sum, as all possible directions are equally populated. The z component of the individual vectors points upwards at 45° angle to the z axis, thus have same positive z component. On adding these together, the net magnetization vector M is attained (figure 4.4). This vector is macroscopic property of the sample and it remain stationary as only x and y components of the individual vectors are moving and all of these cancel due to random distribution around the upper cone. The RF pulse can cause change in position of the stationary z component of the vector, which can induce a measurable voltage in the probe coil.

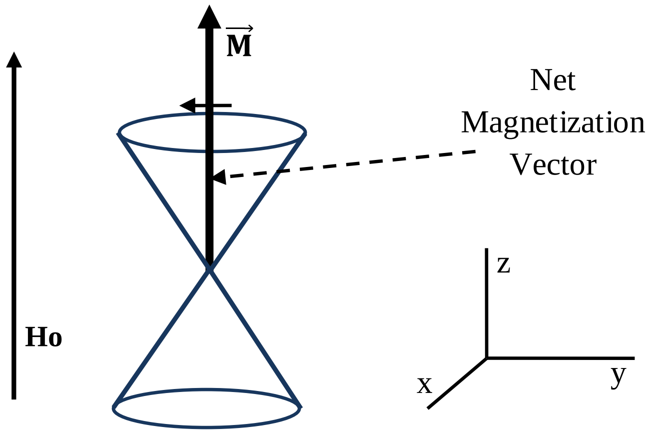


Figure 4.4: Net Magnetization Vector

The stationary net magnetization vector ‘M’, oriented in +z axis at equilibrium is fundamental property of the system on which advance NMR methods like 2D-NMR are based. The magnitude of this net magnetization vector (M) is ‘Mo’ and it is proportional to the population difference ‘p’ and length of individual magnetic vectors i.e. gyromagnetic ratio ‘γ’, as shown in equation (17):

$$M_o = k_1 \times p \times \gamma \qquad \dots(17)$$

where k_1 is a constant, the equilibrium population difference p is proportional to the energy difference between the two states ΔE (α and β) and inversely proportional to absolute temperature T (in degree Kelvin), i.e.

$$p = k_2 \times N \times \frac{\Delta E}{T} \qquad \dots(18)$$

where k_2 is a constant and N is the number of identical nuclei spinning in the sample (concentration of molecules), on inserting equation (18) in (17),

$$M_o = k_1 \times k_2 \times N \times \frac{\Delta E}{T} \times \gamma \qquad \dots(19)$$

The energy difference in above equation is related to applied magnetic field and gyromagnetic constant γ as:

$$\Delta E = h \times \gamma \times \frac{H_o}{2\pi} \qquad \dots(20)$$

Using equation (20) and (19),

$$M_o = k_1 \times k_2 \times N \times h \times \gamma \times \frac{H_o}{2\pi T} \times \gamma \qquad \ldots(21)$$

Combining all constant terms together (as constant k), equation (21) can be simplified as:

$$M_o = k \times N \times \gamma^2 \times \frac{H_o}{T} \qquad \ldots(22)$$

Thus the net magnetization at equilibrium is proportional to the number of identical spins in the sample (concentration), square of nuclear magnet strength, strength of the NMR magnet and inversely proportional to the absolute temperature. Now consider the effect of 180° pulse on a population of nuclei, which exert a torque on the individual nuclear magnets due to its magnetic field. This RF pulse can be viewed as a magnetic field (H_1) oriented in the x-y plane, perpendicular to H_o field. This causes rotation of the net magnetization vector by 180° with respect to original position, i.e. this vector is now pointing along –z axis (in high energy β-state), with same magnitude Mo.(fig 4.5)

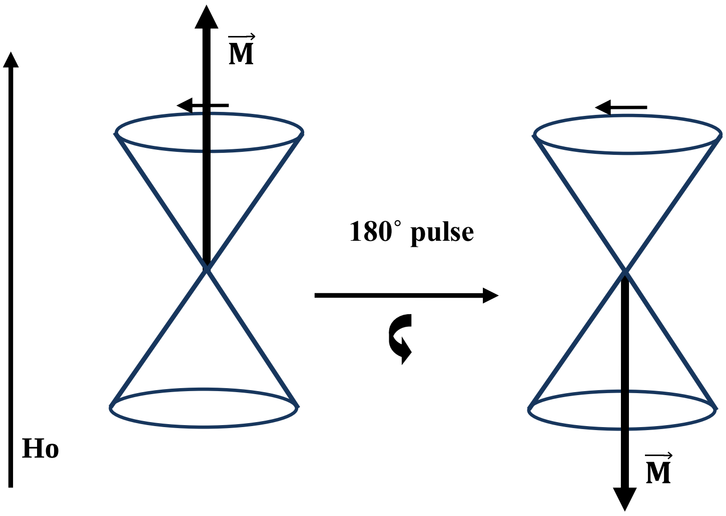


Figure 4.5: Rotation of the Net Magnetization Vector by 180° pulse

Coherence-Net Magnetization in x-y plane: The 90° pulse causes the rotation of net magnetization vector in anti-clockwise direction by 90° angle in left side with respect to original position and has same magnitude (Mo). Thus there is a coherence to attain net magnetization vector in x-y plane that rotates at Larmor frequency. This rotating magnetic vector induces a sinusoidal voltage in the probe coil of spectrometer that on amplification is detected to give free induction decay (FID). The signal is thus attained from Fourier transformation of FID in frequency domain at Larmor frequency .fig 4.6 .

It is more convenient to consider NMR experiment from a rotating frame of reference, so that the fundamental resonant frequency is removed from consideration. The reference axes in the rotating frame are x' and y' axes, rotates anticlockwise about the z axis at a fundamental resonant frequency. The nuclei which precess at exactly this fundamental frequency appears to be stationary, but more shielded nuclei appears to be rotating faster and deshielded nuclei appears to be slowly rotating. The fundamental frequency chosen for the rotating frame of the reference is the reference frequency in the spectrum and it is the centre of spectral window. The nuclei that has chemical shift just at the exact centre of the spectral window, is said to be 'on resonance' and its magnetization vector will stand still in the x'-y' plane. The other nuclei will rotate at a frequency and direction depending on their 'offset', the chemical shift is determined relative to zero at the central of spectral window.

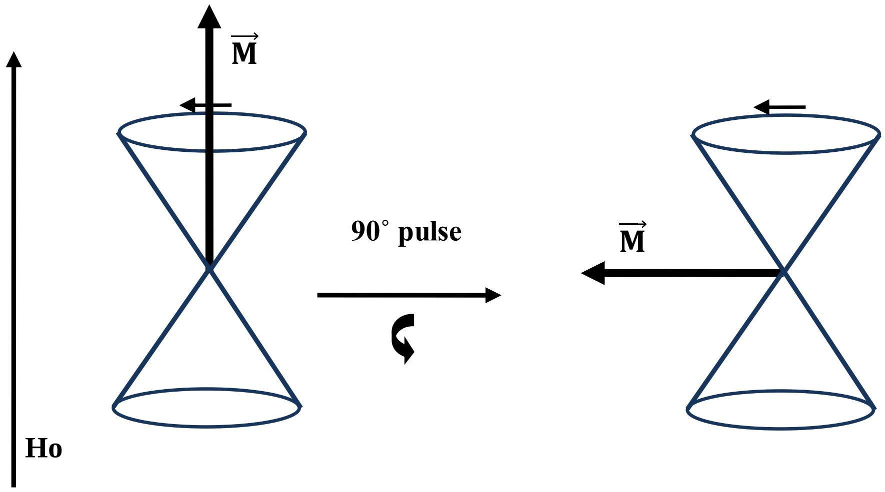


Figure 4.6: Rotation of the Net Magnetization Vector by 90° pulse

The radiofrequency (RF) pulse is a short burst (~10 µs pulse width) of a very high power (50-300 W), with specific frequency, amplitude and phase. The phase of the pulse depends on the starting point in the sine function, which can be precisely controlled by hardware and the software program of pulse sequence. The pulse sequence code that instructs the hardware are referred as 0, 1, 2 and 3 for 0°, 90°, 180° and 270° respectively. The pulse width and different pulse phases are illustrated in figure 4.7.

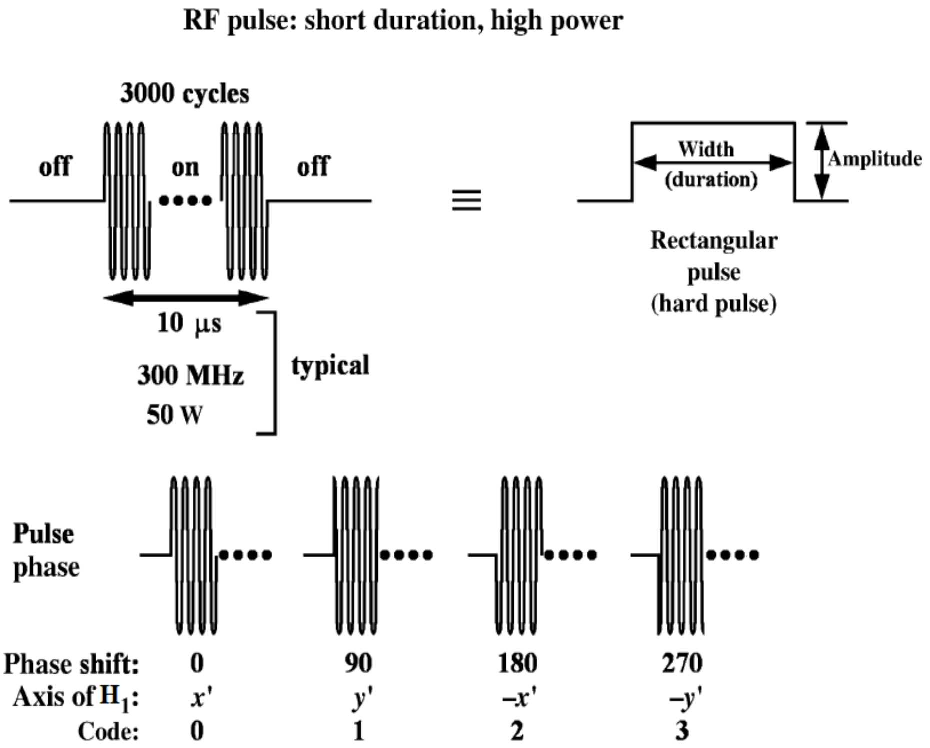


Figure 4.7: RF pulse width and Pulse Shifts

In rotating frame of reference, the position of H_1 vector (representation of RF pulse) is such that 0° phase corresponds to x' axis, 90° phase corresponds to y' axis, 180° phase corresponds to −x' axis and 270° phase corresponds to −y' axis. The effect of pulse on the position of H_1 vector helps to control the pulse, also change in pulse amplitude helps to adjust length of H_1 vector. This H_1 vector interacts with the net magnetization vector Mo, such that it exert a torque on Mo (originally along +z axis) resulting in rotation of Mo in a plane perpendicular to H_1 vector. For example if the H_1 vector is along x' axis then the Mo vector will rotates anticlockwise from +z axis to –y' axis in rotating frame. Thus RF pulse leads to rotation of net magnetization vector around the H_1 vector, in anticlockwise direction. This rotation results in change in actual excess population in α and β nuclear spin states and thereby affecting signal detection and its phase. For example, 90° pulse results in population difference in α and β states, with excess population in lower energy α state (represented as $-I_y$ spin state) and thus normal signal with positive absorptive peak is obtained. The 180° pulse results in excess population in higher energy β nuclear spin states (represented as $–I_z$ spin state) so no absorption of RF takes place and no signal is attained in NMR spectrum. In same way 270° pulse results in excess population in lower energy α state (represented as I_y spin state), thus signal is attained with negative absorptive peak. It must be remembered that the RF pulse sequence is followed by some delays that may be chemical shift evolution and scalar coupling constant J evolution. The FID attained thereafter is converted to frequency domain signal by using Fourier transform.

Chemical shift evolution is a delay between the end of the 90° pulse and the beginning of FID. In chemical shift evolution the net magnetization vector Mo changes is rotating frame of reference axis, say x' to y' axis or y' to –x' axis depending upon the delay time and peak in the spectrum will be dispersive with respect to reference axis x'. The scalar coupling constant J evolution is crucial for magnetization transfer process, in which the net magnetization transfer from ^{13}C to the ^{1}H atoms bonded with it.

4.4 ATTACHED PROTON TEST: APT

In this method ^{1}H-decoupled ^{13}C spectra is attained, in which the phase (up or down) of the ^{13}C peaks is used as method to attain information about the number of protons attached to a carbon atom. In this way, the number of attached protons i.e. methyl (CH_3, three hydrogen atoms), methylene (CH_2, two hydrogen atoms), methine (CH, one hydrogen atom) and quaternary (Cq, carbon with no hydrogen atom) can be easily identified.

In APT method the pulse sequence is 90° ^{13}C pulse, 1/(J) delay without ^{1}H decoupling (J is one bond ^{13}C-^{1}H coupling constant ~150 Hz), which helps in spectral editing of the ^{13}C-NMR spectra. In this way, the phase is modified relative to normal ^{13}C spectrum in order to attain additional information related to number of protons attached with carbon atoms.

Consider the example of 4-hydroxy-3-methyl-2-butanone, for which the normal ^{13}C-NMR spectrum and APT spectrum is shown below in figure 4.8. The APT spectrum of 4-hydroxy-3-methyl-2-butanone shows all carbons, but in comparison to normal ^{13}C-NMR spectrum, in APT spectrum the CH and CH_3 carbons have up peaks and CH_2 and Cq has down peaks.

This additional information is quite useful with complicated spectrum, where proton-decoupling is a necessity to attain simplified spectra, yet the information related to nature of proton substitution on carbon is essentially required. Thus APT spectrum is quite simplified due to ^{1}H-decoupled ^{13}C spectra, with up phase peaks presenting CH and/or CH_3 carbons and down peaks presenting CH_2 and/or Cq carbons.

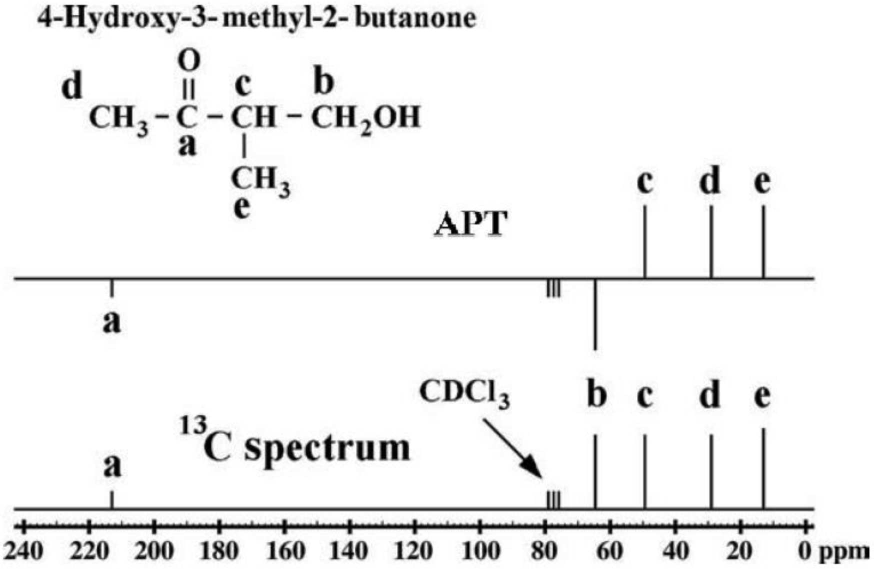


Figure 4.8: APT spectrum of 4-hydroxy-3-methyl-2-butanone

The APT method has two important limitations:

a) The signal-to-noise ratio of normal ^{13}C-NMR is almost three to 2.5 times in comparison to APT method. This is a major limitation of APT method and it is largely attributed to the result of T_2 relaxation during the 2/J delay of the spin echo.

b) In APT spectra, the up-phase peaks are due to CH and CH_3 carbons, but this does not confirm whether it is due to CH or CH_3 (similarly for CH_2 and Cq carbons). But in normal ^{1}H-coupled ^{13}C-NMR spectra, the peak multiplicity clearly indicates which carbon is CH_3 or which carbon is CH_2 and so on.

4.5 BRIEF INTRODUCTION OF INSENSITIVE NUCLEI ENHANCED BY POLARIZATION TRANSFER: INEPT

The magnetization transfer is the fundamental process involved in all advanced NMR experiments i.e. both 1D and 2D-NMR methods. On applying appropriate combination of pulses and delays it is possible to control transfer of net magnetization from one nucleus in a molecule to another. There are two types of net magnetization i.e. z-magnetization and coherence, there are two ways of magnetization transfer: transfer of z magnetization (NOE) and transfer of coherence (INEPT). In INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) method the magnetization transfer occurs via J couplings, means in this effect atoms that are two or three bonds apart are involved in magnetization transfer. The INEPT involves transfer of ^1H coherence to ^{13}C coherence, by pulse sequence 90° pulse on ^1H, delay of 1/(2J), simultaneous 90° pulses on ^1H and ^{13}C. The main advantage of INEPT over APT and normal ^{13}C-NMR is enhancement of sensitivity of by almost four times by increasing the energy difference between α-state and β-state of ^{13}C nucleus. INEPT spectra have following characteristics:

- The quaternary Cq carbon does not show signal in INEPT spectrum.
- The signal intensity is increases four times with respect to signal intensity in normal ^{13}C-NMR spectra.
- The multiplicity due to ^{13}C-^1H coupling in CH, CH_2 and CH_3 carbons is retained, but the multiplets are anti-phase. For example, CH resonances give anti-phase doublets with each component four times as intense as the corresponding signals in the ^{13}C spectrum.fig 4.9.

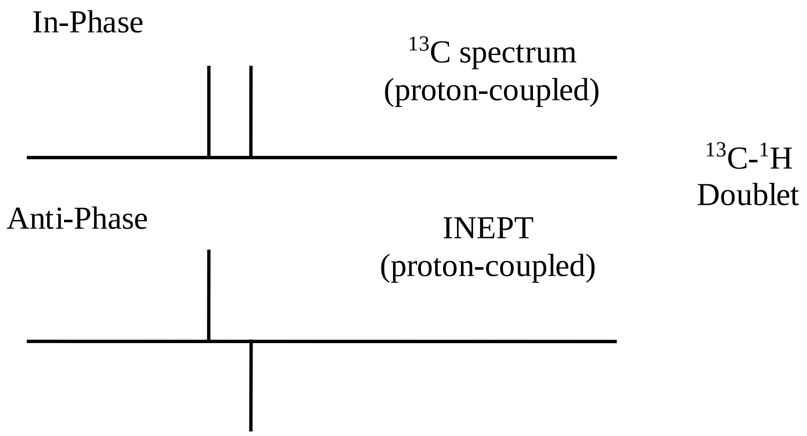


Figure 4.9: ^{13}C-^1H Doublet in ^{13}C-NMR spectrum and INEPT

The two other advanced NMR methods are derived from INEPT experiments. First is DEPT i.e. Distortionless Enhancement by Polarization Transfer (DEPT) in which the pulse sequence of INEPT is altered by incorporating the APT pulse sequence. The second method is 2D-NMR experiment termed as Heterogeneous Correlation (HETCOR) in which anti-phase to anti-phase coherence transfer takes place between ^1H and ^{13}C.

4.6 DISTORTIONLESS ENHANCEMENT BY POLARIZATION TRANSFER: DEPT

DEPT is an advanced 1D-NMR method is an advance method used to distinguish CH, CH_2 and CH_3 carbons. The pulse sequence for DEPT is presented in figure 4.10.

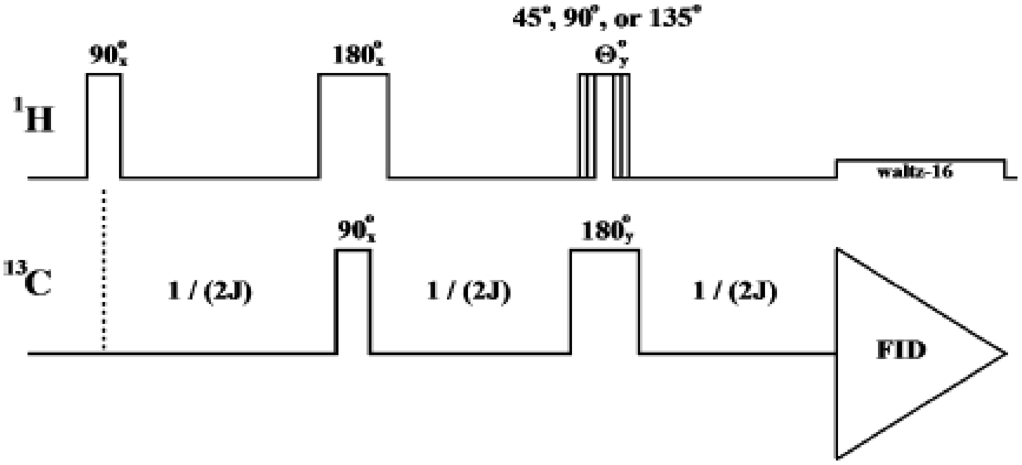


Figure 4.10: Pulse sequence in DEPT

DEPT method involves the coherence transfer technique i.e. 90° pulse on ^{1}H, delay of 1/(2J), simultaneous 90° pulses on ^{1}H and ^{13}C (as in INEPT), with the spin-echo technique used in APT i.e. 1/(2J) delay, 180° pulse, 1/(2J) delay to achieve both signal enhancement and spectral editing with distinction between CH, CH_2 and CH_3 carbons by phase labeling. The three 'flavors' of the DEPT experiment are known as 'DEPT-45', 'DEPT-90' and 'DEPT-135' depending on the pulse width of the final ^{1}H pulse in the sequence i.e. 45°, 90° and 135° respectively. The ^{1}H-decoupling is required in these experiments, which is attained by either using ^{1}H decoupler separated from ^{1}H and ^{13}C pulse transmitters or by rapidly switching the power level from high power ^{1}H pulse to low power pulse (required for ^{1}H decoupling) and repeating again.

When normal ^{13}C experiment is performed along with a DEPT-90 and a DEPT-135 experiment, it is possible to attain following information about carbons like quaternary Cq, methine CH, methylene CH_2 and methyl CH_3, as:

- All carbon atoms that are bonded with one or more hydrogen atoms give positive signal in DEPT-45.
- Quaternary Cq carbons signal are present in ^{13}C spectrum, but absent in all DEPT spectra (DEPT045, DEPT-90 and DEPT-135).
- Methine CH carbons are observed as positive signal in DEPT-90 and DEPT-135.
- Methylene CH_2 carbons give negative signal in DEPT-135 and remains absent in DEPT-90.

- Methyl CH_3 carbons gives positive signals in DEPT-135 and remains absent in DEPT-90.

Consider the ^{13}C-NMR and DEPT experiments for 4-hydroxy-3-methyl-2-butanone, which has one quaternary carbon, one methine, one methylene and two methyl groups. The carbon of carbonyl carbon, which appears at 213 ppm in ^{13}C-NMR spectra (and in APT spectra), but it is missing in the DEPT spectra, as it has no attached proton. The peak at 64 ppm is negative in the DEPT-135 as it is CH_2 carbon but it is absent in DEPT-90. The only peak in the DEPT-90 spectrum is due to CH proton, it is absent in DEPT-135. The most upfield positive peaks in DEPT-135 are due to two CH_3 groups, but these peaks are missing in DEPT-90 spectrum. Fig 4.11.

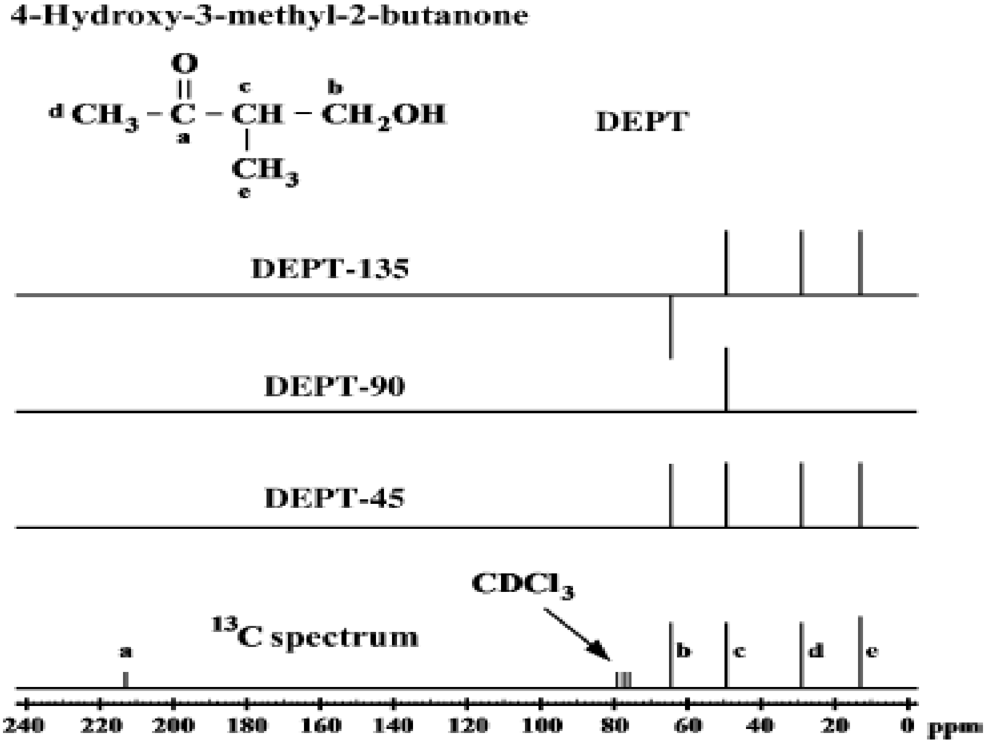


Figure 4.11: DEPT spectrum of 4-hydroxy-3-methyl-2-butanone

On comparing DEPT method with ^{13}C-NMR, we can conclude following points:

- The ^{13}C-NMR spectrum is quite complicated, due to ^{13}C-^{1}H spin-spin coupling. The close peaks in proton-coupled spectra hinder the identification of Cq, CH, CH_2 and

CH_3 carbons in a molecule. The DEPT method provides accurate information regarding number of hydrogen atoms attached with various carbon atoms in the compound under study.

- The sensitivity of ^{13}C-specta is very low as compared to that of DEPT spectra.

In DEPT-135 spectrum is similar to APT spectrum as in both the signal phase changes from up (positive) for CH to down (negative) for CH_2 to up (positive) for CH_3. The advantage of APT method is the signal of quaternary Cq carbon is also observed in APT but in DEPT spectra Cq carbon signal is not observed. But here the DEPT method has advantage over APT as the sensitivity in DEPT is much higher than that attained in APT method.

4.7 PRINCIPLE OF 2D NMR EXPERIMENTS:

In general a 2D NMR experiment involves transfer of magnetization from nucleus A (F1 frequency presented on the vertical axis) to nucleus B (F2 frequency presented on horizontal axis). The different 2D methods can be classified on the basis of (a) two types of nuclei detected in the direct (F2) and indirect (F1) dimensions and (b) method of magnetization transfer during the mixing step. As already discussed the magnetization transfer process can be of two types:

a) Magnetization transfer based on a J-coupling interaction and

b) Magnetization transfer based on a NOE interaction.

The 2D-NMR experiments can be further classified into (i) Homonuclear experiments in which magnetization transfer takes place from one nucleus to another nucleus of the same type i.e. ^1H-^1H, (ii) Heteronuclear experiments in which magnetization transfer takes place between two different types of nucleus ^1H-^{13}C (as presented in table 1).

Table 4.1: Homonuclear and Heteronuclear 2D-NMR

Homonuclear Experiments (^1H-^1H)

Name of Experiment	F_1 Nucleus	F_2 Nucleus	Mixing
COSY	^1H	^1H	J (Single transfer)
TOCSY	^1H	^1H	J, J, J,… (Multiple transfer)
NOESY	^1H	^1H	NOE
ROESY	^1H	^1H	Spin Lock NOE

Heteronuclear Experiments (^{1}H-^{13}C)

Name of Experiment	F_1 Nucleus	F_2 Nucleus	Mixing
COSY	^{1}H	^{13}C	$^1J_{CH}$
TOCSY	^{13}C	^{1}H	$^1J_{CH}$ (^{13}C SQC during t_1)
NOESY	^{13}C	^{1}H	$^1J_{CH}$ (^{13}C MQC during t_1)
ROESY	^{13}C	^{1}H	$^1J_{CH}$ (Long range coupling)

In homonuclear experiments, a diagonal is defined by $F_1 = F_2$ and pairs of crosspeaks appears at symmetrical positions across the diagonal. This is because both ^{1}Ha → ^{1}Hb and ^{1}Hb → ^{1}Ha magnetization transfer can be observed. The heteronuclear experiments do not have diagonal and diagonal symmetry.

The most fundamental step in 2D experiment is transfer of magnetization from nucleus A to nucleus B. The data from a 2D-experiment consist of a series of FIDs, each obtained with a slightly longer t_1 delay that the previous one. Now for each FID, Fourier transform is used to attain signal in frequency domain. The resulting spectra are loaded into a data matrix, in which horizontal axis is labeled as F_2, which is the chemical shift observed directly in each FID and vertical axis is t_1 i.e. the evolution delay. Each row in the 2D matrix represents a spectrum attained with different t_1 delay and each column in the matrix represents either noise (when the F_2 value of that column is in a noise region of the spectrum) or, if F_2 is the frequency of nucleus B, the column is a t_1 FID with maximum intensity at the bottom and oscillating in a decaying fashion as t_1 values are increased. The frequency of this oscillation is chemical shift of nucleus A. The second step in processing the 2D data is to perform a second Fourier transform on each of the columns of the matrix, as most of columns present noise, but when a column which falls on an F_2 peak, transformation of the t_1 FID gives a spectrum in F_1, with a peak at the chemical shift of nucleus A. The final 2D spectrum is a matrix of numerical values that has a pocket of intensity at the intersection of the horizontal line F_1 and the vertical line F_2 and it has an overall intensity depending upon the efficiency of transfer of magnetization from nucleus A to nucleus B.

4.7 HETEROGENEOUS CORRELATION: HETCOR

In INEPT experiment the magnetization transfer is performed by coherence transfer between ^{1}H to ^{13}C, along with this ^{1}H chemical-shift evolution is required. This is attained the complex steps involving varying a evolution delay time t_1, successively recording ^{13}C FID and performing successive Fourier transform. The coherence transfer is thereby attained along with the a correlation between the chemical shift of ‘H’ and chemical shift of ‘C’ is also obtained, which helps to establish that these two atoms are directly bonded to each other.

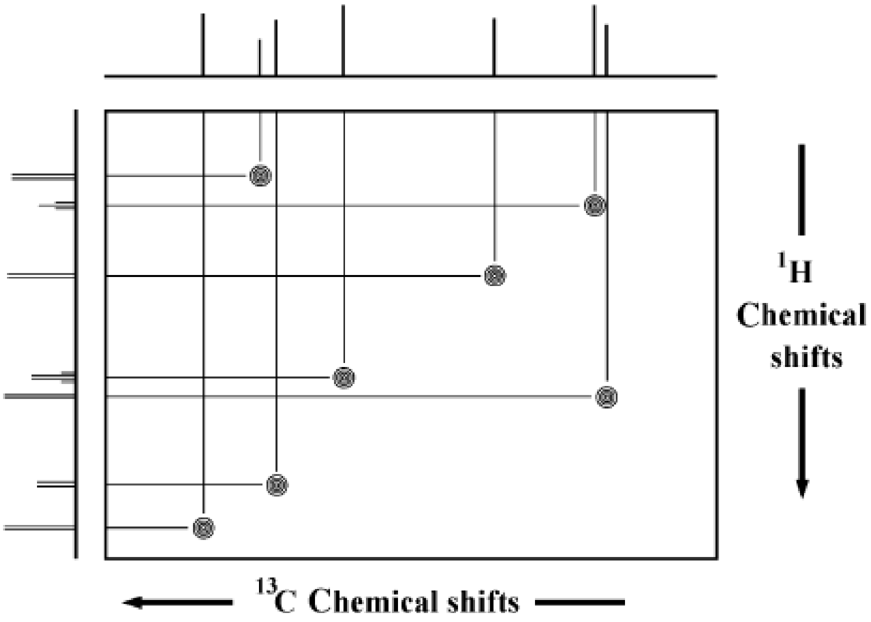


Figure 4.12: HETCOR Spectrum

The 2D spectrum in HETCOR is a construct in which column data corresponds to ^{13}C-NMR spectrum (also displayed horizontally) and row data corresponds to ^{1}H-NMR spectrum (also displayed vertically). The important point is the ^{13}C which is interacted by spin-spin coupling with ^{1}H i.e. the ^{1}H atoms bonded with ^{13}C are correlated in this spectrum. The 2D HETCOR spectrum is presented in figure 4.12, in which any signal in ^{1}H spectrum is observed as a spot or blob of intensity in the 2D data matrix. These clusters of intensity represent correlations in 2D spectrum and known as crosspeaks. A crosspeak when flow in vertical line gives the corresponding ^{13}C signal in the 1D ^{13}C spectrum corresponding to that carbon atom with which that proton is bonded. Thus each proton signal in ^{1}H spectrum is paired with a carbon peak in the ^{13}C spectrum, which is known as chemical shift correlation.

The HETCOR pulse sequence involves following steps:

1. Preparation: 90° nonselective ^{1}H pulse, which rotated ^{1}H z magnetization into x-y plane.

2. Evolution: ^{1}H magnetization vector precesses in x-y plane for t_1 time period, encoding of ^{1}H chemical shift as a function of t_1.
3. Mixing: INEPT sequence converts the ^{1}H magnetization into anti-phase magnetization with respect to the bonded ^{13}C nucleus. Then transfers of ^{1}H magnetization to ^{13}C magnetization by simultaneous 90° pulses on both ^{1}H and ^{13}C.
4. Detection: ^{13}C FID is recorded.

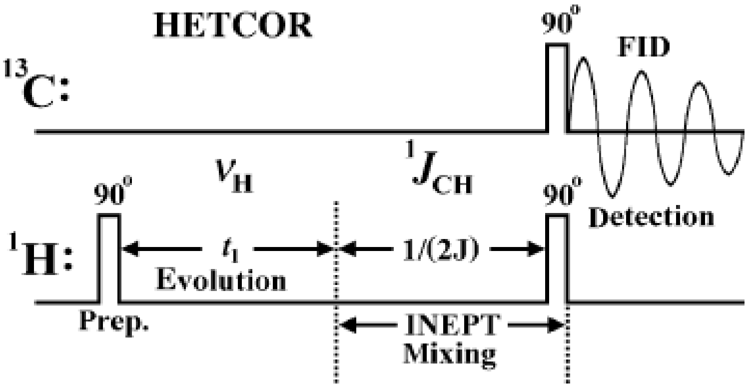


Figure 4.13: Pulse sequence in HETCOR experiment

4.8 2D CORRELATION SPECTROSCOPY: COSY

Correlation spectroscopy (COSY) is the simplest 2D experiment, in which one proton (Ha) is correlated to another (Hb) via single J coupling. On the basis of bond separation between Ha and Hb the J coupling may be 2-bond (geminal), 3-bond (vicinal) or may be long-range coupling. The pulse sequence in COSY is simply 90° ^{1}H pulse, followed by t_1 delay then again 90° ^{1}H pulse and FID, as shown in figure 4.14.

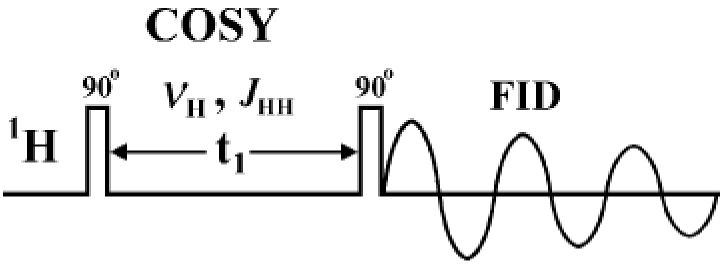


Figure 4.14: Pulse sequence in COSY

The appearance of a homonuclear 2D spectrum is different with that obtained in HETCOR experiment (^{13}C-^{1}H correlation). It is because both frequency scales i.e. F_2 and F_1 are ^{1}H chemical shift scales. In COSY experiment, the transfer on coherence from Ha to Hb is observed at the crosspeak, also the opposite sense of transfer from Hb to Ha is also observed at asymmetrical crosspeaks (upper left crosspeak). While in HETCOR ^{1}H (in F_1) coherence is transferred to ^{13}C (in F_2) with which it is bonded. The coherence transfer from ^{13}C to ^{1}H is not possible because the pulse sequence starts with ^{1}H excitation and detector is set to observe only signals at the radio frequency of ^{13}C.

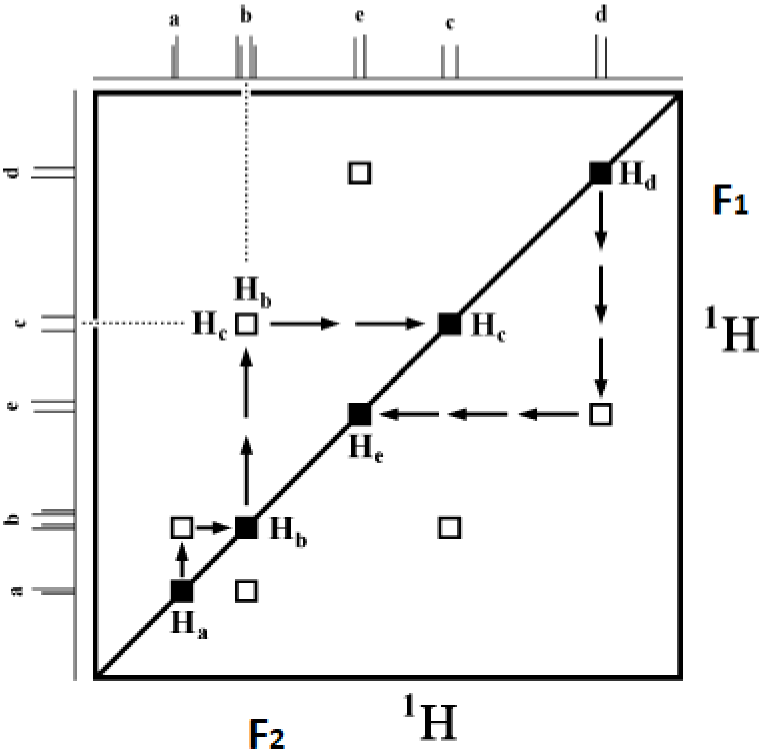


Figure 4.15: 2D COSY Spectrum

Consider a molecular segment, consisting of two simple spin systems separated by a quaternary carbon, presented as –CH_a-CH_b-CH_c-Cq-CH_d-CH_e-. The 2D COSY spectrum of this molecular segment is illustrated in figure 4.15, as you visualize through each spin system by moving from diagonal to cross-peak vertically, back to diagonal horizontally and repeating this process, then you notice that either of the two symmetrical crosspeaks can be used for this visualization. The H_b-H_c upper left crosspeak shows the correlation between H_b

signal in the 1D spectrum (at the top) with H_c signal in the 1D spectrum (on the left).This crosspeak represents the coherence transfer from H_c proton to H_b proton.

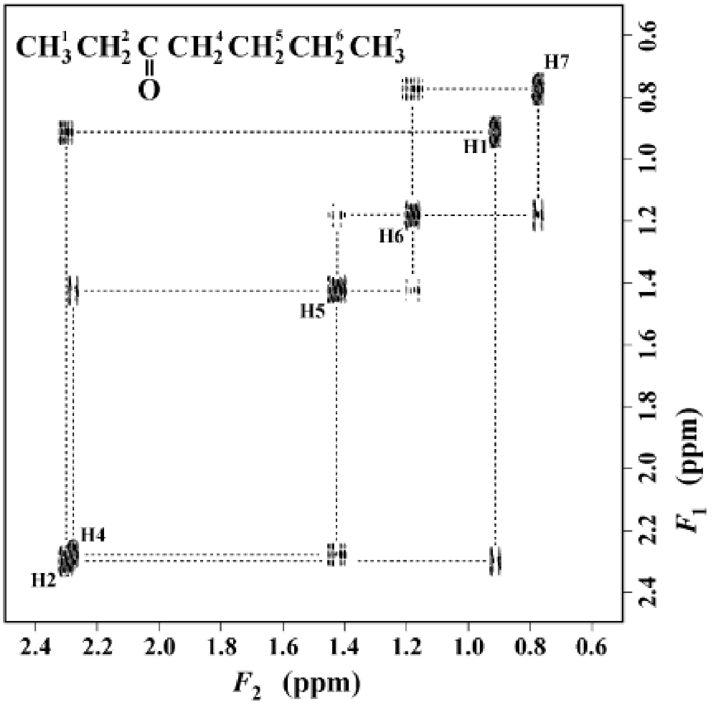


Figure 4.16: 2D COSY Spectrum

Now consider the example of 600 MHz 2D-COSY spectrum of 3-heptanone (figure 4.16). The protons of two α-CH_2 groups with respect to carbonyl carbon are downfield shifted in the region of 2–2.5 ppm (compare to acetone at 2.1 ppm). The two overlapped peaks on the diagonal (lower left) that are identified as separate peaks as their crosspeaks do not line up i.e. the downfield peak lines up vertically and horizontally with crosspeaks at 0.90 ppm, whereas the upfield peak lines up with crosspeaks at 1.42 ppm. For the spin system from the downfield diagonal peak (2.30 ppm), you can move to the right and up (or up and to the right) to the diagonal peak at 0.90 ppm. Thus two signals can be assign to H_2 (2.30 ppm) and H_1 (0.90 ppm). This spin system represents the ethyl group: CH_2–CH_3. The upfield peak of the overlapped pair (2.27 ppm) is for H4 on the other side of the carbonyl carbon. The crosspeaks on either side of the diagonal (dotted lines) on moving to H5 (1.42 ppm), then to H6 (1.18 ppm) and lastly to the methyl group H7 (0.77 ppm). The dotted lines show how this spin system of four resonances (n-butyl group) is distinct from the spin system of two resonances (ethyl group).

4.9 NUCLEAR OVERHAUSER EFFECT SPECTROSCOPY: NOESY

As the term suggest, NOESY is based on Nuclear Overhauser effect (see section 1.16). Nuclear Overhauser effect involves spin relaxation by energy transfer from nucleus in excited spin state to the adjacent nucleus. NOESY correlate protons with other protons via their homonuclear NOE interactions. NOESY spectrum is similar to COSY, with the exception that the crosspeaks correspond to pairs of protons that are close in space (< 5 Å) and not necessarily close in the bonding network. The intensities of crosspeaks are roughly proportional to $1/r^6$, where r is the direct through-space distance between the two protons correlated by the crosspeak.

NOESY experiment is simply the extension of COSY pulse sequence, with one additional delay and one additional 90° ^{1}H pulse (figure 4.17).

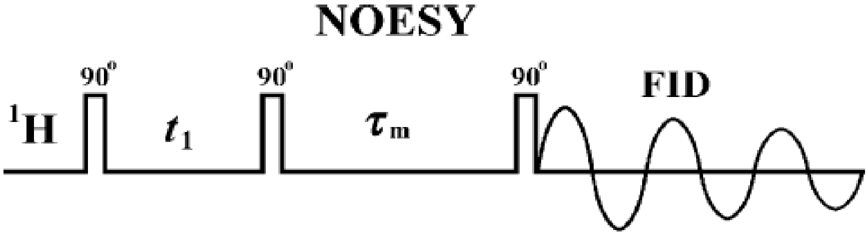


Figure 4.17: NOESY Pulse Sequence

The intensity of the crosspeak will increase by increasing mixing time τ_m, which eventually increases to a maximum and drop off to zero. The NOESY spectrum is also very similar to COSY spectrum but there are additional crosspeaks as compared to COSY. These additional crosspeaks provide information regarding pairs of protons that are close in space but far away in terms of bonding network between them. The important examples of this are 1,3-diaxial protons in rigid cyclohexene chairs, CH-O-CH protons interaction across a glycosidic linkage, etc.

4.10 *INCREDIBLE NATURAL ABUNDANCE DOUBLE QUANTUM TRANSFER EXPERIMENT: INADEQUATE :*

The 2D INADEQUATE is an advanced NMR method which used to deduce chemical structures of small organic molecules. This method provides a connectivity map of all carbon atoms in the molecule. This method in based on ^{13}C-^{13}C coupling, for which both adjacent carbon atoms must be ^{13}C isotope. As the natural abundance of 13C isotope is only 1.1%, so probability of having two adjacent ^{13}C atoms is approximately 1 in 8300 carbon atoms, which adversely affects the sensitivity of the analysis. Thus high concentration, with sample size 100 to 500 mg, is primary requirement for INADEQUATE experiments. The sensitivity increases with high magnetic field strengths.

The pulse sequence in INADEQUATE is 90˚ ^{13}C pulse (proton decoupled), $1/4J_{C\text{-}C}$ delay, 180˚ ^{13}C pulse (proton decoupled), $1/4J_{C\text{-}C}$ delay and 90˚ ^{13}C pulse (proton decoupled). The INADEQUATE spectrum does not appear as COSY spectrum. The ^{13}C chemical shift of one carbon is presented in F_2 axis and sum of the two correlating frequencies in F_1 axis. In INADEQUATE 2D spectrum there are no diagonal signals.

4.11 SUMMARY:

- correlated to another (Hb) via single J coupling. Advanced 1D and 2D NMR methods provide more details about the molecular structure.
- The nuclear magnetic moment vectors of individual nuclei can be visualise collectively as net magnetization vector.
- RF pulse brings coherence in precession of nuclear magnetic moment vectors of population of nuclei.
- The net magnetization depends on number of identical spins in the sample, nuclear magnet strength, applied field and temperature.
- RF pulse is a short burst of radiation of a very high power (50-300 W) with specific frequency, amplitude and phase.
- The position of net magnetization vector changes on irradiation of RF pulse, affecting population in α and β state.
- APT experiment pulse sequence is is 90° ^{13}C pulse, 1/(J) delay without ^{1}H decoupling (J is one bond ^{13}C-^{1}H coupling constant ~150 Hz).
- The intensity of signals in APT is too low.
- INEPT involves transfer of ^{1}H coherence to ^{13}C coherence, by pulse sequence 90° pulse on ^{1}H, delay of 1/(2J), simultaneous 90° pulses on ^{1}H and ^{13}C.
- DEPT and HETCOR methods are based on INEPT method.

- Pulse sequence of DEPT is 90° pulse on ^{1}H, delay of 1/(2J), simultaneous 90° pulses on ^{1}H and ^{13}C (as in INEPT), with the spin-echo technique used in APT i.e. 1/(2J) delay, 180° pulse, 1/(2J) delay.
- Quaternary Cq carbons signal are present in ^{13}C spectrum, but absent in all DEPT spectra (DEPT045, DEPT-90 and DEPT-135).
- Methine CH carbons are observed as positive signal in DEPT-90 and DEPT-135.
- Methylene CH_2 carbons give negative signal in DEPT-135 and remains absent in DEPT-90.
- Methyl CH_3 carbons gives positive signals in DEPT-135 and remains absent in DEPT-90.
- 2D NMR experiment involves transfer of magnetization from nucleus A (F1 frequency presented on the vertical axis) to nucleus B (F2 frequency presented on horizontal axis).
- 2D NMR spectrum is a matrix of numerical values that has a pocket of intensity at the intersection of the horizontal line F_1 and the vertical line F_2.
- HETCOR spectrum is 2D representation of INEPT data in which ^{13}C nuclei is correlated with attached ^{1}H.
- In COSY experiment one proton (Ha) is
- NOESY correlate protons with other protons via their homonuclear NOE interactions.
- INADEQUATE experiment is based on ^{13}C-^{13}C coupling, for which both adjacent carbon atoms must be ^{13}C isotope.

4.12 SUGGESTED READING

- NMR Spectroscopy: Basic Principles, Concepts and Applications in Chemistry 3rd edition by H. Gunther, Wiley Publication.
- Organic Structures from 2D NMR Spectra by L. D. Field, H. L. Li and A. M. Magill, Wiley Publication.
- Spectrometric Identification of Organic Compounds 7th edition by R. M. Silverstein, F. X. Webster and D. J. Kiemle, J. Wiley & Sons Publication.
- NMR Spectroscopy explained by N. E. Jacobsen, Wiley Publication.
- Introduction of Spectroscopy 5th edition by D. L. Pavia, G. M. Lampman, G. S. Kriz and J. R. Vyvyan, Cengage Publication.

- Introduction to NMR Spectroscopy by R. J. Abraham, J. Fisher and P. Loftus, Wiley Publication.

4.13 TERMINAL QUESTIONS:

1. Write short notes on the following:

a) Net magnetization.

b) Coherence

c) Magnetization transfer

2. Explain the APT method? What are the main features of APT NMR method?

3. Discuss the method of DEPT? Describe its pulse sequence and spectrum?

4. Discuss the main features of 2D-NMR?

5. Explain the method of Heterogenous Correlation (HETCOR)?

6. What is COSY? What are differences between COSY and HETCOR?

7. Write short note on the following:

a) NOESY

b) INADEQUATE

UNIT 5 : BASIC PRINCIPLES OF ESR SPECTROSCOPY

Content :

5.1 Objectives

5.2 Introduction

  5.2.1 Comparison between electron spin resonance (ESR) and nuclear magnetic resonance (NMR) spectroscopy

5.3 Theoretical background of electron spin resonance (ESR) spectroscopy

  5.3.1 Principle of electron spin resonance (ESR) spectroscopy

5.4 Instrumentation of electron spin resonance (ESR) spectrometer

5.5 Intensity of electron spin resonance (ESR) signals

5.6 Line width of ESR signal

5.7 Lande's splitting factor (*g*) or, spectroscopic splitting factor or *g*-factor

  5.7.1 Factors affecting the 'g' values

5.8 Hyperfine structures

5.9 Hyperfine splitting: (The hyperfine interaction in hydrogen atom and free radicals)

5.10 The McConnell equation

5.11 Zero field splitting

5.12 Applications of electron spin resonance spectroscopy

5.13 Summary

5.14 Terminal questions

5.1*OBJECTIVES*:

After studying this unit students shall be able to:

- Discuss the principal of ESR spectroscopy
- Understand the condition for absorption of radiation in ESR spectral studies
- Describe the salient features of instrumentation involved in ESR spectrum recording
- Explain the nuclear hyperfine splitting in simple radicals
- Explain the significance of 'g'-values
- Explain the zero-field splitting and
- Illustrate the use of ESR spectral studies in the structure elucidation of some simple molecules

5.2 *INTRODUCTION:*

The present unit is designed to make the student accustomed with paramagnetic species such as free radicals, transition metal ions, ions and molecules having odd number of electrons, and other molecules that carry angular momentum of electronic origin; and their identification by using the technique of electron spin resonance spectroscopy. In 1944-45 the physicist E. K. Zavoisky performed his magnetic resonance experiments in a solid. Where, he observed the strong electron spin resonance absorption for some paramagnetic salts for very first time. After his observation this technique was remained as an interesting tool only to solid state physicist until the applications of molecular orbital theory by Stevens. The application of electron spin resonance in chemistry was first introduced by Owen and McCarvey.Electron spin resonance spectroscopy is one of the important tools to study the paramagnetic species. It is a branch of absorption spectroscopy in which radiation frequency belongs to microwave region (10^4-10^6 MHz) is absorbed by the paramagnetic species to induce transitions between magnetic energy levels of electrons having unpaired spins. The splitting of magnetic energy is achieved under the influence of a static magnetic field.

The electron spin resonance (ESR) spectroscopy is also known as electron paramagnetic resonance (EPR) spectroscopy and electron magnetic resonance (EMR) spectroscopy. All three names carry the same meaning and simply describe the different aspects of same phenomenon. The electron spin resonance (ESR) is another kind of magnetic resonance i.e., nuclear magnetic resonance (NMR). Since, both spectroscopies deal with the interaction of electromagnetic moments of particles. However, the nuclear magnetic resonance (NMR) spectroscopy can-not be used for paramagnetic species. In both spectroscopies the transition of electrons occurs from one energy state to another energy state. The electron spin resonance (ESR) spectroscopy is observed in microwave region whereas the nuclear magnetic resonance (NMR) spectroscopy observed in radiofrequency region of electromagnetic spectrum. In comparison to the nuclear magnetic resonance (NMR) spectroscopy, the electron spin resonance (ESR) spectroscopy has limited applications, since it is observed primarily in the systems containing unpaired electrons. The organic or inorganic free radicals and ions of transition metals which contains unpaired *d*- or *f*- electrons. One of the most important applications of electron spin resonance (ERS) spectroscopy is in the detection of the extremely short-lived (transient) free radical intermediates in chemical reactions.

5.2.1 Comparison between electron spin resonance (ESR) and nuclear magnetic resonance (NMR) spectroscopy:

Although,electron spin resonance (ESR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy are collectively referred to as magnetic resonance phenomena, yet there are many differences between two spectroscopies. Some major differences are listed below:

1. The electromagnetic radiation used in electron spin resonance (ESR) spectroscopy is confined to the microwave region in the frequency range 10^4-10^6 MHz, whereas, the nuclear magnetic resonance (NMR) spectroscopy is typically performed using radiowaves in the frequency range of 10 – 10^3MHz.

2. The electron spin resonance (ESR) spectroscopy is concerned with the magnetically induced electronic spin states, while the nuclear magnetic resonance (NMR) spectroscopyinvolves the splitting of nuclear spin states.

3. In electron spin resonance (ESR) spectroscopy the radiation frequency is typically held constant, and the magnetic field strength is varied. Whereas, in nuclear magnetic resonance

(NMR) spectroscopy the radiation frequency is varied while the magnetic field strength is kept constant.

4. The electron spin resonance (ESR) spectroscopy is about 1000 times more sensitive than the nuclear magnetic resonance (NMR) spectroscopy due to higher frequency of electromagnetic radiation used in electron spin resonance (ESR)spectroscopy in comparison to nuclear magnetic resonance (NMR) spectroscopy.

5. The electron spin resonance (ESR) spectroscopy has short relaxation time in comparison to the nuclear magnetic resonance (NMR) spectroscopy. Therefore, the ESR is usually performed under cryogenic condition using liquid helium as coolant to attain a very low temperature (below 10 K).

The table 1.1 summarises the comparison between ESR and NMR spectroscopy.

Table 5.1: Comparison between ESR and NMR

Property	**ESR**	**NMR**
Radiation source	Microwave	Radiowave
Resonance frequency	10^4-10^6 MHz	$10 – 10^3$ MHz
Spin under investigation	Electron spin	Nuclear spin
Spin quantum number	$S \geq 1/2$	$I \geq 1/2$
Magnetic quantum number	$m_l = \pm 1/2, \pm 1, \pm 3/2, \ldots..$	$m_s = \pm 1/2, \pm 1, \pm 3/2, \ldots..$
Characteristic property	*g values*	Chemical shift
Sensitivity	µM concentrations required	mMconcentrations required
Relaxation time	~µs	~s
Linewidths (~1/relaxation time)	MHz	Hz
Time resolution	~ns	~ms

5.3 *THEORETICAL BACKGROUND OF ELECTRON SPIN RESONANCE (ESR) SPECTROSCOPY:*

An electron is a negatively charged particle with certain mass; it mainly has two kinds of movements. The first one is spinning around the nucleus, which brings orbital magnetic moment. The other is spinning around its own axis, which brings spin magnetic moment. The electron spin resonance (ESR) spectrum of an unpaired electron is the simplest of all forms of spectroscopy. In the absence of external magnetic field, both electron spin states (i.e., ↑ and ↓) are degenerate and it is characterised by the quantum number, m_s=±1/2. When a magnetic field is applied, atoms with unpaired electrons spin either in the same direction of or in the opposite direction of the applied field. These two possible spin alignments will have different energies and therefore will no longer remain degenerate. The spin alignments are directly proportional to the strength of applied magnetic field. This effect is known as the ***Zeeman effect***. The unpaired electron interacts with its environment and produces the ESR spectrum. The details of such ESR spectrum depend on the nature of those interactions. The energy associated with these transitions is expressed in terms of the *applied magnetic field* ***B***, the *electron spin* ***g****-factor*, and the constant μ_B, which is known as ***Bohr magnetron***.

5.3.1 Principle of electron spin resonance (ESR) spectroscopy:

As discussed, the electron is a negatively charged particle which possesses orbital angular momentum (due to circular motion around nucleus) and spin angular momentum (due to spinning motion around its own axis). The spin quantum numberof this spinning electron is considered as *s*= ±½. Due to its spin, the electron possesses spin angular momentum S, which may be given as:

$$S = \frac{h}{2\pi}\sqrt{s(s+1)} \qquad \ldots\ldots\ldots\ldots (1)$$

Where, *h* = Plank’s constant; and *S* = spin quantum number.

If *s* = ½; then

$$S = \frac{h}{2\pi}\sqrt{\frac{1}{2}(\frac{1}{2}+1)} = \frac{\sqrt{3}}{2}\frac{h}{2\pi} \qquad \ldots\ldots\ldots\ldots (2)$$

The spin angular momentum quantum number for an electron will have values m_s = ±½. In the absence of external magnetic field, the two values of m_s i.e., +½ and -1/2 will give rise to a doubly degenerated spin energy state. Since, the spin angular momentum is a vector quantity and in the presence of external magnetic field the degeneracy is removed (Figure 5.1).

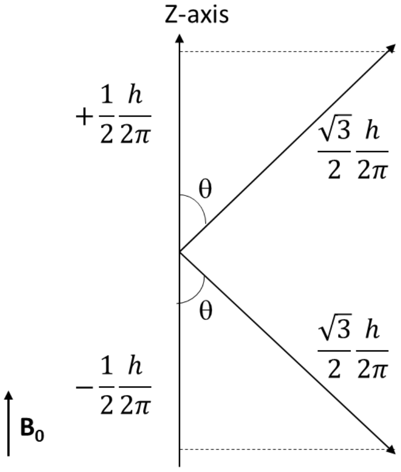


Figure 5.1: Two orientations of spin angular momentum vector under the influence of applied magnetic field*B₀*along z-axis

This leads to two non-degenerated energy levels. In the influence of external magnetic field, the spin angular momentum takes only those orientations in which its components align in the direction of the external applied magnetic field (Figure 5.1).The component of spin angular momentum vector along z-axis is $m_s \frac{h}{2\pi}$; where m$_s$has a value either $+\frac{1}{2}$ or $-\frac{1}{2}$ .Thus, the spin angular momentum vector can take only two orientations in space in the presence of an external applied magnetic field (***B₀***) along the z-direction. In such case the equation (1) may be written as:

$$S_z = \frac{h}{2\pi}\sqrt{s(s+1)}.cos\theta \qquad \ldots\ldots\ldots\ldots (3)$$

$$S_z = m_s \frac{h}{2\pi} \qquad \ldots\ldots\ldots\ldots (4)$$

Where, $\theta$is the orientation angle, and $\sqrt{s(s+1)}.cos\theta = m_s$

According to the classical mechanics principle the electron spinning around its own axis generates a magnetic field and hence it behaves as a tiny bar magnet. Thus, the magnetic moment associated with the spinning electron is given as:

$$\mu = -g\left(\frac{e}{2m}\right)\sqrt{s(s+1)}\frac{h}{2\pi} \qquad \ldots\ldots\ldots\ldots (5)$$

$$= -g\left(\frac{eh}{4\pi m}\right)\sqrt{s(s+1)} \qquad \ldots\ldots\ldots\ldots (6)$$

$$= -g\mu_B\sqrt{s(s+1)} \qquad \ldots\ldots\ldots\ldots (7)$$

Where, $\mu_B = \frac{eh}{4\pi m}$; the μ_B is known as basic unit of magnetic moment of the electron and it is known as ***Bohr magnetron***. The 'g' is known as ***Lande splitting factor*** and it is a dimensionless constant. The value of constant 'g' for the free electron is 2.0023. The value of μ_B can be calculated as:

$$\mu_B = \frac{eh}{4\pi m} = \frac{(1.602\ \times 10^{-19}C)(60626 \times 10^{-34}Js)}{4 \times 3.14 \times (9.1 \times 10^{-31}kg)}$$

$$= 9.2741 \times 10^{-24}JT^{-1}$$

The negative sign indicates that the spin angular momentum vector and the associated magnetic moment vector orient in opposite directions (Figure 5.2).

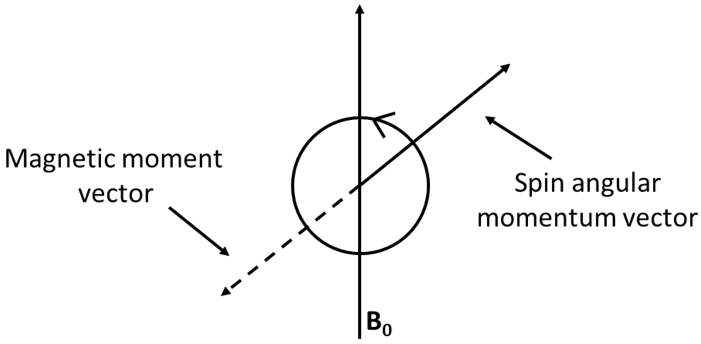


Figure 5.2: The relative orientation of thespin angular momentum vector and magnetic moment vector under the influence of applied magnetic field

When a spinning electron is subjected under the external magnetic field (***B₀***) (along z-axis), the interaction energy between the magnetic moment vector and external appliedmagneticfieldcan be given as

$$E = -\mu_z.B_0 \qquad \ldots\ldots\ldots\ldots\ldots (8)$$

$$E = -\mu cos\theta.B_0 \qquad \ldots\ldots\ldots\ldots\ldots\ldots (9)$$

Substituting the value of $\mu$from equation (7) in to equation (9) we have

$$E = -\left(-g\mu_B B_0\sqrt{s(s+1)}\right).cos\theta \qquad \ldots\ldots\ldots\ldots\ldots (10)$$

$$E = \left(g\mu_B B_0\sqrt{s(s+1)}\right).cos\theta \qquad \ldots\ldots\ldots\ldots\ldots (11)$$

From equation (3) and (11) we have

$$E = g\mu_B B_0 m_s \qquad \ldots\ldots\ldots\ldots.. (12)$$

Since $m_s$has a value either $+\frac{1}{2}$ or $-\frac{1}{2}$, therefore, equation (12) may also be written as

$$E_{+\frac{1}{2}} = +\frac{1}{2} g\mu_B B_0 \qquad \ldots\ldots\ldots\ldots.. (13)$$

$$E_{-\frac{1}{2}} = -\frac{1}{2} g\mu_B B_0 \qquad \ldots\ldots\ldots\ldots.. (14)$$

From equation (13) and (14), it is clear that by increasing the strength of externally applied magnetic field the energy corresponds to $E_{+\frac{1}{2}}$ increases while the value of $E_{-\frac{1}{2}}$ decreases. Thus,m_s=$-\frac{1}{2}$ corresponds to lower energy state, whereas, m_s=$+\frac{1}{2}$ corresponds to relatively higher energy state. Figure 5.3 shows the splitting of energy level of spinning electron under the influence of externally applied magnetic field.

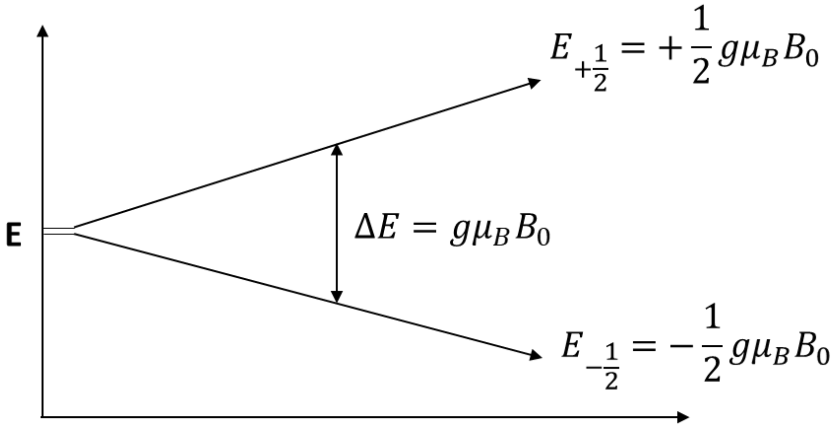


Figure 5.3:Effect of magnetic field strength on the energy of the spinning electron.

The energy difference between two energy states of a spinning electron under the influence of external magnetic field can be given as

$$\Delta E = E_{+\frac{1}{2}} - E_{-\frac{1}{2}} \qquad \ldots\ldots\ldots\ldots.. (15)$$

$$= +\frac{1}{2} g\mu_B B_0 - \left(-\frac{1}{2} g\mu_B B_0\right)$$

$$\Delta E = g\mu_B B_0 \qquad \ldots\ldots\ldots\ldots (16)$$

Since E = $h\nu$, then equation (16) may also be written as

$$\Delta E = h\nu = g\mu_B B_0 \quad \ldots\ldots\ldots\ldots (17)$$

Equation (17) gives rise the resonance condition for electron spin resonance (ESR) spectrum. In the presence of the electromagnetic radiation of required frequency (ν) transition of electron from lower energy spin state (*i.e.*, $m_s=-\frac{1}{2}$) to higher energy spin state (i.e., $m_s=+\frac{1}{2}$) takes place with absorption of this radiation giving rise to the electron spin resonance (ESR) spectrum. If the external magnetic field strength is 0.34T, then the resonance frequency for ESR spectrum can be calculated as follow:

$$\nu = \frac{g\mu_B B_0}{h}$$

$$= \frac{(2.0023)(9.2741 \times 10^{-24} JT^{-1})(0.34T)}{60626 \times 10^{-34} Js}$$

$$= 9.530 \times 10^{9} Hz$$

$$= 9530 \times 10^{6} Hz$$

$$\nu = 9530\ MHz$$

This frequency falls under the microwave region of electromagnetic radiation. Therefore, the electron spin resonance (ESR) spectrum is recorded using microwave radiation source and related techniques. The ESR spectrometers operating at the external magnetic field strength is 0.34T, and the resonance frequency 9530 MHz are referred as X-band spectrometers. However, Spectrometers operating at higher magnetic fields ($\boldsymbol{B_0}$ = 1.3 T) with corresponding higher microwave frequency (~35000 MHz) are also available. They are called Q-band spectrometers.

5.4*INSTRUMENTATION OF ELECTRON SPIN RESONANCE (ESR) SPECTROMETER:*

Electron spin resonance (ESR) can be obtained by the sweeping the microwave frequency at constant magnetic field or by sweeping the magnetic field at constant microwave frequency. However, it is always more convenient to linearly sweep the magnetic field at constant microwave frequency. The most commonly used ESR spectrometer operates in the X-band microwave region. The block diagram of a typical ESR spectrometer is shown in figure 5.4 including various components. The ESR spectrometer generally consist of the following subsystem.

- An electromagnet and power supply with magnetic field control and magnetic field sweep.
- A microwave bridge and resonant cavity.
- A detection system which is normally a detector and signal amplifier followed by phase-sensitive detection at the magnetic field modulation frequency and recorder display.

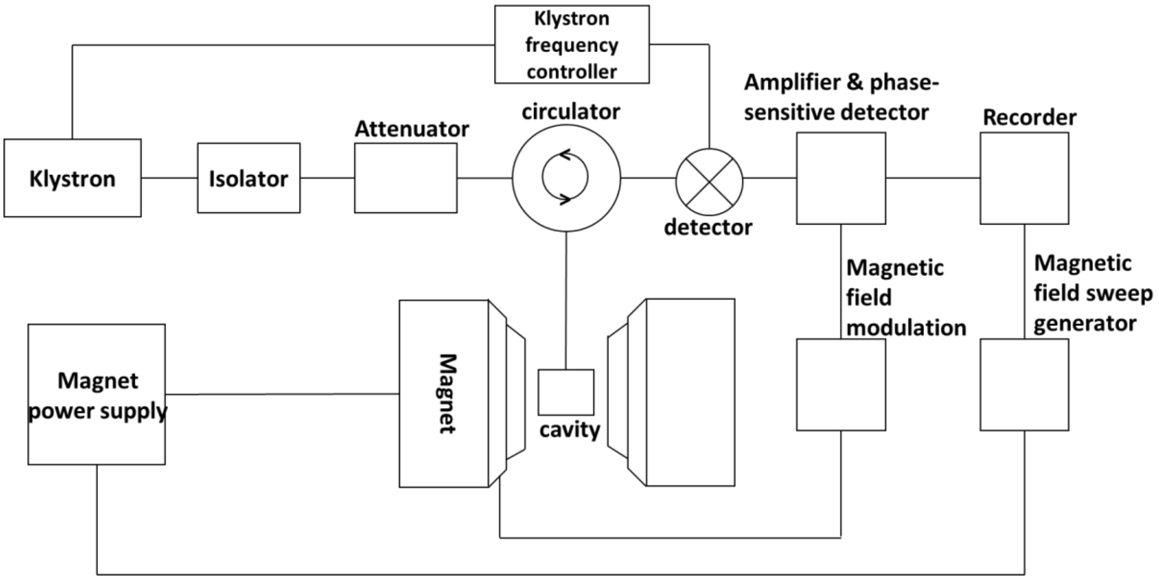


Figure 5.4: Block diagram of ESR spectrometer

The description of various components of ESR spectrometer is as follow:

1. Radiation source: The various components of a source are:

(*i*). *The Klystron*:TheKlystron (vacuum tube) is generally used as the microwave source for ESR spectrometer that provides a monochromatic coherent source of electromagnetic radiation.The frequency of radiation can be swept between -100 MHz and +100 MHz for the purpose of tunning the microwave circuit and cavity. It is always critical to maintain the microwave frequency at constant value so that the signal will not drift on a constant magnetic field. A klystron oscillator is generally operated at 9500Mc/sec. The microwave source is connected to a reference arm type homodyne bridge. The microwave passes from microwave source through an isolator then through an attenuator and finally through the circulator to resonant cavity containing sample.

(*ii*). *Isolator*: The isolator is a non-reciprocal device which minimizes the vibrations in the microwave frequency produced by the Klystron oscillator. It also serves to decouple the microwave source from reflection or other noise generated in the bridge that might cause frequency instabilities. The isolator is a ferrite material that has low insertion loss in the forward direction but heavy loss in the backward direction.

(*iii*). *Attenuator*:The attenuator is placed between the isolator and circulator. It adjusts the level of the microwave power incident upon the sample. It possesses and absorption element and corresponds to a neutral filter in light absorption measurement.

(*iv*). *Circulator*:The microwave radiations produce by the source are allowed to pass through the isolator, and the attenuator and finally enter in the circulator through a balance mixer by a loop of wire that couples with oscillating magnetic field and sets up a corresponding field in the balance mixer.Abalance mixer is generally made up of hollow rectangular copper brass tube having silver or gold coating inside to produce a highly conducting flat surface. The operation of a four-port circulator is shown in figure 5.5. The microwave radiations enter at arm 1. The arm 2 is connected to resonant cavity and sample. The arm 3, generally having a terminating load, and it absorbs any power which might be reflected from the detector arm. The arm 4 is attached to detector.

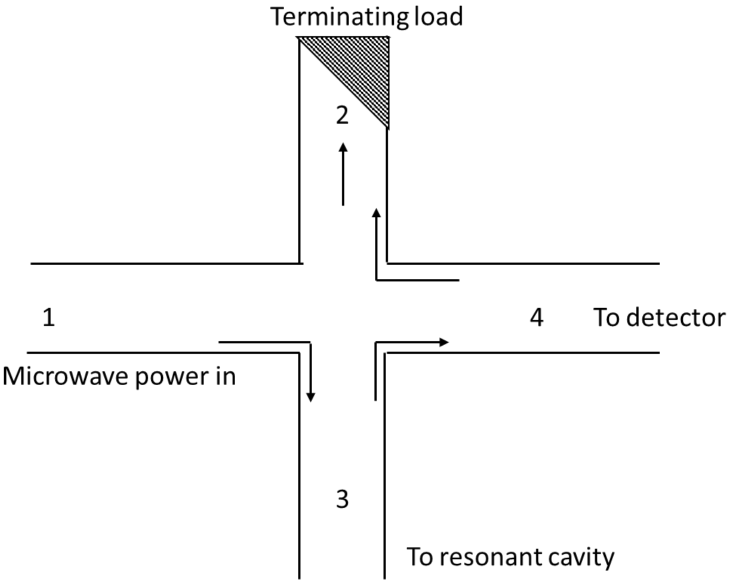


Figure 5.5: A four-port circulator showing the directions of microwave transmission

(*v*). *Sample Cavity*:It is the most important part of ESR spectrometer. The cavity is designed in such a way to maximize the applied magnetic field along the sample dimensions. The sample is placed inside a microwave resonant cavity which is kept between the pole pieces of the electromagnet. The cavity has two important functions: a) it stores the microwave energy so that the intensity of microwave field at the sample is maximized; and b) the design of cavity results the standing wave pattern which maximizes the magnetic field component of the microwave field and minimizes the electric field component at the sample. This minimizes the dielectric loss by the sample causesdue to the electric dipole absorption. The dielectric losses would lead to the cavity failing to resonate as a result sensitivity is lost.For ESR experiment the sample volume to samples which do not possess a high dielectric constant are used in excess (0.15 to 0.5 mL) in comparison to those which have high dielectric constant (for those, sample volume required 0.05 mL). In most of the ESR spectrometers, dual sample cavities are being used for the simultaneous observation of a sample and a reference material.

(*vi*). *Magnate System*: The Sample cavity is placed between the pole pieces of an electromagnet. This provides a homogeneous magnetic field and can be varied from 0 gauss to 500 gauss. The field strength should be stable and uniform over the sample volume. The stability of magnetic field can be achieved by energising the magnet with a well-regulated power supply.

(*vii*). *Detector*: The most commonly used detector is a crystal diode incorporating a silicon crystal attached to a tungsten whisker.The detector acts as a microwave rectifier, and it converts the microwave power into a direct-current output.

(***viii***). ***Amplifier and phase sensitive detector*:**After passing the detector the waves are then amplified by the preamplifier. However, the amplified signal contains a lot of noise. The reduction in noise is achieved by the phase sensitive detector or phase shifter. Phase sensitive detectorsare usually used to detect ESR signals which are represented as absorption or first derivativecurves. The reference line is adjusted to the same phase as the signal line by phase shifter (or phase sensitive detector). The phase sensitive detector not only give the high sensitivity, it also takes the derivative of the signal.

(*ix*). *Recorder*:The signal is then recorded by the use of suitable filtering time constant.

5.5 *INTENSITY OF ELECTRON SPIN RESONANCE (ESR) SIGNALS:*

When the applied magnetic field has a value that corresponds to the resonance energy, at this stage the energy is absorbed and transition between two levels takes place. The separation between two levels increases linearly with the magnetic field. Under the influence of applied magnetic field the energy separation between two spin states of an electron is generally greater than that of between two nuclear spin states. The transition between two energy levels results the relative population difference of two energy states and it is governed by the Boltzmann’s distribution law. Which is given as:

$$\frac{N_{+\frac{1}{2}}}{N_{-\frac{1}{2}}} = e^{-\frac{\Delta E}{kT}}$$

or

$$\frac{N_{+\frac{1}{2}}}{N_{-\frac{1}{2}}} = e^{-\frac{h\nu}{kT}}$$

The above mathematical representation it is clear that the lower energy state is relatively more populated than the higher energy state. To get a strong signal, ΔE must be large, which inturn implies that the sensitivity increases with increasing energy difference ΔE, thatis with the microwave frequency.

5.6 *LINE WIDTH OF ESR SIGNAL:*

The line width of ESR resonance depends upon the relaxation time of the spin state under study. As mentioned, the ESR absorption phenomenon depends upon the difference between relative population of two energy states. The transition from lower spin state to higher spin state results due to the absorption of radiation, as a result the increase in population of higher spin state. This process will continue until $N_{-\frac{1}{2}} = N_{+\frac{1}{2}}$ and net absorption of energy tends to be zero. However, this situation does not occur, it means that there must therefore be some other mechanism by means of which energy absorbed and stored in the upper level can be dissipated in such a way as to allow return to lower spin state to maintain the relative population difference. These mechanisms are called the relaxation mechanisms or processes. Usually there are two important relaxation processes: a) spin-lattice relaxation; and b) spin-spinrelaxation;

The spin-lattice relaxation can be interpretated as the interaction between the spins and the lattice vibrations. The spin magnetic moment is not influenced directly by the vibrations of the lattice. However, the coupling of the lattice vibrations and the magnetic spin states occurs indirectly through the residual spin-orbit coupling. The spin-lattice relaxation can be achieved by the direct process, Raman process and Orbach process. The spin-spin relaxation generally contains all the mechanism where by the spin can exchange amongst themselves, rather than giving it back to the lattice. The spin-spin relaxation can be achieved by diploe interaction and exchange couplingprocesses.Out of these two possible relaxation processes, the spin-spin relaxation is comparatively more efficient, unless the sample is extremely dilute. For most of the samples the spin-spin relaxation times comes out to be 10^{-6} to 10^{-8} sec., which is much shorter than the spin-spin relaxation time in the case of NMR. At room temperature thespin-lattice relaxation is efficient and gives the relaxation time about 10^{-6} sec, but it becomes progressively less efficient at reduced temperature. Therefore, for most of the samples a 10^{-7} sec is taken as a typical relaxation time, and if we substitute this value in the Heisenberg's relation ($i.e., \Delta E = \frac{h}{4\pi} \times \frac{1}{\Delta t}$), we can calculate a frequency uncertainty which is known as line width. The frequency uncertainty ($\Delta \nu$) can be calculates as:

$$\Delta E = \frac{h}{4\pi} \times \frac{1}{\Delta t}$$

or

$$h\Delta \nu = \frac{h}{4\pi} \times \frac{1}{\Delta t}$$

or

$$\Delta \nu = \frac{1}{4\pi \Delta t} = \frac{1}{4 \times 3.14 \times 10^{-7}} Hz = 0.796 MHz$$

The line width can be further increases with shorter relaxation time. Thus, there is a much wider line in ESR in comparison to the NMR, where the width of a normal line for a liquid is ≅0.1MHz. The greater line width in ESR spectrum has its advantages and disadvantages. As an advantages, in ESR a lower magnetic field is employed than the NMR. However, the major disadvantage of greater line width is that these are more difficult to observe and measure than sharp line. For this reason, the ESR signals are recorded as the derivative of absorption curve with respect to the magnetic field.The ESR spectrum peak and its first derivative with respect to the magnetic field are shown in figure 5.6.

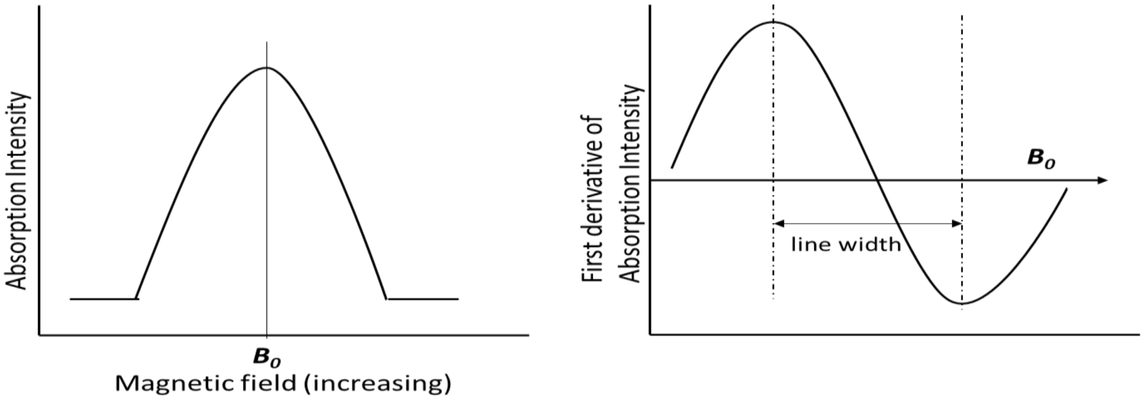


Figure 5.6 : The ESR spectrum peak and its first derivative with respect to the magnetic field

5.7 *LANDE'S SPLITTING FACTOR(g) OR,SPECTROSCOPIC SPLITTING FACTOR OR g-FACTOR:*

The Lande's g-factor is a geometric factor which arises in the evaluation of magnetic interaction which gives the Zeeman effect. It is the ratio of magnetic moment of the electron to the total spin angular momentum of the electron. An electron with no angular momentum has a g value equal to that of a free electron spin and equal to 2.0023. On the other hand, if the electron is moving in an atomic orbital associated with a single atom, it may possess considerably orbital angular momentum and this will shift the g value away from that 2.0023. The reason for this shift in g value is that relationship between magnetic moment and the angular momentum is different for spin motion and for orbital motion. In the case of an electron associated with a free atom, which has no external magnetic or electric field acing on it, the resultant ‘g’ value can be derived directly in terms of the quantum numbers defining the total spin and orbital magnetic moments. In most of the free radicals, the value of the odd electron of close to that of a free electron, however, on metal ions ‘g’ values are often quite different from the free electron values.

5.7.1 Factors affecting the ‘g’ values:

Generally, the magnitude of ‘g’ depends upon the orientation of the unpaired electron with respect to the externally applied magnetic field. In solution or in the gas phase the ‘g’ is averaged over all orientations because of the free motion of the electrons. However, in the crystalline state, such motions are restricted.If the paramagneticradical or ion is located in a perfectly cubic crystal site, *i.e.*, an octahedral or tetrahedral site,the g-value is independent of the orientation of the crystal and is said to be isotropic. In acrystal site of lower symmetry, the value of ‘g’ depends upon the orientation of the crystaland is said to be anisotropic.The value of g for an unpaired electron in gaseous atom/molecule/ion for whichRussel Saunders coupling (L-S coupling) is applicable, is given by the expression

$$g = 1 + \frac{J(J+1) + S(S+1) - L(L+1)}{J(J+1)}$$

For a free electron, $= \frac{1}{2}, L = 0\ and\ J = \frac{3}{2}$, the values of ‘g’ comes out to be 2.0 using above equation. The actual value of ‘g’ for a free electron is 2.0023 where the contribution 0.0023 is due to a relative correction. In most of the free radicals, the orbital contributions to the magnetic moment are verysmall and the ‘g’ values are nearly equal to free electron value of 2.0023. The small deviations(±0.05) often observed for most free radicals are accounted for by the mixing of low-lyingexcited states with the ground state.The properties of transition metals are determined to a large extent by the relativemagnitudes of the crystal field and spin-orbit coupling. These two interactions have oppositeeffects on orbital degeneracy and these can be generalized as follow:

1. **The effect of spin orbit coupling is much larger than that of the crystal field-** The rare earth ions fall in this category, since the *f*-electron are well shielded from crystal field effect so that the spin-orbit coupling is not disturbed.
2. **The effect of crystal field is strong enough to destroy the spin-orbit coupling**– The first-row transition metals fall in this category. The orbital degeneracy is not removed completely because of the effect of some spin-orbit coupling. Consequently, a net orbital magnetic moment results, giving rise to a g value different from the free electron value.
3. **The effect of crystal field is strong enough to destroy the spin-orbit coupling completely** – This corresponds to covalent bonding and is applicable to the complexes of 4d and 5d transition metals and to the strong field complexes of 3d transition metals such as cyanides

5.8 *HYPERFINE STRUCTURES:*

Interaction of magnetic moment of an unpaired electron with neighbouring magnetically active nuclei results in the splitting of ESR spectrum. This splitting usually occurs into $2nI+1$ components, where n is the number of neighbouring magnetically active nuclei and I is their respective nuclear spins. Such splitting of ESR signals under the influence of the neighbouring magnetically active nuclei is known as the hyperfine structure of ESR signals.

The difference in energy between the magnetic moment of unpaired electron and that of neighbouring magnetically active nuclei depends upon the direction of the magnetic moment of the electron relative to that of the neighbouring magnetically active nucleus. The interaction energy is expressed as Am_sm_I, where, 'A' is a constant and known as hyperfine coupling constant of hyperfine splitting constant. It is the measure of the extent of the interaction between magnetic moment of the electron and that of the interacting magnetically active nuclei. Thus, the energy of the electronic spin states under the influence of a nearby magnetic nucleus isgiven by

$$E_{m_sm_I} = g\mu_B B_0 m_s + Am_sm_I \ldots\ldots\ldots\ldots\ldots \quad (18)$$

Thus, based on equation (18) is can be stated that for a given value of m_s, the energy of electronic spin state also depends upon the value of m_I.

5.9*HYPERFINE SPLITTING*: (*The hyperfine interaction in hydrogen atom and free radicals*).

Hydrogen atom consists of an unpaired electron (s = $\frac{1}{2}$) and a magnetic nucleus (I = $\frac{1}{2}$). In the absence of external magnetic field, the single electron (with s = $\frac{1}{2}$) gives rise a doubly degenerated spin energy level. However, under the influence of external magnetic field, the degeneracy is removed and two different energy levels will be obtained, in which one energy level corresponds to $m_s = -\frac{1}{2}$ aligned with the applied magnetic field and the other energy level corresponds to $m_s = +\frac{1}{2}$ aligned opposite to the applied magnetic field (Figure 5,7). Figure 5.7a shows the effect of external magnetic field on the energy states for an electron, and figure 5.7b shows the effect of nuclear spin of proton on these energy states.

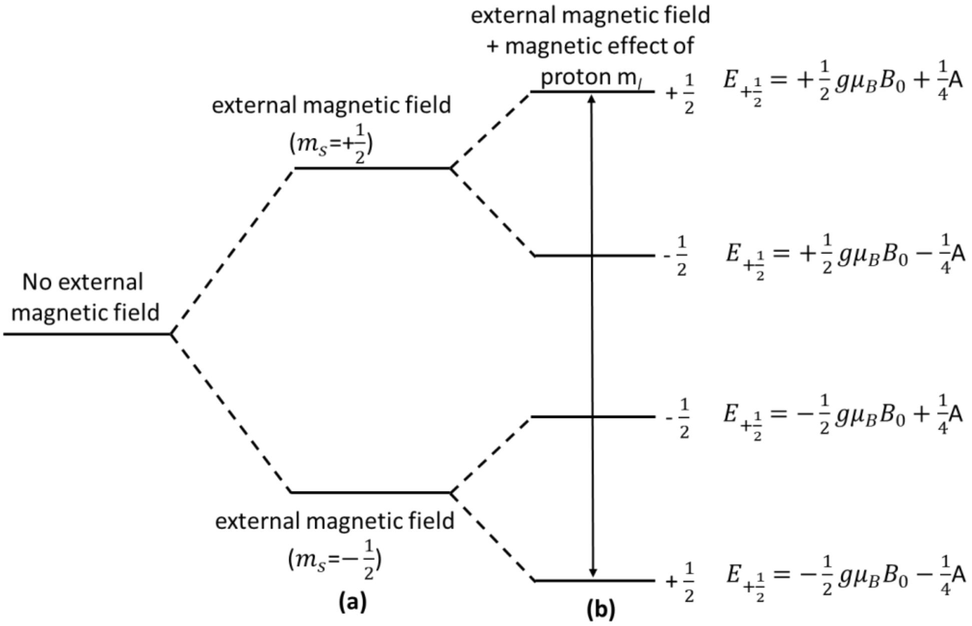


Figure 5.7: (a) effect of external magnetic field on the energy states for an electron; (b) effect of nuclear spin of proton on these energy states.

The ESR spectrum of hydrogen atom would consist of a single peak corresponding to a transition between these energy levels (Figure 5.8).

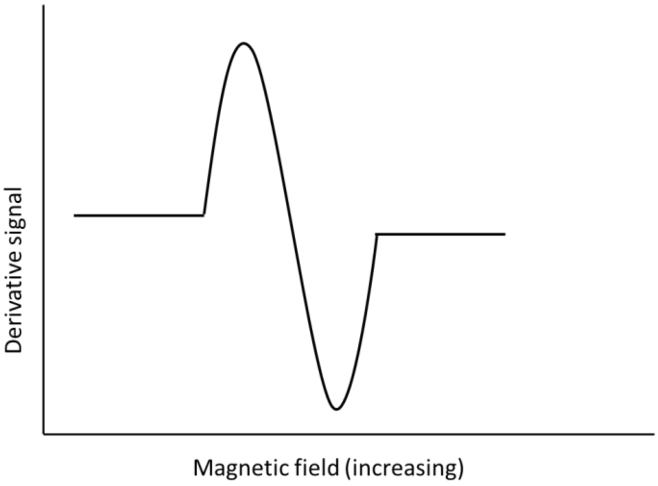


Figure 5.8 ESR signal of a free electron of hydrogen atom

However, further splitting of these energy states corresponding to $m_l = +\frac{1}{2}$ and $m_l = -\frac{1}{2}$ (where m_l is the nuclear spin angular quantum number) is observed when the interaction between two energy states and the nuclear spin due to proton is considered. Thus, four different energy states are obtained correspond to two energy states (Figure 5.7b). Because of such splitting the ESR spectrum of hydrogen would consists of two peaks corresponding to two transitions shown in figure 5.7. The ESR peaks due to splitted energy states are shown in figure 5.9.

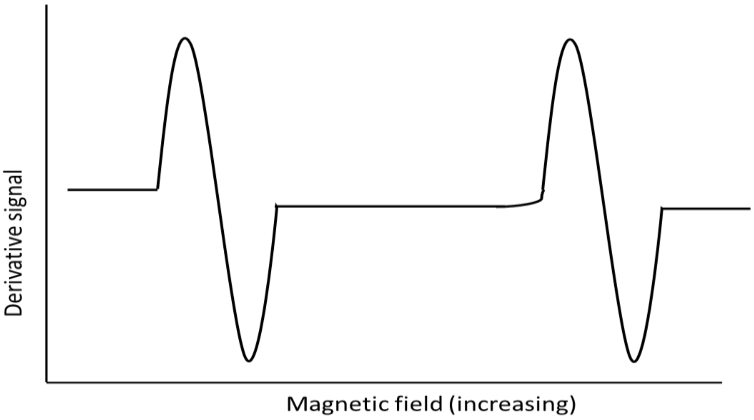


Figure 5.9 ESR signal after splitting of energy states due to their interaction with nuclear spin of hydrogen atom

Based on equation (18), the mathematical expressions for energies of four energy levels (shown in figure 5.7) may be written as follows:

$$E_{+\frac{1}{2},+\frac{1}{2}} = +\frac{1}{2}g\mu_B B_0 + \frac{1}{4}A \quad \ldots\ldots\ldots\ldots (19)$$

$$E_{+\frac{1}{2},-\frac{1}{2}} = +\frac{1}{2}g\mu_B B_0 - \frac{1}{4}A \quad \text{............ (20)}$$

$$E_{-\frac{1}{2},-\frac{1}{2}} = -\frac{1}{2}g\mu_B B_0 + \frac{1}{4}A \quad \text{............ (21)}$$

$$E_{-\frac{1}{2},+\frac{1}{2}} = -\frac{1}{2}g\mu_B B_0 - \frac{1}{4}A \quad \text{............ (22)}$$

By putting the values of 'g', the Bohr magnetron (μ_B), and applied magnetic field (B_0) in the above equations (eq. 19 to 22) the values of energies indicated in figure 5.7 can be calculated for a given values of m_s and m_l. The selection rules for ESR spectroscopy are

$$\Delta m_l = 0 \text{ and } \Delta m_s = \pm 1$$

Hyperfine splitting of methyl free radical **($^{\bullet}CH_3$):** For a methyl radical s = ½ ,and I = $^3/_2$. When the interaction between unpaired electron of carbon atom and three hydrogen nuclei is considered, there are four values of m_l corresponding to $m_s = +\frac{1}{2}$ and four value of m_l corresponding to $m_s = -\frac{1}{2}$, *i.e.*,

For $\quad m_s = +\frac{1}{2}, m_l = +\frac{3}{2}, +\frac{1}{2}, -\frac{1}{2}, -\frac{3}{2}$

And for $\quad m_s = -\frac{1}{2}, m_l = -\frac{3}{2}, -\frac{1}{2}, +\frac{1}{2}, +\frac{3}{2}$

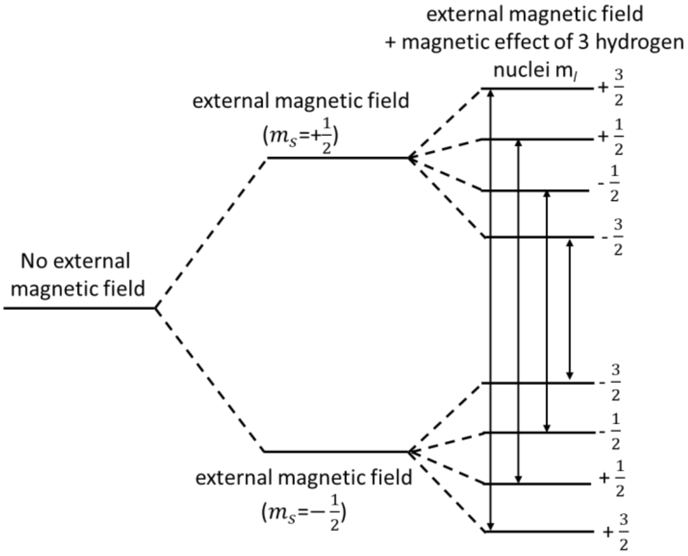


Figure 5.10 ESR spectrum of methyl radical under the influence of three hydrogen nuclei

Applying the selection rule, we may have $\Delta m_l = 0$ and $\Delta m_s = \pm 1$, and four transitions are possible that result in the formation of four lines in the ESR spectrum of methyl radical. The relative intensities of four spectral lines are observed in the ratio of 1:3:3:1. The four transitions that occur in the ESR spectrum of methyl radical are shown in Figure 5.10; and its derivative spectrum is shown in Figure 5.11.

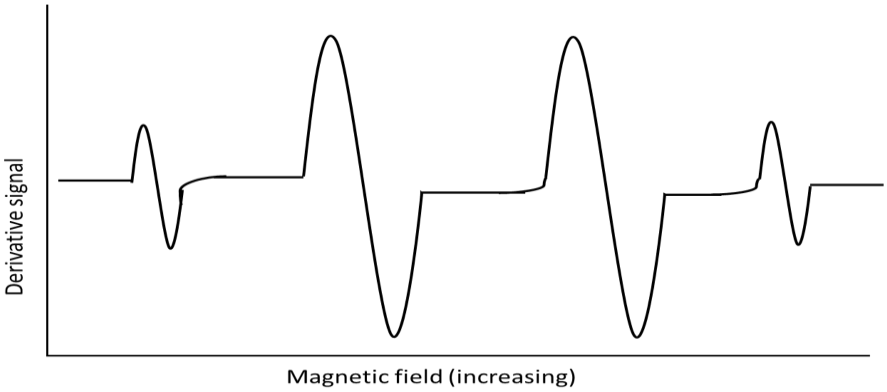


Figure 5.11Derivative spectrum of methyl radical in presence of three hydrogen nuclei

5.10*THE McConnell EQUATION*:

The hyperfine structure of an EPR spectrum is a kind of fingerprint that helps to identify the radicals present in a sample. Moreover, because the magnitude of the splitting depends on the distribution of the unpaired electron near the magneticnuclei present, the spectrum can be used to map the molecular orbital occupied by theunpaired electron. In aromatic radicals, the odd electron occupies a molecular orbital which is delocalisedover the atomic framework of the radical. Being in a π orbital, it has zero probabilityof being in the plane of the ring where the H atoms lie - the π orbital has a nodethere. However, despite our conclusion above on 's' character, hyperfine interactionswith the H-atoms are observed. For example, because the hyperfine splitting in $C_6H_6^-$ is 0.375 mT,and one proton is close to a C atom with one-sixth the unpaired electron spin density(because the electron is spread uniformly around the ring), the hyperfine splittingcaused by a proton in the electron spin entirely confined to a single adjacent C atomshould be 6 × 0.375 mT = 2.25 mT. If in another aromatic radical we find a hyperfinesplitting constant a, then the spin density, ρ, the probability that an unpaired electronis on the atom, can be calculated from the McConnell equation:

$$a = Q\rho$$

with Q = 2.25 mT (Q is a proportionality constant). In this equation, ρ is the spin density on a C atom and a is thehyperfine splitting observed for the H atom to which it is attached.

Thus, the McConnell's equation describes the relation between the hyperfine splitting constant (a) and the spin density (ρ) [spin density can be defined as the probability of an unpaired electron being on a particular atom] in aromatic radical compounds such as benzene radical anion.

5.11*ZERO FIELD SPLITTING*:

The splitting of spin levels in the absence of external magnetic field is known as zero-field splitting. It occurs mainly in transition metal complexes that contain more than one unpaired electron. When a transition metal complex (or metal ion) is placed in a crystal field, the degeneracy of d-orbital's is lifted by the electrostatic interactions, but the spin degeneracy remains, until a magnetic field is applied. However, the spin degeneracy would be destroyed by crystal field when the species contains more than one unpaired electron. Thus, the spin levels may be split even in the absence of a magnetic field. This phenomenon is known as ***the zero-field splitting***. For the transition metals with an odd number of unpaired electrons, the spin degeneracy of every level remains doubly degenerated. This is known as Kramer's degeneracy; whereas, in case of even number of unpaired electrons, the spin degeneracy may be destroyed completely by the crystal field.

Consider an example of a molecule or ion having two unpaired electrons. Thus, the total spin would be S = 1, forming the triplet state because the spin multiplicity would be 3 (s = 2S + 1). The triplet state can be treated for most ESR purposes as a single particle with spin 1. Thus, the angular momentum vector corresponding to S = 1 is given as:

$$S = \sqrt{S(S+1)}\frac{h}{2\pi}$$

$$S = \sqrt{1(1+1)}\frac{h}{2\pi}$$

$$S = \sqrt{2}\frac{h}{2\pi}$$

In the absence of external magnetic field, the angular momentum vector orients itself in all three directions in space. However, when the external magnetic field is applied, the angular momentum vector canorient in any one of the three directions. The orientation is either may align the external magnetic field or may oppose it. The components of the angular momentum vector in the direction of applied magnetic field (***B₀***) are $m_s = -1, 0\ and + 1$. Under the influence of applied magnetic field the +1 state correspond to the raise in energy and -1 state correspond to decrease in energy; whereas, the 0 state remains unaffected. The splitting of spin states under the influence of magnetic field are shown in figure 5.12:

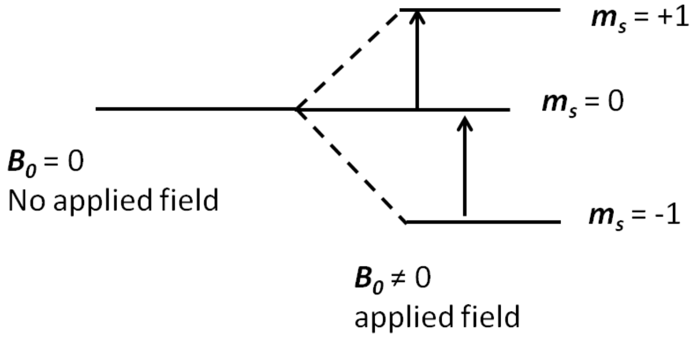


Figure 5.12: Splitting of spin states in a magnetic field

According to the selection rules of ESR for spin allowed transitions only two transitions are allowed $i.e., m_s = -1\ to\ m_s = 0$; and $m_s = 0\ to\ m_s = +1$. Both the transitions have identical energy hence gives rise only one line in ESR spectrum. However, actually two spectral lines of different energy areinvariably observed. This is because of the following reason.

Since, the triplet state arises when the two electrons have parallel spins. Each spin produces a small magnetic field in the vicinity of other and as result it affects the net magnetic field experienced by its neighbour. This phenomenon is known as the dipolar spin-spin coupling. Thus, both spins in $m_s = +1$ experience an applied field greater than the external magnetic field (***B₀***), as a result this state is raised in energy. Similarly, for the spin $m_s = -1$, both experience an applied field smaller than the external magnetic field (***B₀***), which again raises their energy. However, in the case of $m_s = 0$ the dipolar field is across the applied magnetic field, hence it experience neither increase nor decrease in energy of spin states. Thus, $m_s = 0$ state remains unaffected by the dipolar spin-spin coupling. Therefore, because of this dipolar spin-spin interaction the degeneracy of spin state is lifted even in the absence of applied magnetic field. Thus, the splitting of spin states even in the absence of applied magnetic field is called zero-field splitting.

When an increasing magnetic field is applied, the $m_s = +1$ level is raised in energy and $m_s = -1$ level is lowered. This results in the formation of an unsymmetrical splitting of spin states, and transition takes place between two different energy states under the influence of applied magnetic field and the spectrum thus obtained will consists two fine structural lines (Figure 5.13).

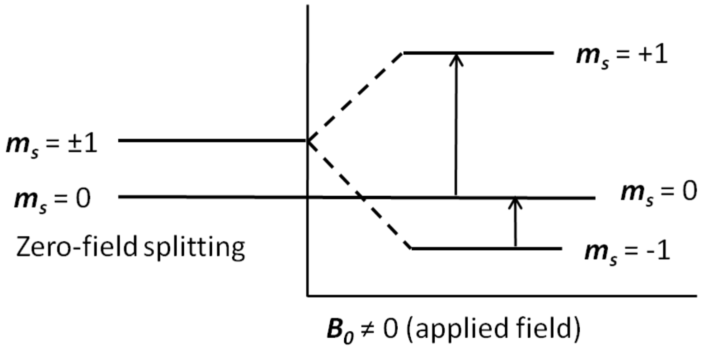


Figure 5.13 Zero-field splitting and unsymmetrical splitting of spin states

5.12 *APPLICATIONS OF ELECTRON SPIN RESONANCE SPECTROSCOPY:*

The interpretation of ESR spectrum of simple substances is very easy because the provided spectrum is fully resolved and absorption lines are narrow. However, in complex substances the ESR spectrum usually does not contains all the expected lines because the ‘g’-factor and coupling values are such that two lines may overlap and thus, the lines are not resolved.It is also observed that many equivalent nuclei may undergo interaction as a result the relative heights of the peaks increases. This results the difficulty to locate the smallest peaks which play important role in the interpretation of ESR spectrum of complex molecules.

An ESR spectrum provides following common information.

1. The ESR spectrum helps t decide the site with unpaired electron.
2. The number of line components in ESR spectrum gives the information about the type and number of nuclei present in the vicinity of unpaired electron.

3. The relative intensities of the spectrum lines in an ESR spectrum confirm the type of nuclei which are responsible for the splitting pattern.
4. The summation of relative intensities can be utilised to evaluate the total number of free electrons in the sample.
5. The ESR spectrum can also be useful to measure the value of land's splitting factor 'g' by comparing the position of the line with that of the standard substance of known 'g' value, e.g., DPPH powder ('g' = 2.0036) or $K_2(SO_3)_2NO$ ('g' = 2.0057).
6. If the electric field is not spherical, the ESR spectrum is anisotropic, i.e., the rotation of the sample shifts the ESR spectrum.

The important applications of ESR spectroscopy are discussed as follow:

1. Study of Free radicals: The free radicals are very short-lived species therefore their precise detection is needed for their study. One of the most important applications of ESR spectroscopy is in the determination of the structure of organic and inorganic free radicals. These radicals may be produced chemically, photochemically or by using high energy radiation. ESR spectroscopy can detect free radicals even at very low concentrations. A good ESR spectrum can be obtained if free radical is produced in a concentration of 10^{-13}mol dm^{-3}. The intensity of an ESR signal is directly proportional to the number of the free radicals present. The hyperfine structure of the ESR spectrum is a kind of fingerprint that helps to identify the radical present in a sample. Besides using nuclear hyperfine splitting to explain the number of magnetic nuclei present nearby the unpaired electron can also help in mapping the unpaired spin density.This can be done by using the McComell Equation:

$$a = Q\rho$$

For example, in case of hydrogen atom $a = 50\ mT$, and ρ for 1s orbital is 1; hence Q = 50 mT. For $^{\bullet}CH_3$ radical, $a = 2.3\ mT$.Using $Q = 50\ mT$,ρcanbe calculatedas

$$\rho = \frac{2.3}{50} = 0.046$$

This signifies that the electron spends 5% time in 1s orbital of each hydrogen atom and remaining 85% of its time near carbon.

2. Study in the Reaction Velocity and Mechanism: In organic chemistry many reactions occur via radical mechanism. Most of the radicals formed during these reactions are unstable and highly reactive. Therefore, the rapid flow system is generally utilised in order to maintain a high and enough concentration of radical generated. In order to obtain this a special cell is designed to permit the compounds to mix together and immediately flow through the observation area. For example, the characterisation of $^{\bullet}CH_2OH$ radical which contains two equivalent protons. This radical may be produced in a flow system by mixing a solution of $Ti^{+3}$and CH_3OH with H_2O_2 solution. At sufficiently low pH the OH proton shows no detectable hyperfine splitting because of the rapid exchange of this proton. The following reactions are proposed to take place to produce $^{\bullet}CH_2OH$ radical:

$$Ti^{+3} + H_2O_2 \longrightarrow Ti^{+4} + HO^{\bullet} + HO^{\ominus}$$

$$Ti^{+3} + HO^{\bullet} \longrightarrow Ti^{+4} + HO^{\ominus}$$

$$HO^{\bullet} + H_2O_2 \longrightarrow H_2O + HO_2^{\bullet}$$

$$HO^{\bullet} + CH_3OH \longrightarrow H_2O + {}^{\bullet}CH_2OH$$

3. Application in Biological systems: ESR studies can also be applied successfully to biological systems. Some important examples are listed below:

i. The presence of free radical has been studied by ESR in healthy and diseased tissue.
ii. With the help of ESR spectroscopy of biological systems it is observed that the a definite correlation exists between the concentration of free radicals and the metabolic activity of the material.
iii. The ESR spectroscopy also confirms that most of the oxidative enzymes function via single-electron redox reactions that involves in the production of either enzyme-bound free radicals or by a change in the valence state of a transition metal ion.
iv. Most of the ESR work on photosynthesis has been carried with photosynthetic bacteria. When these bacteria are irradiated with near IR light (λ = 700-900 nm). A single-line ESR signal with '*g*' = 2.0025 and ΔH ~ 10G is observed. However, a weak ESR signal is obtained for the photosynthetic bacteria in dark.

5.13*SUMMARY:*

In present unit, you studied that

1. ESR transitions occur in the microwave region of the electromagnetic spectrum and are accompanied by a change in the spin of the unpaired electron.
2. The ESR spectra are recorded as derivative curves.
3. The coupling of the electron and nuclear spins results in nuclear hyperfine interaction. This results in the splitting of ESR line.
4. The splitting is denoted by the hyperfine splitting constant, 'a'. The splitting pattern helps in the determination of structure of free radical.
5. The 'g'-factor for the electron is a universal constant and has the value 'g' = 2.00232. The derivation from this 'g'-value indicates the departure from the free radical behaviour (*i.e.*, delocalization).

5.14*TERMINAL QUESTIONS/ANSWERS:*

Q.1. Calculate the ESR frequency of an unpaired electron in a magnetic field of 3000 G (0.3 T).

Solution: Given that: g = 2.0023;$\mu_B = 9.2741 \times 10^{-24}\, JT^{-1}$; B_0 = 0.3T; and h = $9.27 \times 10^{-34}\, Js$

We know that $$\Delta E = h\nu = g\mu_B B_0$$

$$\nu = \frac{g\mu_B B_0}{h}$$

$$= \frac{(2.0023)(9.2741 \times 10^{-24} JT^{-1})(0.3T)}{6.626 \times 10^{-34} Js}$$

$$\nu = 8.397\ \times 10^{9} Hz$$

Q.2. Calculate the value of g id the methyl radical show ESR at 0.329T and the ESR spectrometer is operating at 9.230 GHz.

Solution: Given that:$\mu_B = 9.2741 \times 10^{-24}\, JT^{-1}$; B_0 = 0.329 T; $\nu = 9.230\ GHz\ or\ 9.23\ \times 10^{9}\ Hz$; and h = $9.27 \times 10^{-34}\, Js$

We know that

$$\Delta E = h\nu = g\mu_B B_0$$

$$g = \frac{h\nu}{\mu_B B_0}$$

$$g = \frac{(6.626 \times 10^{-34} Js)(9.23 \ \times 10^{9}\ Hz)}{(9.2741 \times 10^{-24} JT^{-1})(0.329T)} = 2.0044$$

Q.3. The benzene anion has g = 2.0025. At what magnetic induction B, would its ESR spectral line be centred at frequency 9.350 GHz?

Hint: Use $\Delta E = h\nu = g\mu_B B_0$ equation and solve accordingly

Q.4. How many ESR spectral lines are observed if an unpaired electron, delocalised on two non-equivalent protons, is placed in magnetic field?

Q.5. Which of the following systems will show electron spin resonance (ESR)spectrum?

(i) H (ii) H_2 (iii) Na^+ (iv) Cl^- (v) $^\bullet CH_3$ (vi) NO_2 (vii) CO_2^-

Hints: (i) '**H**' has one unpaired electron. Therefore, it will show ESR spectrum.

(ii) 'H_2' has no unpaired electron. Therefore, it will not show ESR spectrum.

(iii) 'Na^+' has no unpaired electron. Therefore, it will not show ESR spectrum.

(iv) 'Cl^-' has no unpaired electron. Therefore, it will not show ESR spectrum.

(v) '$^\bullet CH_3$' has one unpaired electron. Therefore, it will show ESR spectrum.

(vi) NO_2has a total 23 electrons, out of which 17 are valance electrons. The electronic distribution among the valance molecular orbital shows that the molecule has one unpaired electron hence, it will show ESR spectrum.

(vii) $CO_2^-$is isoelectronic with NO_2, therefore, it will also show ESR spectrum

Unit 6: BASIC PRINCIPLES OF MOSSBAUER SPECTROSCOPY

Content:

6.1. OBJECTIVES:

After studying this unit students shall be able to:

- Know about the Mossbauer spectroscopy and its principle
- Learn about the recoilless emission and absorption
- Understand the nuclear γ-resonance or the Mossbauer effect
- Learn the important characteristics of a Mossbauer nuclei
- Describe the various kinds of hyperfine interactions such as isomer shift, magnetic hyperfine splitting and quadrupole splitting
- Know about the various application of Mossbauer spectroscopy

6.2. INTRODUCTION:

The Mossbauer spectroscopy deals with the nuclear properties to obtained the information regarding the environment surrounding the nucleus. This technique was first introduced by Rudolf L. Mossbauer in 1958. R. L. Mossbauer while working on doctoral thesis under the supervision of Professor Maier-Leibnitz at Heidelberg/Munich, discovered the *recoilless nuclear resonance absorption (fluorescence) of γ-rays.* This discovery is later known as the *Mossbauer effect*. This technique has now valid applications in diverse fields, such as solid-state physics, metallurgy, chemistry and biochemistry. Thus, Mossbauer spectroscopy is primarily based on the recoilless emission and resonant adsorption of γ-radiation by atomic nuclei. Before we describe the experiments and relevant electric and magnetic hyperfine interactions related to Mossbauer spectroscopy, let us first understand the concept of nuclear γ-resonance and Mossbauer effect.

6.3*NUCLEAR γ-RESONANCE or THE MOSSBAUER EFFECT*:

As we are familiar with the phenomenon of resonant absorption of electromagnetic radiation from the observation of light-induced electronic transitions. The most common reason for the electronic transitions in transition metal ions in inorganic coordination compounds is because of the absorption of the visible light at exactly the energies ofthe splitting of *d*-electrons or at the energies correspondingto metal-to-ligand charge transfer transitions.Only when the quantumenergy of the light matches the energy gap between the electronic states involveddoes such resonant absorption occur. Such transitions are the primary reason of colour in inorganic complexes.

Similar process is also possible for γ-radiation, for which nuclear states are involved as emitters and absorbers. In such experiments, the emission of the γ-rays is mostly triggered by a preceding decay of a radioactive precursor of the resonance nuclei with Z protons and N neutrons. The nuclear reaction (*i.e*,α–, or β– decay, or K-capture) yields the isotopes (Z, N) in the excited state (e) with energy Ee. The excited nucleus has a limited mean lifetime τ and will undergo a transition to its ground state (g) of energy Eg, according to the exponential law of decay. Such transition leads with a certain probability to the emission of a γ-photon with quantum energy $E_0= E_e - E_g$, if the process occurs without recoil. The emitted γ-photon my be reabsorbed by the nucleus of the same kind in its ground state, whereby a transition to the excited state of energy $E_e$takes place. This phenomenon is known as the ***nuclear resonance absorption of γ-rays*** or the ***Mossbauer effect***. The pictorial representation of the Mossbauer effect is shown in figure 6.1.

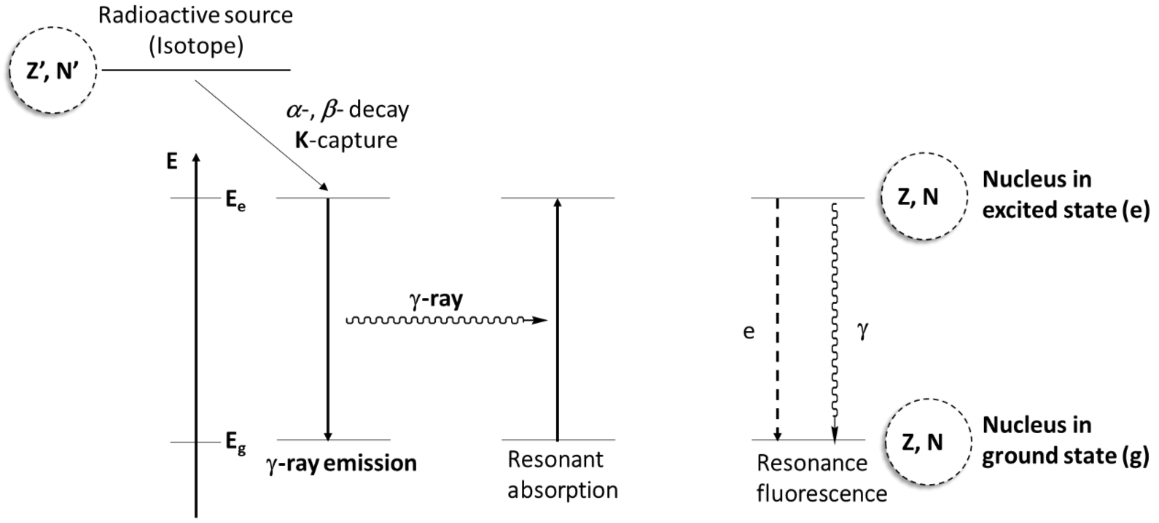


Figure 6.1: Nuclear resonance absorption of γ-rays (Mossbauer effect) for nuclei with Z protons and N neutrons. The Left part of figure shows the population of the excited state of the emitter by the radioactive decay of a source isotope (Z', N') via-α- or β-emission, or K-capture (depending of the isotope).The right part of the figure shows the de-excitation of the

absorber by re-emission of a γ-photon or by radiationless emission of a conversion electron (thin arrows labelled "γ-" and "e", respectively).

Resonant γ-ray absorption is directly connected with nuclear resonance fluorescence. This is the re-emission of a (second) g-ray from the excited state of theabsorber nucleus after resonance absorption. The transition back to the ground stateoccurs with the same mean lifetime t by the emission of a g-ray in an arbitrarydirection, or by energy transfer from the nucleus to the K-shell via internalconversion and the ejection of conversion electrons. Nuclearresonance fluorescence was the basis for the experiments that finally led to R. L.Mossbauer's discovery of nuclear g-resonance in ^{191}Ir and is thebasis of Mossbauer experiments with synchrotron radiation which can be usedinstead of γ-radiation from classical sources.In order to understand the Mossbauer effect and the importance of recoilless emission and absorption, one has to consider a few factors that are mainly related tothe fact that the quantum energy of the g-radiation used for Mossbauer spectroscopy($E_0 \cong 10–100$ keV) is much higher than the typical energies required in optical spectroscopy (1–10 eV). Although, the absolute line width of the energy levels involved in both spectroscopies are quite similar; however, the relative width of the nuclear energy levels is very small due to high mean energies ($\Delta E/E_0 \cong 10^{-13}$ or less). Consequently, the recoil connected with any emission or absorption of a photon is a particular problem for nuclear transitions in gases and liquids, because the energy loss for the γ-quanta is so large that emission and absorption lines do not overlap and nuclear g-resonance is virtually impossible.

6.4*RECOIL-FREE EMISSION AND ABSORPTION:*

If a γ-ray is emitted from a nucleus of a free atom at rest, the nucleus recoils with an energy $R = \frac{\rho^2}{2M}$; where ρrefers to the recoil momentum and M the mass of the nucleus. This recoil energy makes the energy of the emitted γ-ray, E_γ^e, smaller than the energyE_0which would have been obtained in the absence of recoil. If the photon is reproduced because of the transition from an excited state $E_{ex}$to the ground state, E_g, of the nucleus, we have

$$E_{ex} - E_g = E_0 = \frac{\rho^2}{2M} + E_\gamma^e = R_0 + E_\gamma^e \; \dots\dots\dots\dots\dots (6.1)$$

In absorption the energy needed by the nucleus for the same transition is E_0+Ras the absorbing atom also recoils. Thus, it is evident that the emission and the absorption lines get separated by 2R as shown in figure 6.2.

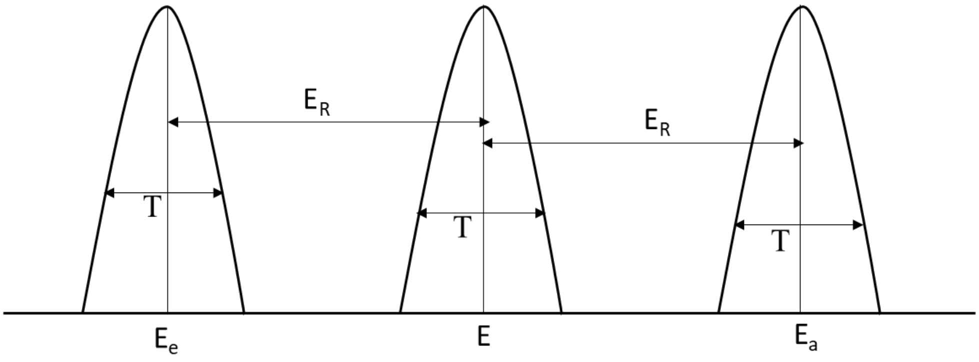


Figure 6.2: Position of E_e and E_a relative to recoil free situation

If these lines are having natural line width T(*tau*) which is small compared to the R a resonance fluorescence experiment in which one nucleus emits and another absorbs the photon does not succeed. To understand the this let us consider an example.

The natural line width T of the 14.4 KeV transition of the ^{57}Fe is of the order of 10^{-9} eV. The recoil energy R for this nucleus is of the order of 10^{-3} eV. Thus, at low temperature where the lines are not broadened became of the thermal motion of the emitting and absorbing nuclei (Doppler broadening) the overlap between the two lines vanishes and the resonance absorption does not occur. As the temperature get increased, the Doppler line width, which is given by $E_0\sqrt{(3k_BT/2Mc^2)}$, also increases. Where, $\frac{3}{2}k_BT$ represents the average kinetic energy of the atom. For the 14.4KeV transition the Doppler line width at room temperature is ≈ 10^{-3} eV. It would then appear that with the increase in temperature the nuclear resonance fluorescence would increase.

In contrast to this, however, the resonance fluorescence in ^{57}Fe get increased when the temperature gets decreased and reaches a maximum as the temperature (T) tends to 0K. Similar observations were reported by Mossbauer on ^{191}Ir nuclei in 1957. To explain these results Mossbauer proposed that some of the ^{191}Ir nuclei are not free in the solid but get firmly embedded in the crystal lattice so that when they decay the recoil momentum is effectively taken up by the whole solid. If this happens the mass M in equation 6.1 has to be replaced by *NM* where *N* is the total number of atoms in the solid and is of the order of 10^{23}. Consequently, the energy of the photon to a sufficiently good approximation is given by the following expression, this confirms the emission is recoilless.

$$E_0 = E_\gamma^e$$

A nucleus embedded in the absorber in a like manner would absorb this recoilless frequency. The process of recoilless emission and absorption in nuclear resonance fluorescence is termed as the ***Mossbauer effect***.

6.5 *CHARACTERISTICS OF MOSSBAUER NUCLEI:*

Nuclei to be used for Mossbauer spectroscopy must have the following characteristics:

1. The radioactive isotope which is utilized as parent nucleus must have fairly long half-life.
2. For a given element a source of gamma ray emission must exist- a parent nucleus whose decay gives rise to the appearance of isomer nuclei.
3. The Mossbauer emission energy lies within the range of a few keV up to lower hundreds of KeV.
4. The lifetime of the isomer excited level should be in the range of ~10^{-6} – 10^{-3} s.The lifetime determines the width T (tau) of lines and ratio T/E ~ 10^{-10} – 10^{-14}. Longer lifetime reduces T/E. Therefore, Doppler velocity becomes too low and measurable only with difficulty. Conversely, with rising T/E, the resonance selectivity diminishes.Some of the characteristics of most important ^{57}Fe nuclei of Mossbauer spectroscopy are given below

1	Isotope abundance	2.17%
2	Energy of the first excited state	14.4KeV
3	Level lifetime (τ)	1.4 x 10^{-7} s
4	Line width (T)	4.6 x 10^{-12} KeV
5	Ratio of line width to energy (T/E)	3 x 10^{-13}
6	Ground state spin	1/2
7	Excited state spin	3/2
8	Ground state magnetic moment	0.095 nm
9	Excited state magnetic moment	- 0.15 nm
10	Quadrupole moment of excited state	0.29 barn

11	Recoil energy E_R	1.9 x 10^{-3}Kev
12	Typical Debey temperature	693 K
13	Doppler velocity due to natural linewidth	0.095 nm/s
14	Recoilless fraction f (with Debey temperature equal to 693 K)	0.92 at 273 K, 0.79 at 573 K

6.6HYPERFINE INTERACTIONS IN *MOSSBAUER SPECTROSCOPY*:

As we have been discussed in chapter 5 that the interaction of magnetic moment of an unpaired electron with neighbouring magnetically active nuclei results in the splitting of energy levels. Which is known as the "Zeeman effect". Similarly, the nuclear states have spin, and associated magnetic dipole moments, and the spins can be oriented with different projections along a magnetic field. In presence of external magnetic field, the energies of nuclear transitions are therefore modified. The energy perturbations caused by this hyperfine magnetic field are sometimes called the "nuclear Zeeman effect," analogous to the more familiar splitting of energy levels of atomic electrons when an atom is placed under the influence of external magnetic field. Three kinds of hyperfine interactions may be observed in a Mossbauer spectrum.

(a). ***Electric monopole interaction***: It is the interaction between the protons of the nucleus and s-electron penetrating the nuclear filed. The observable Mossbauer parameter is the "isomer shift". The isomer shift values give information about the oxidation state, spin state, and bonding properties such as covalency and electronegativity.

(b). ***Magnetic dipole interaction***: It is the interaction between the nuclear magnetic dipole moment and a magnetic field at the nucleus. The observable Mossbauer parameter is the "magnetic splitting ΔE_M. This quantity gives information on the magnetic properties of the material under study.

(c). ***Electric Quadrupole interaction***: It is the interaction between the nuclear quadrupole moment and a heterogeneous electric field at the nucleus. The observable Mossbauer parameter is the "quadrupole splitting ΔE_Q". The information derived from the quadrupole splitting refers to oxidation state, spin state and site symmetry.

The various hyperfine interactions and corresponding Mossbauer parameters are summarized in Table 6.1.

Table 6.1: Various hyperfine interaction and corresponding Mossbauer parameters

Types of Interaction	**Mossbauer Parameter**	**Chemical Information**
Electric monopole (Coulombic) interaction between nucleus and electrons	Isomer Shift	Oxidation state Electronegativity of ligands Nature of bonds Spin state (high spin, low spin, etc.)
Magnetic dipole interaction between nuclear magnetic dipole moment and magnetic field	Magnetic splitting (ΔE_M)	Magnetic properties such as ferromagnetism, antiferromagnetism
Electric quadrupole interaction between nuclear quadrupole moment and heterogeneous electric field	Quadrupole splitting (ΔE_Q)	Molecular symmetry Oxidation state Nature of bond Spin state (high spin, low spin, etc.)

6.6.1 MOSSBAUER ENERGY LEVELS WITH ISOMER SHIFT:

The Coulombic interaction between the nuclear charge and the electron charge alters the energy separation between the ground state and the excited state of the nucleus. Therefore, it causes a slight shift in the position of the observed resonance line. This shift will be different in different chemical compounds. Therefore, it is generally known as the isomer shift or chemical shift or isomer chemical shift. The isomer shift in the Mossbauer spectroscopy reflects the difference in the s-electron density around the emitter and absorber nuclei. As the hyperfine splitting of line width are of the order of ~10^{-9} eV, which changes in chemical environment. Thus, technique is of great importance in the investigation of molecular structure. Generally, the isomer shift decreases as the oxidation state (s-electron density) increases. For example, isomer shifts for iron and tin compounds in their different oxidation states of oxidation are given below:

(1) $_{26}Fe^0 = 1s^2, 2s^2, 2p^6, 3s^2, 3p^6, 4s^2, 3d^6$

Oxidation state of Fe	**+2**	**+3**	**+4**	**+5**
	Fe^{+2} ($3d^6$, $4s^0$)	**Fe^{+3} ($3d^5$, $4s^0$)**	**Fe^{+4} ($3d^4$, $4s^0$)**	**Fe^{+5} ($3d^3$, $4s^0$)**
Isomer shift (mms^{-1})	**1.5**	**0.7**	**0.2**	**-0.6**

(2) $_{50}Sn^0 = 1s^2, 2s^2, 2p^6, 3s^2, 3p^6, 3d^{10}, 4s^2, 4p^6, 4d^{10}, 5s^2, 5p^2$

Oxidation state of Sn	**+2**	**+4**	**+4**
	Sn^{+2} ($5s^2$, $5p^0$)	**Sn^{+4} (sp^3)** **covalent**	**Sn^{+4} ($5s^0$, $5p^0$)**
Isomer shift (mms^{-1})	**3.7**	**2.1**	

The isomer shift in above example can be explained quantitatively by assuming that s-electron shielding is governed by the formal d-electron charge state. Moreover, if the actual or effective charge state of the ion in the crystal is known the above correlation could be more precise. However, it is difficult to know, because the exact calculation of actual or effective charge state depends upon several factors such as nature and number of ligands, the co-ordination number, the molecular structure, electronegativity difference etc.

Thus, the main factor responsible for affecting the magnitude of isomer shift is the electron density at the nucleus. The electron density at nucleus for p- and d- orbitals is zero, hence electron density of s-orbital is only important for isomer shift. The observation of isomer shift permits measurement of relative s-electron density and this gives an idea about the bond character of atoms or ions chemically attached to the Mossbauer nuclei. For example, the ground state valence shell electronic configuration of ^{119}Sn is **$5s^2$, $5p^0$.** The Sn^{+4} state of tin has no outer s-electron ($Sn^{+4} = 5s^0, 5p^0$), while Sn^{+2} state of tin has 2 s-electrons ($Sn^{+2} = 5s^2$, $5p^0$). InSn^{+4}covalent state, where the compound obtained tetrahedral structure (sp^3 hybridization), there is only one s-electron. The isomer shift reflects these structures almost linearly. Thus,from Mossbauer spectrum the valance state of an unknown tin compounds can be determined. The schematic representation of isomer shift is shown in figure 6.3.

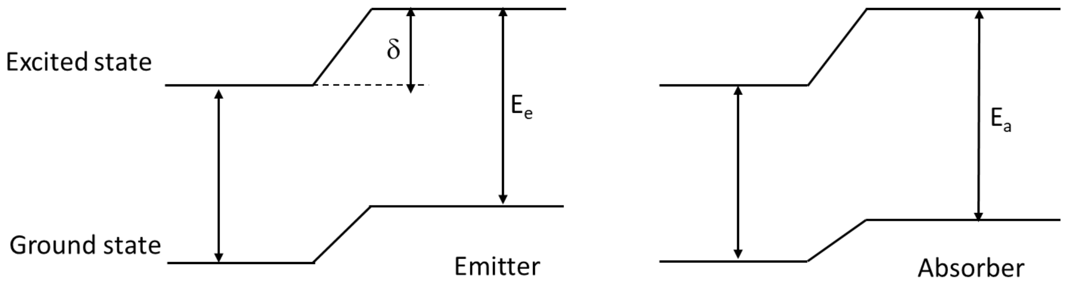


Figure 6.3: Schematic representation of Mossbauer energy levels with isomer shift

6.6.2 MOSSBAUER ENERGY LEVELS WITH MAGNETIC HYPERFINE SPLITTING:

The nucleus possessing a magnetic moment when placed under the influence of external magnetic field the energy levels get perturbed. In the presence of external magnetic field, the nuclear spin magnetic moment experiences a dipolar interaction with magnetic field*i.e.*, nuclear Zeeman effect. The magnetic field at the nucleus dose not extend over the whole crystal but arises due to interaction of the nucleus itself with its own electrons. The internal magnetic field at the nucleus is

$$B = B_{contact} + B_{orbital} + B_{dipole}$$

$B_{contact}$ arises due to the interaction of the nucleus with s-electrons, $B_{orbital}$is due to orbital magnetic moment of the atom, and B_{dipole} is dipolar field due to spin of these electrons. The magnetic field is almost completely linked with the interaction of the nucleus with s-electrons, this is known as the Fermi contact term. The s-electrons are subject to the action of unpaired electron d electrons, which polarize the s-electrons even of the occupied shells. The greatest number of unpaired d-electrons (e.g., five d-electrons) in Fe^{+3} causes a strong polarization of the s-shell and large magnetic field ~40-60 T. Decrease in the number of unpaired d-electrons (e.g., four unpaired d-electrons in Fe^{+2}) leads to decrease of the magnetic field ~20-40T. When the nuclear magnetic moment μ_n, interacts with the filed then the corresponding Hamiltonian (H) and Energy(E) is given be given by $H = -\mu_n B$

$$H = -g_I \beta_N I B$$

Where $\mu_n = g_I \beta_N I$

$$E = -g_I \beta_N m_I B$$

Where $m_I = I, I-1 \ldots\ldots\ldots\ldots -I$. Thus magnetic field splits the nuclear level with spin I into 2I+1 components. For Fe^{57}, g_I= 0.1806 and -0.1033 for ground state and the excited state, respectively. Figure 6.4 shows the magnetic hyperfine splitting for Fe^{+3}. The transitions between these sublevels gives rise to six lines according to the selection rules $\Delta m_I = 0. \pm 1$.

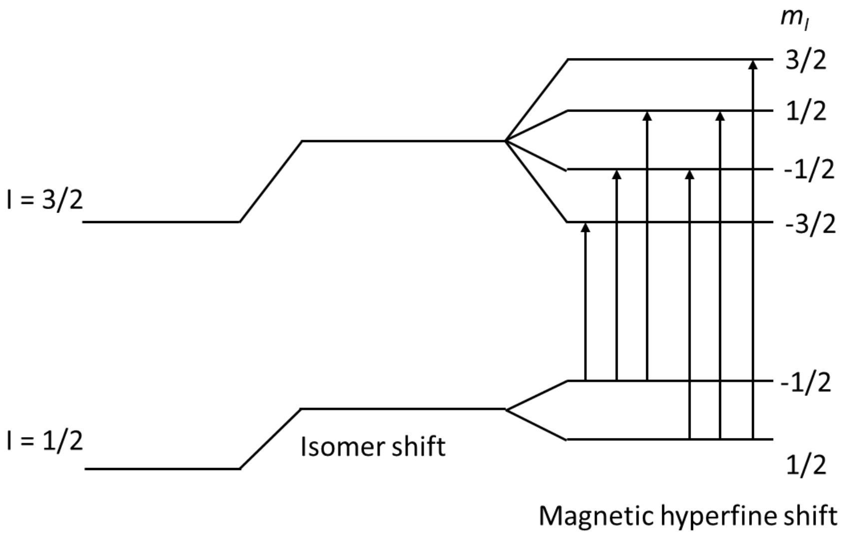


Figure 6.4: Magnetic hyperfine splitting of ground state and first excited state of Fe^{57}

The line positions are related to the splitting of the energy levels, but the line intensity is related to the angle between Mossbauer gamma rays and nuclear spin moment. The splitting of the nuclear levels in the magnetic field is quite similar to the splitting of the levels in nuclear magnetic resonance (NMR). However, there are differences between the NMR and Mossbauer effect as states below:

S. No	Nuclear Magnetic Resonance (NMR)	Mossbauer effect
1.	The magnetic field in NMR is superimposed outer field generated by the magnet ~1T.	Intensity of atomic magnetic field is -20-60T.
2.	The ground state is only considered	Both ground state and excited state is considered in Mossbauer effect
3.	NMR results from transition between neighbouring magnetic sublevels during absorption of a radio frequency quantum ~MHz (10^{-7}-10^{-8} eV).	Mossbauer results Gamma transition from magnetic sublevels of the ground level to magnetic sublevels of the excited level occurs
4.	Selection rules for NMR are $\Delta m_I = \pm 1$	Selection rules for Mossbauer are $\Delta m_I = 0. \pm 1$

6.6.3 MOSSBAUER ENERGY LEVELS WITH QUADRUPOLE SPLITTING:

Quadrupole splitting is a salient feature of Mossbauer spectra. However, othertechniques, such as perturbed-angular correlation spectroscopy, nuclear quadrupoleresonance (NQR), solid-state nuclear magnetic resonance (NMR), and electron-paramagnetic or electron-nuclear double resonance (EPR/ENDOR) spectroscopy,can also detect electric quadrupole interaction. Quadrupole splitting arises from the presence of electric field gradient at the Mossbauer nucleus, exactly as the Nuclear Quadrupole Resonance spectroscopy. This is due to the asymmetry of the electron cloud around the nucleus. Any effect that gives a different population in the p_z orbital from that in the (p_x, p_y) orbitals, or in the d_{z^2}, or $d_{x^2-y^2}$ from (d_{xz}, d_{yz}) will produce a splitting of the spectrum which increases as the population imbalance increases. Such imbalance may occur directly through the presence of lone pairs of electrons, *i.e.*, non-bonding electrons, or indirectly through the bonding electrons. The lone pairs of electronsmake the larger contribution to the electric field gradient, *i.e.*, for example in Sn(II) or Sb (III) compounds. For transition metals atoms there is often an imbalance in the d-shell: in octahedral geometry only d^3, d^8, d^{10}, high-spin d^5 and low-spin d^6 configurations make no contribution to Quadrupole splitting. In Mossbauer spectra, the quadrupole splitting becomes apparent in the presence of two conditions:

a. When the Mossbauer nucleus displays the quadrupole moment in the ground state or excited state.
b. The presence of electric field gradient ‘q’ at the site of nucleus position.

The electric field gradient acting on the nucleus is given by

$$q = q_v(1-R) + q_L(1-\gamma_\infty)$$

Where; q = electric field gradient

q_v= Charge contributed by unfilled valance electron shell in transition element

q_L= Charge contributed by non-spherical charge distribution in lattice

(1-R) = Nucleus shielding factor

$(1-\gamma_\infty)$ = Sternheimer factor

The interaction between the nuclear electric quadrupole moment Q and electric field gradient is expressed by the Hamiltonian

$$H = Q.\nabla E$$

And the energy is given by

$$E_Q = \frac{e^2qQ[3m_1^2 - I(I+1)]}{4I(2I-1)}\ eV$$

In ground state the Fe^{57} nucleus has a spin I = ½ and therefore have no quadrupole moment. In the excited state, Fe^{57}have a nuclear spin I = 3/2and therefore have a quadrupole moment. The isomer level (excited level with I = 3/2) splits into two sublevels with m_I= ±3/2, ±1/2. Reversal of the sign of $m_I$not change the nuclear charge distribution. Therefore, electric field gradient will not completely lift the four-fold degeneracy of the I = 3/2 state. The quartet will split into two doublets while I = ½ state will remain degenerate. For I = 3/2, the energy of the sublevelsm_I= ±3/2, ±1/2 are

$$E_{Q\,(\pm\frac{3}{2})} = \frac{e^2qQ}{4}\,eV$$

$$E_{Q\,(\pm\frac{1}{2})} = \frac{e^2qQ}{4}\,eV$$

And

$$\Delta = E_{Q\,(\pm\frac{3}{2})} - E_{Q\,(\pm\frac{1}{2})}$$

$$\Delta = \frac{e^2qQ}{2}\,eV$$

The splitting of the level is shown in figure 6.5. The transition between these energy levels take place according to selection rule $\Delta m_I = 0, \pm 1$ and resulting in two lines. The separation between the transitions$m_I = \pm 1/2 \leftrightarrow \pm 1/2$ (σ-transition) and $m_I = \pm 1/2 \leftrightarrow \pm 3/2$ (π-transition) can be calculated by the energy equations given above. The relative probability of the transitions$m_I = \pm 1/2 \leftrightarrow \pm 1/2$ and $m_I = \pm 3/2 \leftrightarrow \pm 3/2$ are identical, but shows an angular dependence. In monocrystal, for transitions $m_I = \pm 3/2 \leftrightarrow \pm 1/2$ the angular dependence is of the from $3/2(1+\cos^2\theta)$ and from $m_I = \pm 1/2 \leftrightarrow \pm 1/2$ is of the from $(1+3/2\sin^2\theta)$.

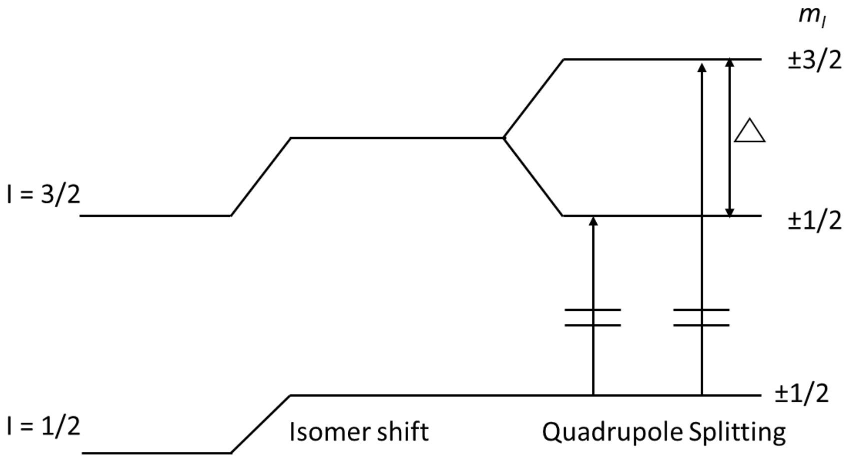


Figure 6.5: Quadrupole Splitting in Fe^{57}

6.7*APPLICATIONS OF MOSSBAUER SPECTROSCOPY:*

Some important applications of Mossbauer spectroscopy are listed below:

1. In Analytical Chemistry: This technique is being used to determine the iron and tin contents in ores, alloys and industrial wastes. With the help of Mossbauer spectroscopy, it is possible to detect the small amounts of iron in complex system such as iron rust and asbestos. Moreover, the concentration of different charge states of the same atoms like Fe+2 and Fe+3 present in a material like Fe2O3 can be readily estimated as these two states have different isomer shift, quadrupole splitting and nuclear Zeemann splitting.
2. Metallurgy: This technique can also be used to study the compositional inhomogeneity in alloys, corrosion mechanism, thermal decomposition processes, etc., at the atomic level.
3. Electronic structure and bonding: The isomer shift and quadrupole splitting data provide information about the electronic state and the character of covalent bonding including the electron acceptor and donor properties of the atom. This also makes the computation of the electron charge cloud distribution in the atom.

6.8 *SUMMARY:*

In present unit, you studied

1. What is Mossbauer effect and Mossbauer spectroscopy?

2.The recoilless emission and absorption are the important factor responsible to generate the Mossbauer spectrum.

3. Important characteristics for Mossbauer nuclei in special reference of $Fe^{57}$and Sn^{119} nuclei.

4. Various kinds of hyperfine interactions their corresponding Mossbauer parameters and the relative chemical information given by these interactions.

5. The important differences between the splitting of energy levels occurs in NMR spectroscopy and Mossbauer effect

6.9*TERMINAL QUESTIONS*:

Q1. Explain Mossbauer spectroscopy. Describe some of its important applications.

Q2. What is recoilless emission? Elaborate the conditions under which Mossbauer effect is most likely to occur.

Q3. What do you understand by isomer shift? Explain isomer shift for iron and tin compounds in different states of oxidation.

Q4. Write a note on

(a). Isomer shift (b). Magnetic hyperfine splitting (c). Quadrupole effect

Q5. Xalculate the recoil velocity of a free Mossbauer nucleus of mass 1.67 $\times 10^{-25}$ Kg when emitting a gamma ray of 0.1 nm wavelength. What is the Doppler shift of the gamma ray frequency to an outside observer?

[Hint: Doppler shift $\Delta\nu = \frac{\mathrm{v}}{\lambda}$; Momentum conservation $\mathrm{Mv} = \frac{h}{\lambda}$] (**Ans: 3.976 x 10^{11} Hz**)

Q6. The lifetime of Fe^{57} (excited state) is 1.5 x 10^{7} s. The excited state is 14.4 keV above the ground state. Determine the linewidth Γ (tau) and ratio Γ/E.

Hint: $\Gamma = \frac{h}{2\pi\tau}$; τ = 1.5 x 10^{7} s; E = 14.4 keV. **(Ans: $\boldsymbol{\Gamma = 4.4 \text{ x } 10^{-9} eV}$; Γ/E = 3.05 x 10^{-3})**

UNIT 7 :MASS SPECTROMETRY-I

7.1 INTRODUCTION:

At first in 1898 Wein stated that positive ions could be deflected by electric field and magnetic field, then after that in 1912 J.J.Thomson firstlyrecordedthe mass spectra of simple molecules of low molecular mass.This technique for the identification of organic compounds do not absorb the electromagnetic radiations by this reason this technique is called as mass spectrometry instead of mass spectroscopy. In this mass spectrometry, electromagnetic radiation is used for excitation of compound being analyzed for identification while it involves 70 eV energy electron for the bombardment of compound by their fragmentation patterns, not by their interaction with the electromagnetic radiation.Mass spectrometry identifies the organic compounds on the basis of following two observations.

1. By measuring the molecular weight,which gives the idea about the molecular formula.
2. By indicating the point of fragmentation, which gives the idea about molecular skeleton.

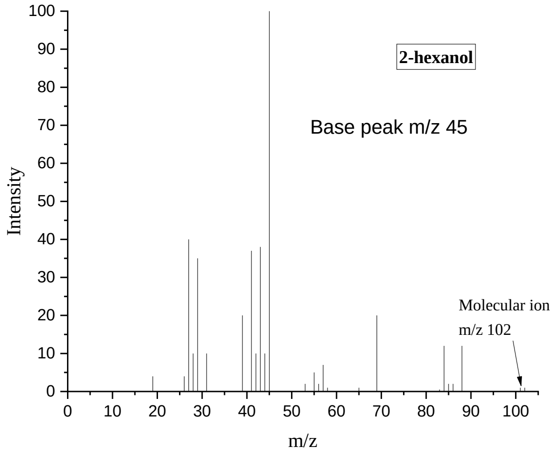


Figure 7.1 Show the molecular ion peak of 2-hexanol

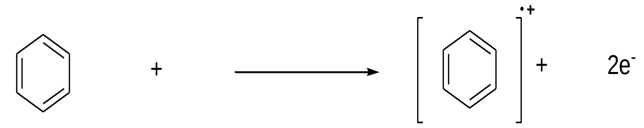
7.2 BASIC THEORY OF MASS SPECTROMETRY:

Mass spectrometry deals with the analysis of molecular ion and fragment ion obtained from the fragmentation of organic molecules. Mass spectra are plotted between relative abundance or intensity of ions and mass/charge ratio.

The mass spectrometry involves the bombardment of the vapour of organic molecules by the beam of high energy electron accelerated from a filament to an energy of 70 eV to generate radical cation ($M^{\cdot+}$) which is called as molecular ion with the ejection of an electron

$$M \quad + \quad e^- \longrightarrow M^{\cdot+} \quad + \quad 2e^-$$

Neutral Molecule — Radical Cation (Molecular Ion)



Neutral molecule — Molecular Ion

Due to the formation of molecular ion from neutral molecule the energy required for electron impact (EI) process having the following order

$$\text{Lone Pair} > \pi \text{ Bonded } e^- \text{ Pair} > \sigma \text{ Bonded } e^- \text{ Pair}$$

This is due to loosely bonded non bonded electron pair and pi bonded electron pair of alkene and aromatic molecules in compare to the electron pair of carbon-carbon and carbon hydrogen sigma bonds .The remaining energy of high energy accelerated lectron is used in breaking of various bonds within the molecular ion . Further fragmentation of molecular ion can generate either even electron cation with the radical or odd electron ion (radical cation) with the neutral molecule in the following fashion .

$$M^{\cdot+} \longrightarrow A^{\cdot+} \quad + \quad D$$

Molecular ion — Radical cation — Neutral Molecule

$$M^{\cdot+} \longrightarrow C^{-} \quad + \quad B^{\cdot}$$

Molecular ion — Even electron ion — Radical

7.3 *MOLECULAR ION AND ITS INTENSITY:*

Radical cation obtained after bombarding high energy accelerated electron beam over the volatile organic molecule is known as molecular ion ($M^{\cdot+}$) . Molecular ion obtained after electron impact (EI) having a particular mass/charge ratio called m/z ratio which is recorded on the graph paper ploted between intensity vs m/z value and called as molecular ion peak . The molecular ion peak gives the idea about the molecular weight of the compound. If lower energy is required for the ionization of molecule then molecular ion will be more stable and more intense will be the peak of molecular ion .Intensity of molecular ion of certain molecules can be given as .

Table 1

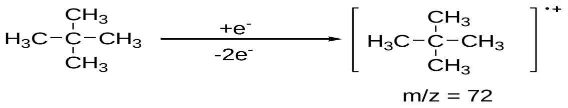
Strong	**Modrate**	**Poor or non-existant**
Aromatic hydrocarbon	Aryl bromide, aryl iodide	Aliphatic alcohol, amines and nitriles
Aryl fluoride, aryl chloride	Conjugated alkenes	Branched hydrocarbons
Fully saturated cyclic compounds	Straight chain aldehyde, ketones, carboxylic acids, esters,amides	Alkyl halides

There are certain factors like extension of conjugation, decrease in branching in parent chain, presence of heteroatom which can easily release the valence shell electron, having the tendency to increase the intensity of molecular ion peak.

7.4 BASE PEAK IN MASS SPECTROMETRY :

Most intense peak in mass spectrum of organic compound is called as base peak. Molecular ion peak or some fragment ion peak can behave as the base peak in mass spectrum according to the nature of compounds .The intensity of base peak in mass spectrum is considered as 100 percent and other peaks intensity will be relative to base peak.Generally most stable cation or radical cation behaves as the most abundance peak or base peak of the molecules.

Example: 1 The mass spectrum of 2,2-dimethyl propane can be represented as



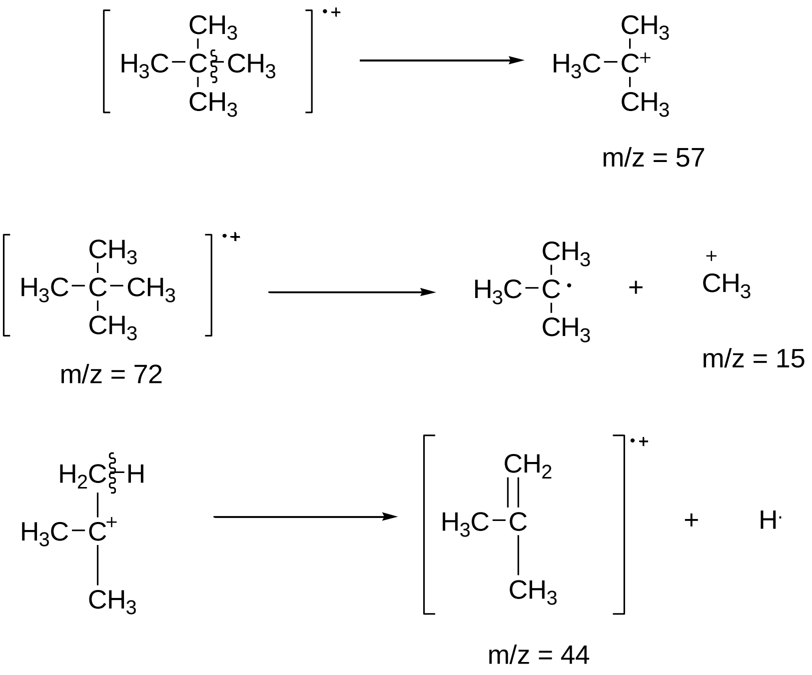


Figure 7.2

In the above mass spectrum of 2,2-dimethyl propane peak corresponding to m/z = 57 behave as most abundance peak or base peak due to most stable nature of 3^o carbocation formed during this fragmentation process.

7.5 DETECTION OF THE ISOTOPES OF ELEMENT:

Some time mass spectrum of the compounds contain(M+1) and (M+2) peak with the molecular ion peak. This peak occurs due to the presence of heavier isotope of elements present in the molecules. In these cases symbol M represent the mass of parent ion or molecular ion while 1 and 2 represent the mass increases due to the presence of heavier isotopes of 1 or 2 mass unit.This indicate that $M^{\cdot +}$ is not the peak of highest m/z value in the mass spectrum.Large no of elements occur naturally in the various isotopic form and mostly it was observed that the lightest of them predominated while heavier isotopes of element occur in lesser extent.

Relative percentage abundance of various heavier isotopes of several elements with respect to the lowest weight isotope can be represented through the following table 3.

Element	**$M^{\cdot +}$**	**M+1**	**M+2**
Hydrogen	^1H (100%)	-	-
Carbon	^{12}C (98.9%)	^{13}C (1.1%)	-

Nitrogen	^{14}N (99.6%)	^{15}N (.4%)	-
Oxygen	$^{16}0$ (99.8%)	-	^{18}O (.2%)
Chlorine	^{35}Cl (75.5%)	-	^{37}Cl (24.5%)
Bromine	^{79}Br (50.5%)	-	^{81}Br (49.5%)
Sulphur	^{32}S (95%)	^{33}S (.8%)	^{34}S (4.2%)

This table conclude that presence of M+1 peak in small proportion with respect to $M^{\cdot+}$ peak indicate the presence of ^{13}C in organic molecule while $M^{\cdot+}$ with M+2 peak in 3:1 indicate the presence of ^{35}Cl with heavier isotopic ^{37}Cl and $M^{\cdot+}$ with M+2 peak in 1:1 indicate the presence of ^{79}Br with heavier isotopic ^{81}Br . Presences of sulphur in compound can also significant M+2 peak.

Example – Mass spectra of methyl bromide shows the peaks at m/z = 94 and m/z = 96 in intensity ratio approximate 1:1 corresponding to $M^{\cdot+}$ and M+2 peak.

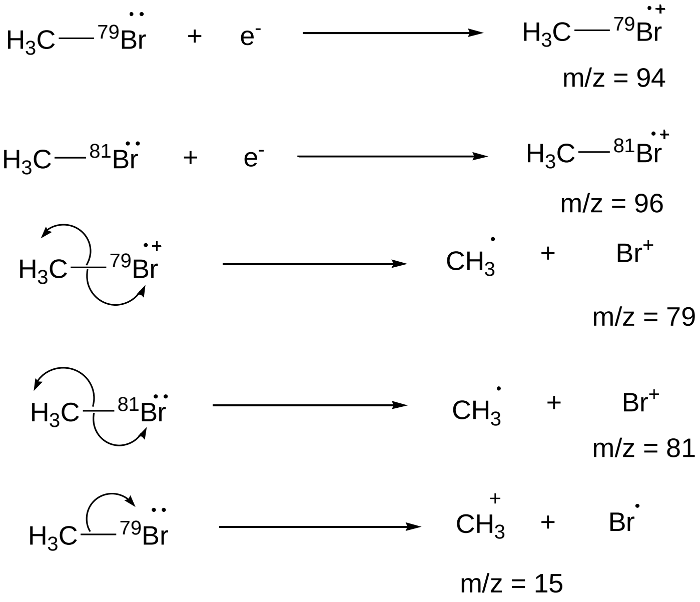


Figure 7.3

7.6 INSTRUMENTATION OF MASS SPECTROMETRY :

General diagrammatic representation for the mass spectrometer is given below –

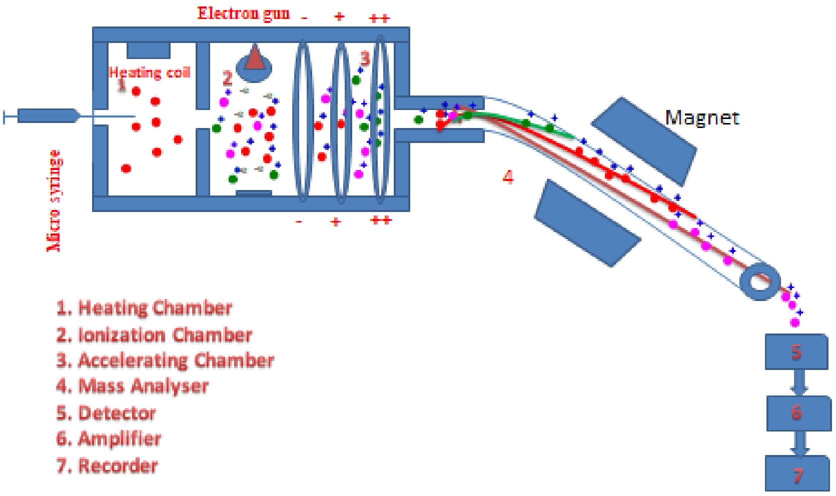


Figure 7. 4

For the mass spectrometric observation at first, sample is introduced into inlet chamber where it is vaporized then after that it is introduced in the high vacume chamber where molecule is ionized into the radical cation or molecular ion ($M^{\cdot+}$) with the help of high energy accelerated electron beam (70 ev) generated by e^- gun ,but energy of 10-15 ev being sufficient to ionize the different type of electron present in the molecules so extra amount of energy of accelerated electron generate fragment ions by the further fragmentation of molecular ion. Now the resulting positive charged ions are accelerated by passing through the positive and negative charged plates into the bent shaped chamber surrounded by magnetic field. By changing the strength of magnetic field radius of curvature of different ions is changed depending on the mass of ion and strength of magnetic field,thus the various ions are separated on the basis of their m/z value.

Derivation-When the cation or radical cation obtained after the ionization process are passed through the accelerating electrodes then the kinetic energy gained by these ions depend on the potential difference (V) according to following relationship

$$\text{Kinetic energy} = \frac{1}{2}mv^2 = zV$$

$$mv^2 = 2zV \qquad \text{------------------ (1)}$$

Where m = mass of ion

V = velocity gained by ions

V = Voltage

Since different ions having, unipositive nature so different ion moves with the different velocity according to their mass. When the ions move from magnetic field then they are deflected and forced into the circular path of which radius depends upon the m/z value according to following equations:

Centripetal force = Bzv ---------------- (2)

Centrifugal force = mv^2/r ---------------- (3)

For the movement of ion in curvature of spectrometer both the centrifugal force and centripetal forces become equal to each other

i.e. $Bzv = mv^2/r$

Since $v = Bzr/m$

Now on putting the value of v in equation (1)

$mv^2 = 2\,zV$

$m(Bzr/m)^2 = 2zV$

$B^2r^2z/m = 2V$

$m/z = \frac{1}{2}B^2r^2/V$

Thus by changing the strength of magnetic field (B), ions with different m/z value are collected successively and resolved according to their m/z value

7.7 IONIZATION TECHNIQUES:

Ionization techniques are two types

1. Soft ionization technique

2. Hard ionization technique

Some of the methods to produce the positive charged ions from organic molecules in the ion source are given below.

7.7.1 Electron ionization method (EI Method)

In this method the vaporized sample molecules are introduced in the ion source where sample molecules are bombarded by high energy electron as a result of which there occur the ejection of electron from the sample molecules to generate the radical cation (molecular ion) according to the following equation

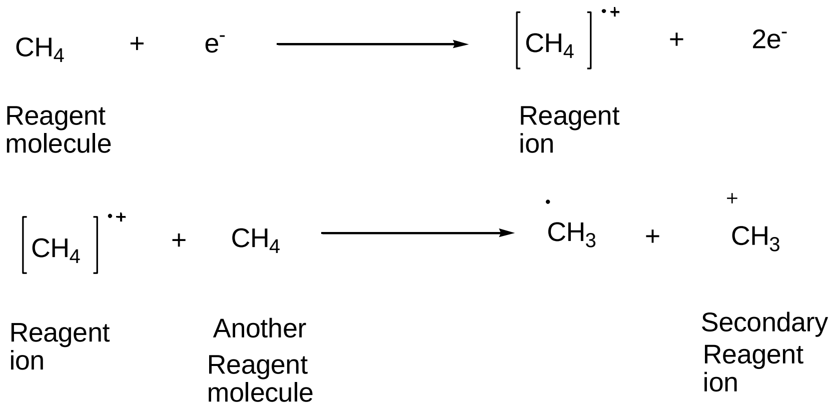
$$M + e^- \longrightarrow M^{\bullet +} + 2e^-$$

Figure 5

The accelerated electron used in this method having high energy (70 ev)which on bombardment with sample molecules produces positive ion with some negative charged ion as well as neutral particles.

7.7.2 Chemical ionization method (CI Method)

In the chemical ionization method reagent like methane, isobutene or ammonia is introduced in the high pressure ion source then they are ionized by electron bombardment. The ion produced from the reagent molecules undergo ion-molecular collision with another molecule of reagent to generate the secondary reagent ions according to following equations.

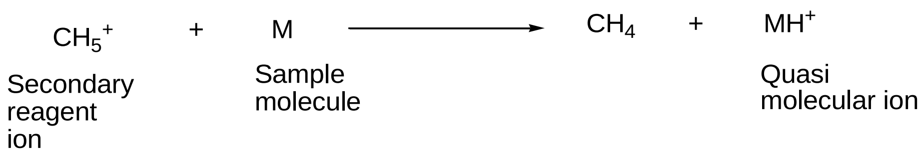


$$CH_4 + e^- \longrightarrow [CH_4]^{\bullet +} + 2e^-$$

$$[CH_4]^{\bullet +} + CH_4 \longrightarrow \overset{\bullet}{C}H_3 + \overset{+}{C}H_3$$

Figure 7.6

Finally introducing a small amount of sample moleculs into ion source in vapour phase generate the cation from sample molecule (M)



$$CH_5^+ + M \longrightarrow CH_4 + MH^+$$

Figure 7.7

The protonated nolecular ion (MH^+)having m/z value one amu greater than that of molecular ion ($M^{\cdot +}$) is called as quasi-molecular ion.

7.7.3 Fast Atom Bombardment Technique (FAB)

This is the soft ionization technique classified under desorption technique. FAB is used to determine the molecular weight of the molecules having size from 300 to 6000 dalton. By this reason this technique is used to determine the molecular weight of peptides. In this method firstly sample is dissolved in non-volatile,viscous nature of liquids like glycerol, thioglycerol, 3-nitro benzyl alcohol etc, known as matrix materialsto generate the sample – matrix mixture .

$$\text{Sample} + \text{Matrix material} \longrightarrow \text{Sample -Matrix mixture}$$

Now ionisation is done by using the bombardment of fast moving neutral atoms of rare gases like xenon or argon. For this purpose to obtain the fast moving neutral atoms at first rare gas atoms are ionised and passed through electric field to obtain the fast moving radical cation according to following equation :

$$Xe + e^- \longrightarrow \overset{\cdot +}{Xe} + 2e^-$$

Now these radical cation colloids with other neutral molecule to generate fast moving neutral according to following equation:

$$\overset{\cdot +}{Xe}\text{ (fast)} + Xe \longrightarrow Xe\text{ (fast)} + \overset{\cdot +}{Xe}$$

‘Finally these fast moving neutral atom on collision with sample- matrix mixture generate the protonated molecular ion with m/z higher by one unit with respect to the m/z value of molecular ion and called as quasi molecular ion . The hydrogen ion (H^+ ion) used to generate quasi molecular ion is obtained from matrix material.

7.7.4 Electron spay ionization mass spectrometry

This spectrometry basically used for determination of the high molecular weight biological compound, labile compound, non- volatile compounds like protein,peptides, noble-metal nanocluster. In electron impact ionisation technique where 70eV energy electron bombardment is applied over the analyte for ionisation while remaining energy used for further fragment so EI technique is hard ionisation that is why molecular ion peak of biological molecule not appear this draw back overcome by electron spray ionization (ESI)technique because it is soft ionisation technique.

Procedure

In electron spray ionization the sample is dissolve in suitable solvent and filled in capillary. In the tip of capillary applied high potential 3-4 KV for oxidation of analyte. This capillary attached with nebulizer to form aerosol (small droplet of solvent and analyte). The aerosol formed is oxidized by high tipped voltage.Now the aerosol solvent is desorbed by heated nitrogen and the desorbed droplet containing cluster of molecular ions or quasi molecular ion. Finally due to the columbic repulsion the cluster of molecular ion converted into the individual molecular ion which moves toward the magnetic analyser through ion accelerating chamber and detected by detector.

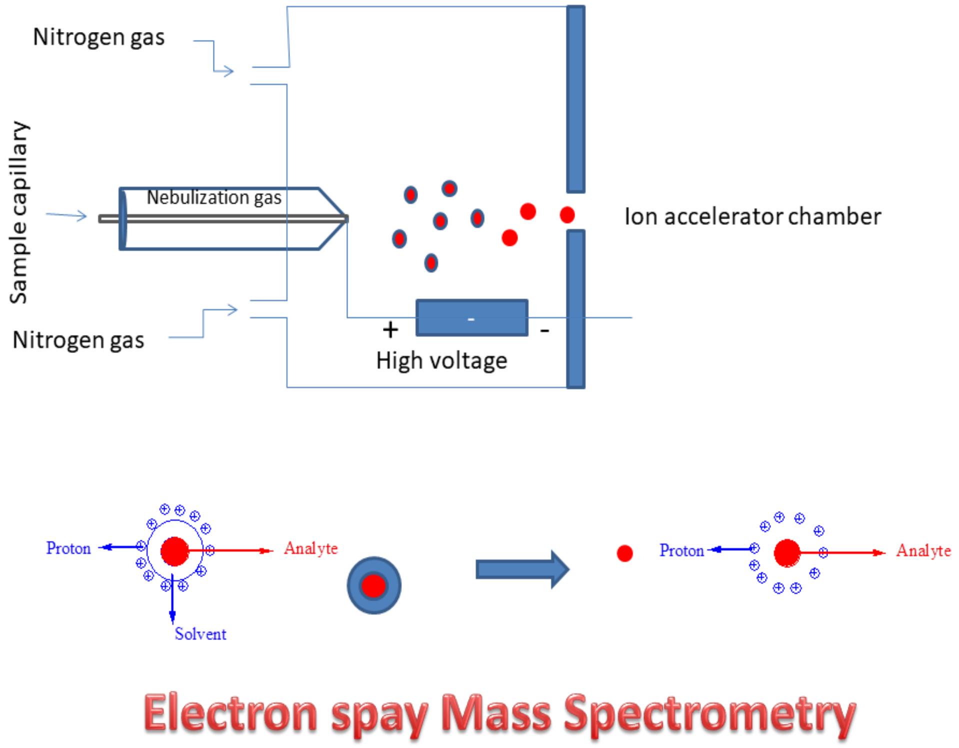


Figure 7.8

7.7.5 MALDI-TOF Spectrometry

MALDI-TOF mass spectrometry is an analytical technique used to detect and characterize the mixture of organic molecules,particularly large biomolecules and synthetic polymers. Full form of MALDI is Matrix Assisted Laser Desorption Ionization. This type of ionization technique involves a laser striking a matrix of small organic molecule to generate the large, breakable analyte molecule into gaseous ions without fragmenting or decomposing them.

TOF means Time Of Flight. This is a mass spectrometric technique that separates the ions according to their mass to charge ratio and determine that mass to charge ratio by the time it takes for the ions to reach the detector.

Procedure

That molecule or mixture which has to be analysed is known as analyte. MALDI-TOF is the best method for analyzing biomolecules like peptides, lipids, nucleotides, saccharides or other organic macromolecules. These macromolecules being so large and decomposed by using traditional ionization technique like electron impact into fragment ions. MALDI is considered as a soft ionization method that ionizes the analye molecules as whole. MALDI ionization process is done by using matrix. The matrix is small organic molecule used to facilitate the ionization process. Urea, 2,5- dihydroxy benzoic acid, 3,5-dimethoxy 4-hydroxycinnamic acid etc are used as matrix molecules. For analysis first sample is co crystallized with matrix

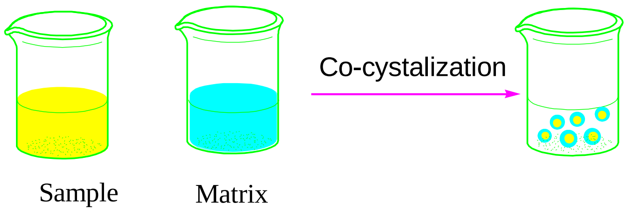


Figure 9

All matrix molecules containing the conjugated pi- system which will be able to absorb the UV light and blast the analyte molecule into gas phase. In this method at first solution of matrix and analyte is deposited onto a sample plate called as target. The target is made up of polished stainless steel and has the spot for several different samples to be applied. The solvent is allowed to evaporate from the sample solution, leaving behind a solid sample spot on the target which will be analyzed by MALDI-TOF spectrometer. Within the spectrometer the target is placed into vaccume and pulse of UV laser having wavelength 337 nm is introduced .The laser energy is absorbed by the matrix molecules which are desorpted from the surface of sample, carrying the analyte molecule into the gas phase. During the desorption process analyte molecules are ionized by proton transfer with the matrix molecule. Now the mass of gas phase analyte ion is determined by using the time of flight method (TOF method).

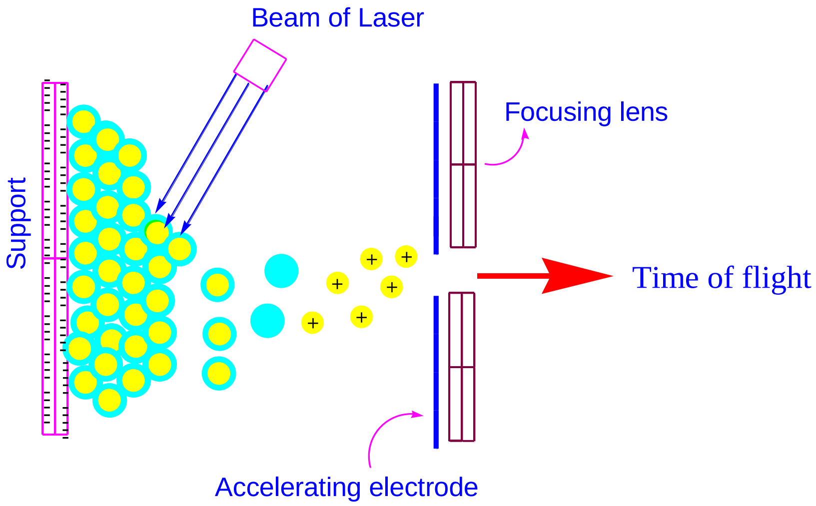


Figure 7.10

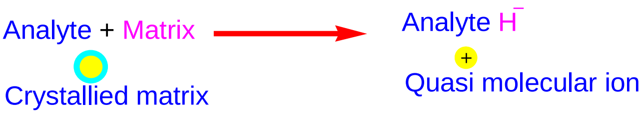


Figure 7.11

The TOF process accelerates the gas phase analyte ion in a high voltage electric field .This high voltage electric field provide a constant amount of kinetic energy over the ions which will cause the smallest ion to travel the fastest . Now the TOF detector records the time it takes for the groups of ions to travel a certain distance. From these time and known constant kinetic energy of electric field the spectrometer will calculate and record mass/charge ratio of each ion it detects

7.8 NITROGEN RULE :

Nitrogen rule is useful for the identification of molecular ion. According to the nitrogen rule even molecular mass containing molecules should contain either zero nitrogen atoms or even number of nitrogen atoms while on the other hand odd number of molecular mass containing molecules will contain odd number of nitrogen atoms. This rule is applicable for all those compounds having carbon, nitrogen, hydrogen, sulfur, oxygen and halogens.

Example1 : 2,4-dinitro phenol containing two nitrogen atoms due to which according to nitrogen rule its m/z value is founded in even number (m/z = 184)

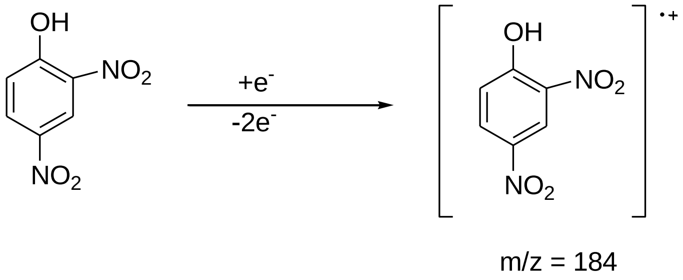


Figure 7.12

Example 3 : Nitrobenzene containing one nitrogen atom due to which according to nitrogen rule its m/z value is founded in odd number (m/z = 123)

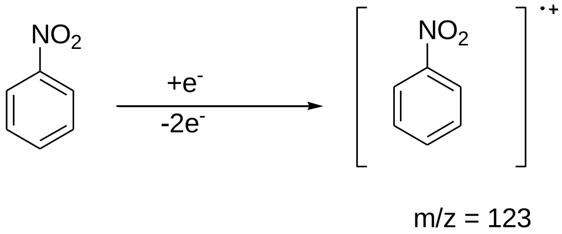


Figure 7.13

When molecule converted to molecular ion the mass remains unchanged so nitrogen rule will be same for molecular ion peak. But the above statement is reverse for cation fragment.

Table 2 gives general idea of mass of radical cation or cation by Nitrogen rule.

Type of ion	Even mass	Odd mass
Molecular radical cation or fragment radical cation (odd no of electron species)	There is no nitrogen or even no of nitrogen(2, 4, 6….)	Odd no of nitrogen 1,3,5….
Cation Fragment (even no of electron)	Odd no of nitrogen 1,3,5….	There is no nitrogen or even no of nitrogen(2, 4, 6….)

Example 3: Radical cation ofpara amino anisolel (molecular ion) has odd no of molecular mass 123 because it contain odd number of nitrogen. This molecular ion on homolytic fission removed methyl radical and became cation, has even number of molecular mass 108 and according to nitrogen rule cation fragment even mass have odd no of Nitrogen.

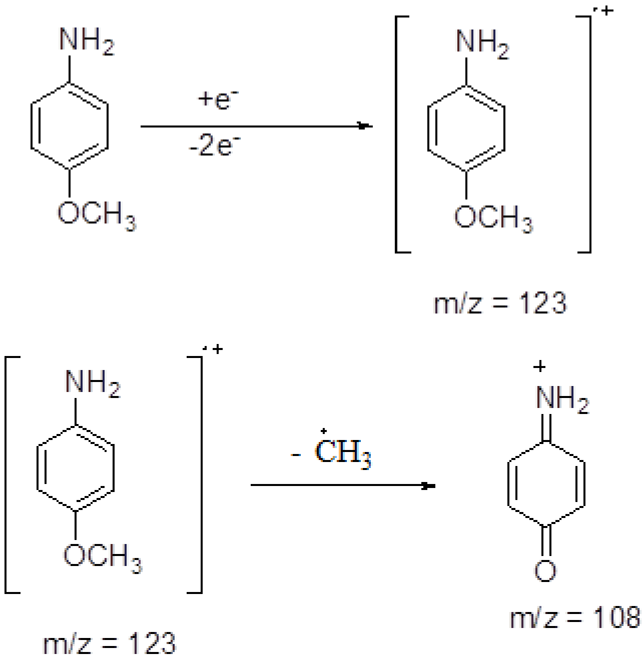


Figure7.14

Nitrogen rule is useful for the identification of molecular ion. According to the nitrogen rule even molecular mass containing molecules should contain either zero nitrogen atoms or even number of nitrogen atoms while on the other hand odd number of molecular mass containing molecules will contain odd number of nitrogen atoms. This rule is applicable for all those compounds having carbon, nitrogen, hydrogen, sulphur, oxygen and halogens.

Example:12,4-dinitro phenol containing two nitrogen atoms due to which according to nitrogen rule its m/z value is founded in even number (m/z = 184

Example: 2 Nitrobenzene containing one nitrogen atom due to which according to nitrogen rule its m/z value is founded in odd number (m/z = 123)

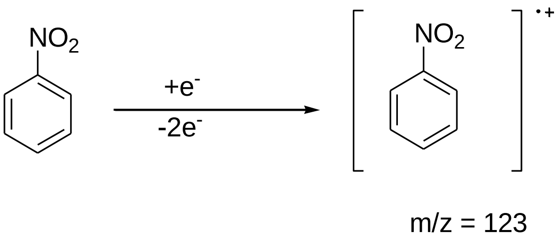


Figure7.15

7.9 METASTABLE ION :

Sometime molecular ion obtained by the ionization of molecular having very shortlife time due to which it undergoes decomposition between the source and collector unit of spectrometer. Thus the ions generated as a result of decomposition are called, metastable ion and the peak corresponding to the metastable ion is known as metastable peak/signal.

Some of the characteristic of the metastable ion can be given as:

1 Metastable peakis being much broader than the normal peak.

2 Metastable ion do not exhibit the integral m/z value.

3 Metastable peak having very low abundance.

Position of metastable peak in mass spectrum can be given by following relationship:

$m^* = \frac{m2}{M}$

Where m* = Position of metastable peak

m = Mass of daughter ion

M = Mass of parent ion

Example : Formation of the metastable ion in the case of p-amino anisol can be represented as:

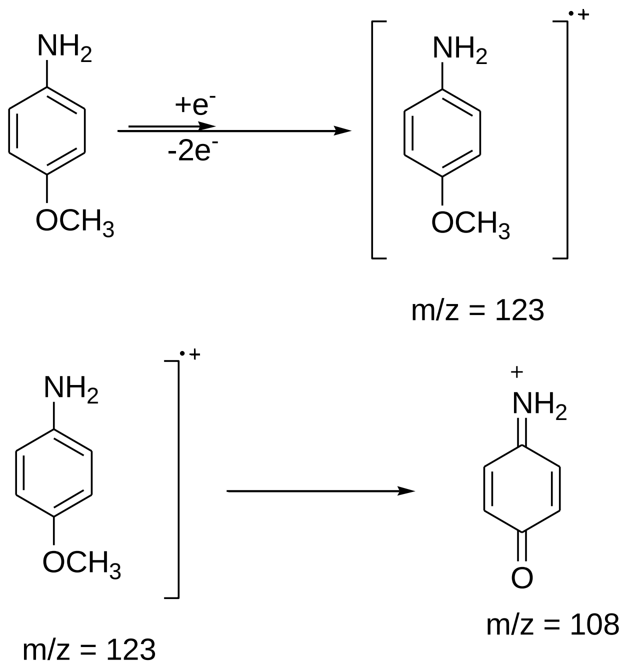


Figure 7.16

The position of metastable peak in this case will be calculated as:

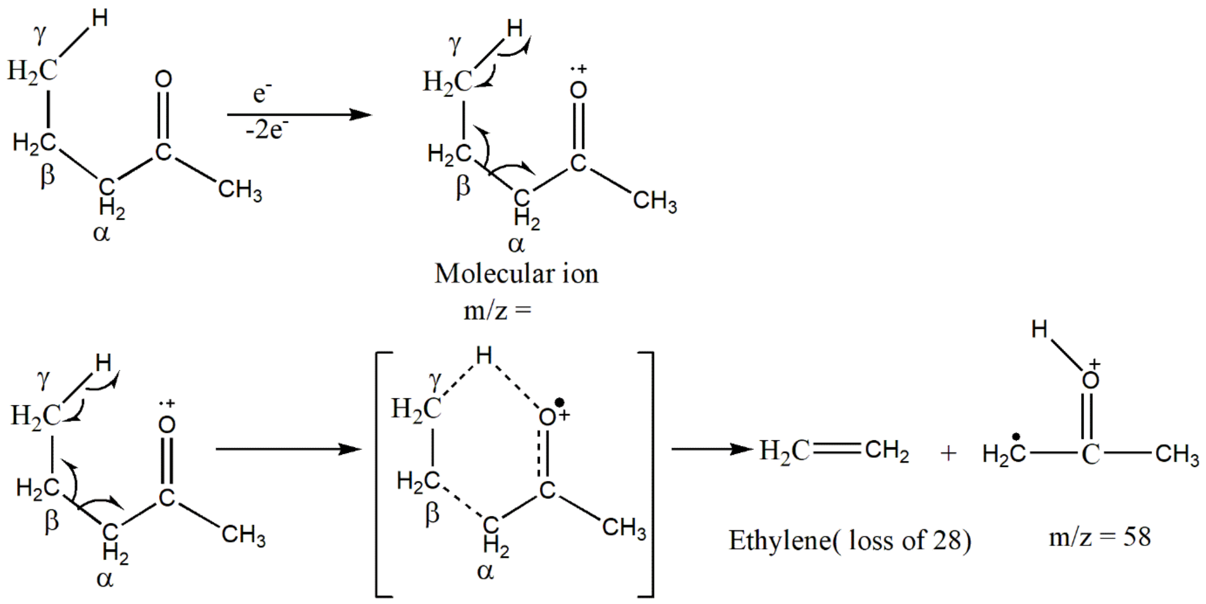
$$m^* = \frac{m2}{M}$$

$$m^* = \frac{(108)(108)}{123} = 94.8$$

7.10 MC LAFFERTY REARRANGEMENT:

β – Cleavage reaction with the γ hydrogen transfer process in the compounds like aldehyde, ketone, amines, unsaturated hydrocarbons, substituted aromatic hydrocarbons etc is known as Mc Lafferty rearrangement reaction, as a result of which there occur the elimination of compounds like alkene with the formation of radical cation through the 6 membered transition state.

Example 1: 2-Pentanone can exhibit the Mc Lafferty rearrangement reaction through the 6 membered transition state that can be represented as:



McLafferty rearrangement shown by one electron movement

Figure 7.17

Example 2: 2-Methyl 1-Pantene exhibit the Mc Lafferty rearrangement reaction through the 6 membered transition state , as a result of which there occur the formation of neutral molecule ethylene with the radical cation (m/z = 56) as a base peak .

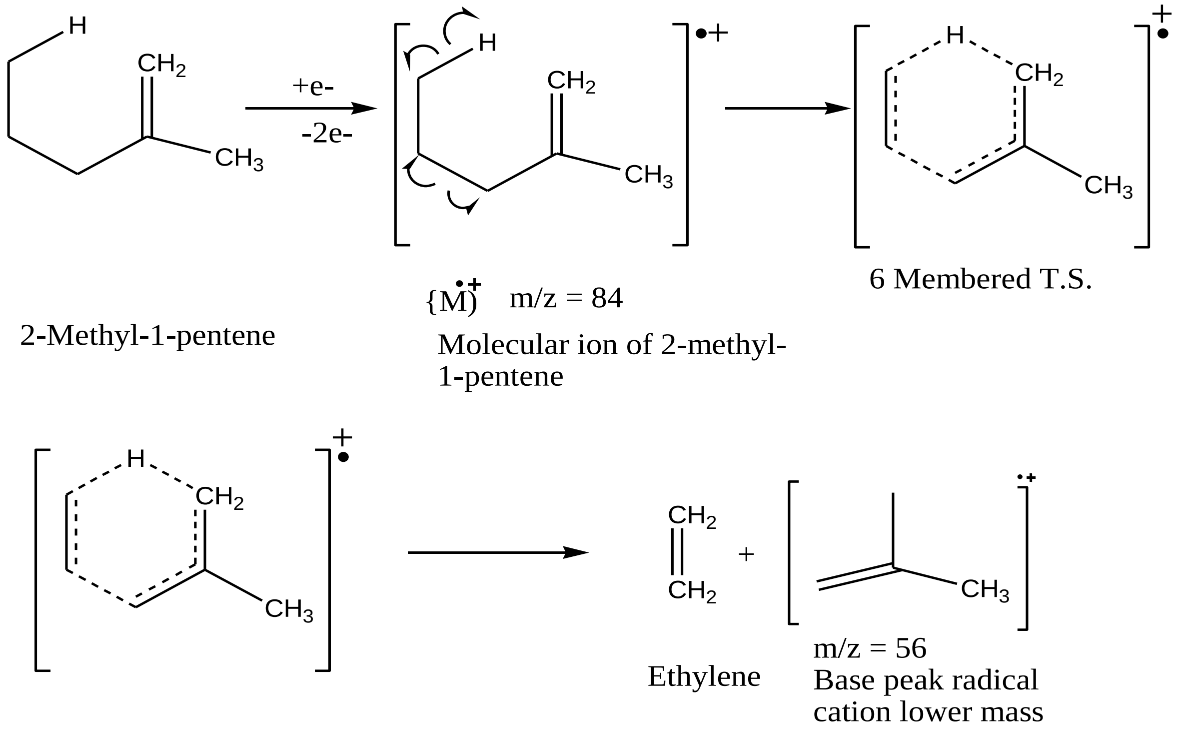


Figure 7.17

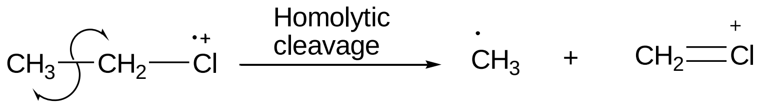
7.11GENERAL FRAGMENTATION MODE IN ORGANIC MOLECULES:

Some of the different types of fragmentation mode which can be possible in various organic species are given below:

7.11.1Homolytic cleavage

This type of cleavage involve the breaking of β bond with respect to the radical cation. This type of cleavage is denoted by half headed arrow and as a result of this type of cleavage covalently bonded atom gain one electron each.

Example:1



7.11.2Heterolytic Cleavage

This type of cleavage involve the breaking of α-bond with respect to the radical cation site, as a result of this type of cleavage there occur the formation of radical and even electron cation. This type of cleavage is denoted by full headed arrow which indicate the movement of both the electron of covalent bond toward an atom.

Example:1

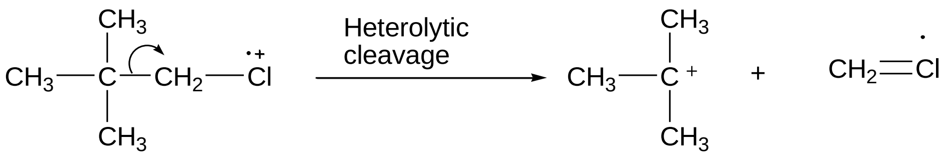


Figure 7.18

These above given fragmentation by homolytic cleavage or heterolytic cleavage can further be specified by certain differen names which are defined as:

7.11.3α – Cleavage

Breaking of the bond α- with respect to the radical cation site either by the homolytic cleavage or heterolytic cleavage is known as α- cleavage.

α – cleavage by heterolytic bond fission is shown in following example:

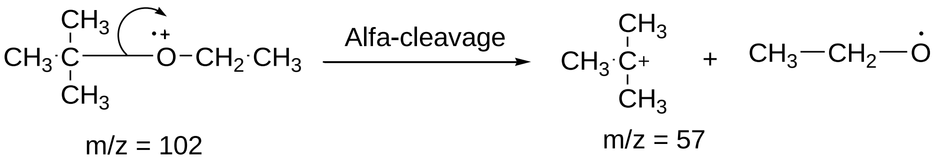


Figure 7.19

α – cleavage by homolytic bond fission is shown by following example:

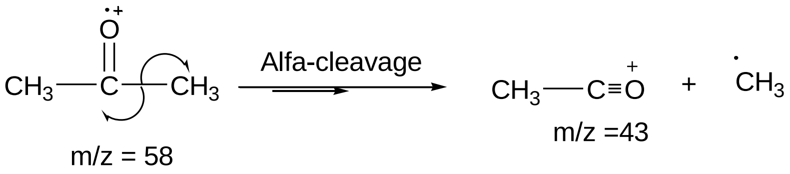


Figure 7.20

7.11.4 β – Cleavage

Breaking of the bond β- with respect to the radical cation site or charged site either by homolytic or heterolytic cleavage is known as β- cleavage. Generally Possibility of the β-cleavage is lesser in compare to the α – cleavage in organic compounds.

β- cleavage in the ethyl amine by homolytic bond fission can be represented as :

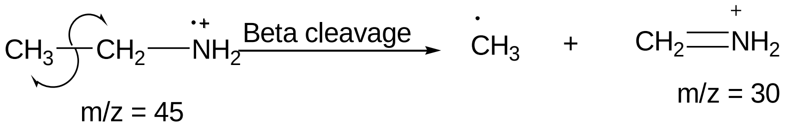


Figure 21

β- cleavage in the 1-methoxy 2,2-dimethyl propane by heterolytic bond fission can be represented as:

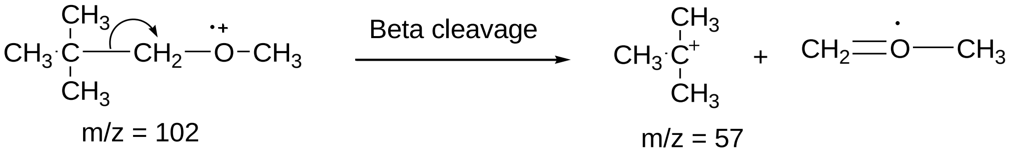


Figure 22

7.11.5 Allylic cleavage

Cleavage of the bond β-with respect to carbon-carbon double bond is knowk as allylic cleavage. This is the best cleavage of alkene because this type of cleavage gives the resonance stabilized allylic cation. This type of cleavage occurs by homolytic fission of the β-bond, with respect to carbon-carbon double bond in alkens.

Allylic cleavage in 1-butene to generate allylic cation is represented as:

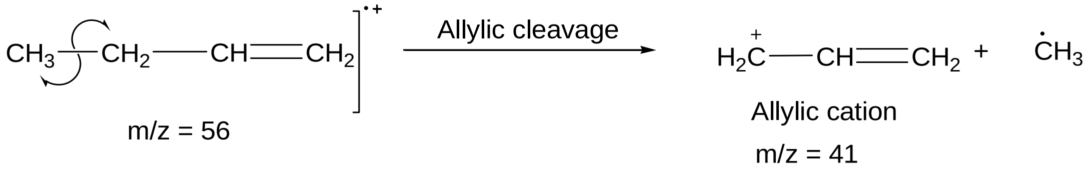


Figure 7.23

7.11.6 Benzylic cleavage

Cleavage of the bond β- with respect to the benzene ring is known as benzylic cleavage. This type of cleavage generates the resonance stabilized benzylic carbocation and finally tropylium ion by this reason this type of cleavage is an energetically favoured cleavage.

Benzylic cleavage in ethyl benzene to obtain the tropylium ion is given as:

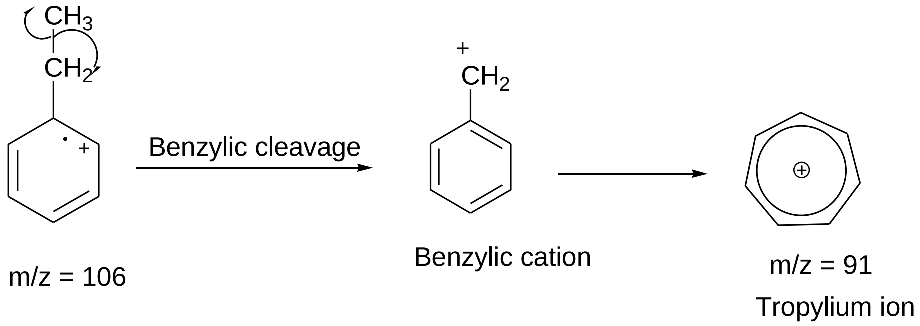


Figure 7.24

7.11.7 Ortho effect

In the ortho substituted aromatic compounds or cis alkenes ,due to close proximity of hydrogen and any substituent ,there occur the elimination of neutral molecules from the molecular ion obtained after the bombardment of high energy accelerated electron. This effect is known as ortho effect.

Elimination of methanol from the molecular ion of methyl crotonate is the ortho effect, that can be represented as:

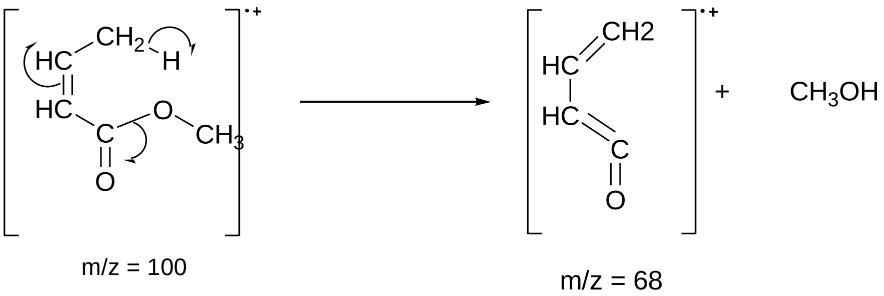


Figure 7.25

Elimination of water molecule from the molecular ion of ortho methyl benzil alcohol can be represented as:

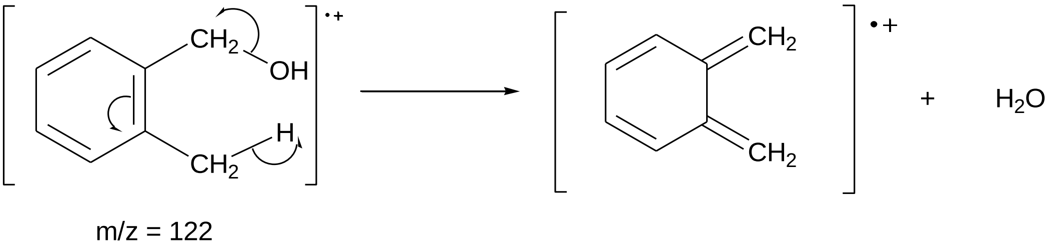


Figure 7.26

7.11.8 Retro Diels-Alder reaction

Formation of two unsaturated molecules after the breaking of molecular ion of cyclic alkenes is known as Retro Diels Alder reaction. This is the most common type of fragmentation of cyclic alkene that can be observed from the following example.

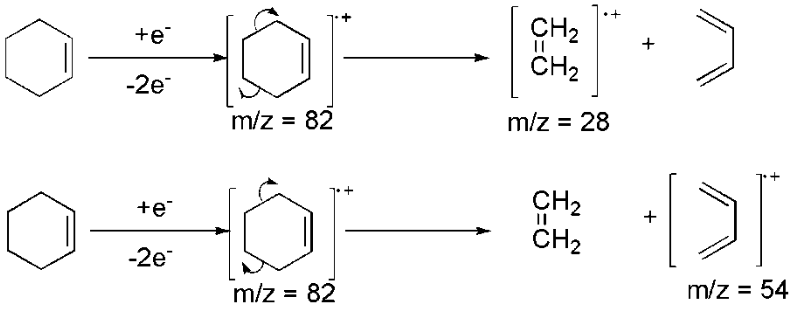


Figure7.27

7.12 SELF ASSESSMENT QUESTION:

1. What will be the structure of organic compound which gives m/z values at 74 (Molecular ion), 56, 43 and 31 (Base peak).
2. What prediction can be done if the organic compound gives M and (M+1) peak in 1:1.
3. Explain why soft ionisation technique like FAB is used in place of hard ionisation technique for generation the molecular ion from of macromolecules like peptides and proteins.
4. What can we predict if the given organic compound gives M and (M+1) peak in 3:1.
5. Define nitrogen rule in mass spectrometry.
6. Explain the following terms used in mass spectrometry
    1. Quasi molecular ion peak
    2. Metastable peak
    3. Isotopic peak
    4. Ortho effect
    5. Even electron rule.
7. What will be the structure of organic compound which gives the peak at m/z 99, 71, 57, 43, 41 and 29.
8. Predict the structure of organic compound which gives peak corresponding to m/z 57 (base peak), 41, 29 and 27.
9. Compound gives peak corresponding to m/z at 106 (molecular ion) and M-1 peak at 105. What can be be the organic compound.
10. In alkane having the molecular formula C_5H_{12} gives base peak at m/z 57. What can be the alkane.

7.13 REFERENCES / SUGGESTED BOOKS :

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2. Introduction to spectroscopy by- Donald L. Pavia, Gary M. Lampman and George S. Vyvyan.
3. Spectroscopy of Organic Compounds by- P. S. Kalsi.
4. Elementry Organic Spectroscopy by- Y.R.Sharma.
5. Organic Chemistry Paula YaukanisBruice.

6. Electrospray Ionization Mass Spectrometry: A Powerful Platform for Noble-Metal Nanocluster Analysis Dr.TiankaiChen,Dr. QiaofengYao,Dr. RiccaRahmanNasaruddin,Prof. JianpingXie ,1,2019.

UNIT 8 :MASS SPECTROMETRY - II

8.1HIGH RESOLUTION MASS SPECTROMETRY:

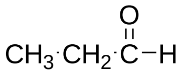
High resolution mass spectrometry is the technique used to differentiate the compound of same m/z value. This particular type of mass spectrometry differentiated the compounds by m/z value up to three to four decimal place.

Example:

$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_3$

n-Butane

m/z = 58



$CH_3 \cdot CH_2 \cdot \overset{\overset{\displaystyle O}{\|}}{C} - H$

Propanal

m/z = 58

When we use low resolution mass spectrometry or normal mass spectrometry then both these compounds exhibit molecular ion peak corresponding to same m/z value (m/z=58). Thus in order to differentiate these two compounds high resolution mass spectrometry is used. The high-resolution mass spectrometry give exact m/z value of butane equal to 58.1222 and that of propanal at 58.0791. On the basis of these exact m/z value butane and propanal can be differentiated.

Table 1: m/z value of certain molecules under low resolution mass spectrometry (LRMS) and high-resolution mass spectrometry(HRMS) are given below:

Molecule	**m/z under LRMS**	**m/z under HRMS**
O_2	32	32.9898
N_2H_4	32	32.0375
CH_3OH	32	32.0262

Thus, these three molecules will be identified only by high resolution mass spectrometry.

8.1.1Limitation: High resolution mass spectrometry does not give the idea about the purity of compounds.

8.2 IMPORTANT CHARACTERISTICS OF THE MASS SPECTRA OF ORGANIC COMPOUNDS:

8.2.1 Mass spectra of alkane

Some important characteristics related to the mass spectra of saturated hydrocarbon (alkane) are given below:

With the increase in the molecular mass in the homologous series of alkane the relative height of the molecular ion peak is decreases.

The most intense peak of alkane being corresponding to general formula C_nH_{2n+1} cation.

In most of cases the intense peak being due to C_4 and C_3 ions corresponding to 3^o and 2^ocarbocations.

Example: Fragmentation pattern in 3-ethyl 3-methyl hexane can be represented:

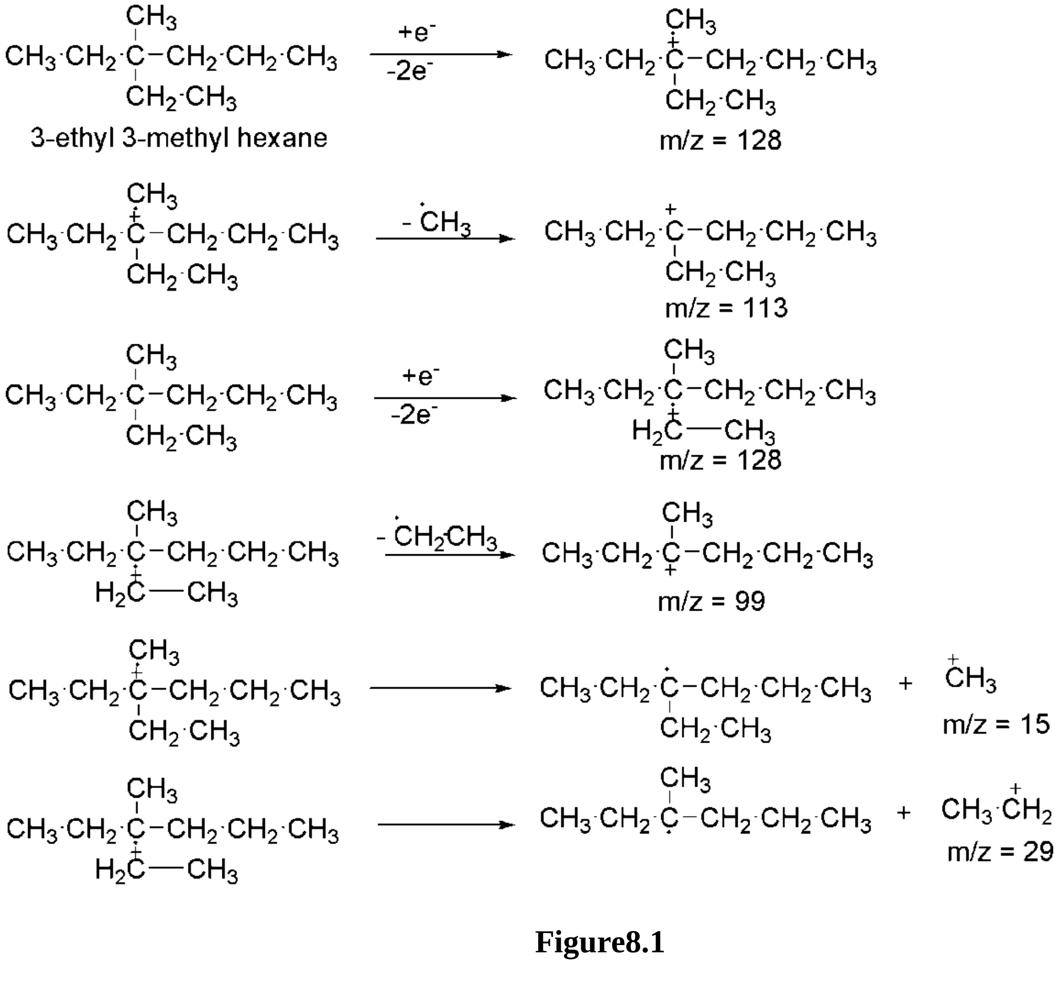


Figure8.1

According to above fragmentation pattern of 3-ethyl 3-methyl hexane the mass spectra of 3-ethyl 3-methyl hexane appeared as:

8.2.2 Mass spectra of cycloalkane

Some of the characteristics related to the mass spectra of cycloalkanes are given below:

1 Relative abundance of the molecular ion of cycloalkane being higher than the corresponding alkane.

2 The most intense peak or base peak of the alkyl substituted cycloalkane being due to the elimination of side chain alkyl free radical.

Example: Fragmentation pattern of n-propyl cyclohexane can be represented as:

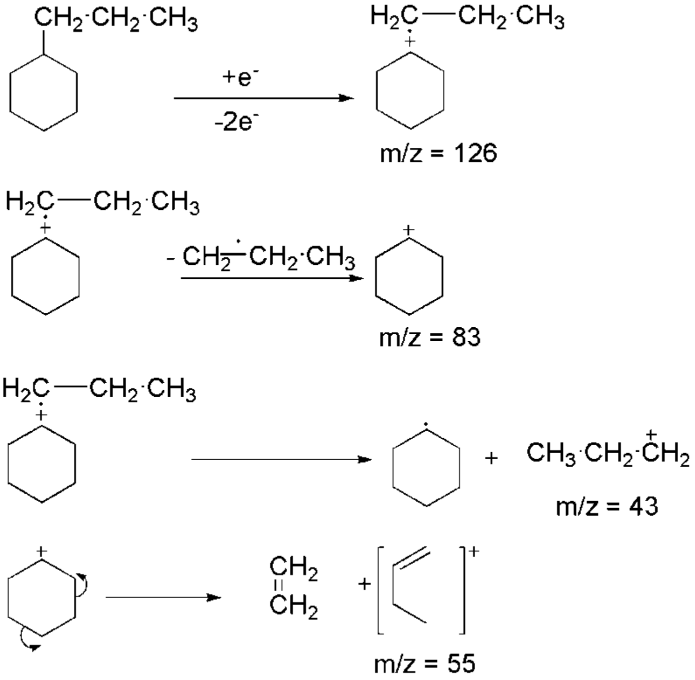


Figure8.2

8.2.3 Mass spectra of alkene

Some of the characteristics related to the mass spectra as well as fragmentation of alkene can be given as:

1. During the formation of molecular ion of olefin by the ionization of neutral molecule electron ejection occur from pi bond and not from sigma bonds due to loosely bonded nature of pi bonded electron pair in compare to sigma bonded electron pair.

2. Molecular ion peak of alkenes being more intense in compare to the saturated hydrocarbons.

3. Possibility of allylic cleavage in alkene being highly favored fragmentation due to the formation of resonance stabilized allylic carbocation.

4. If the alkene containing γ- hydrogen with respect to double bond then Mc Lafferty rearrangement reaction can also be possible in alkene.

Example: Mass fragmentation of 1- butane can be represed as

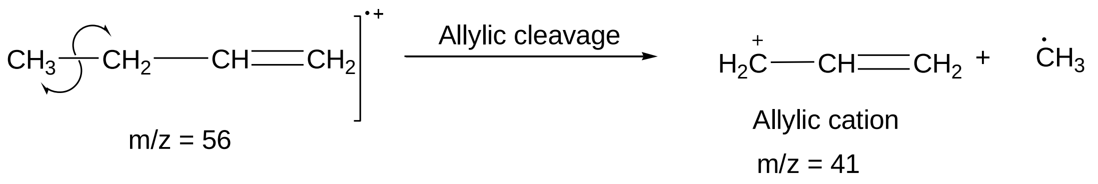


Figure8.3

Example 2:

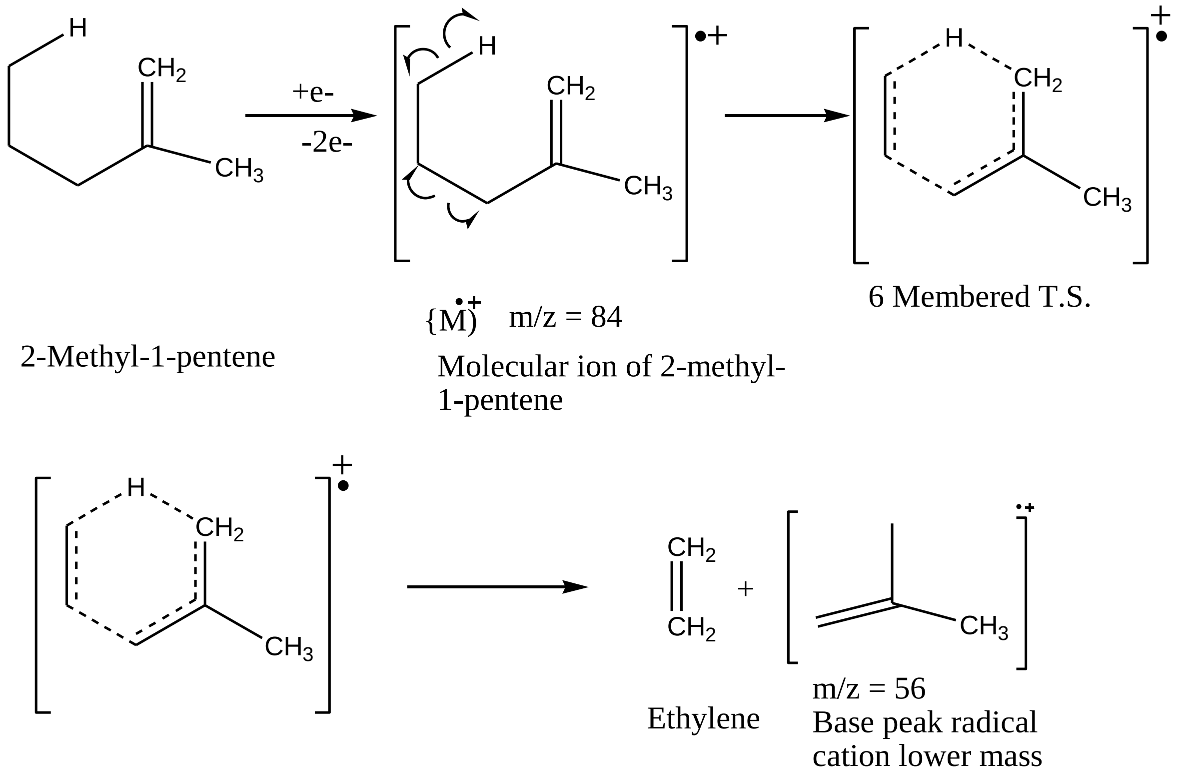


Figure8.4

8.2.4Mass spectra of cycloalkene

Alicyclic compound containing one double bond in the ring like cyclohexene exhibit the retro Diels Alder reaction as the most important type of fragmentation path that can be represented as:

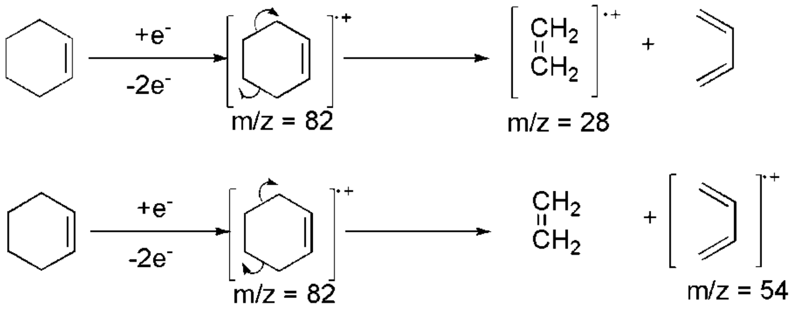


Figure8.5

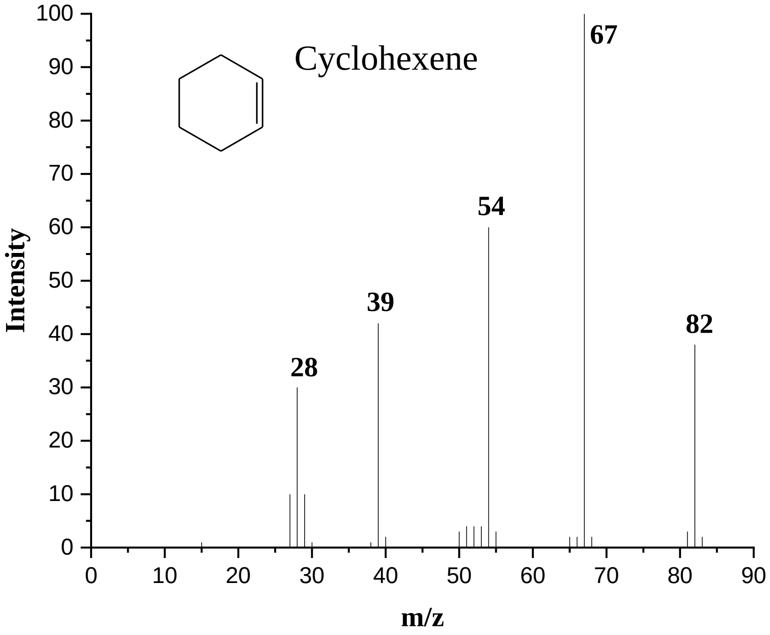


Figure8.6

8.2.5 Mass spectra of aromatic hydrocarbon

Molecular ion peak of the aromatic hydrocarbon being highly intense due to resonance stabilized radical cation obtained after the ionization of neutral aromatic hydrocarbon.

Example 1: Mass spectra of benzene exhibit molecular ion peak at m/z 78. Other peak in mass spectra also occur at 77, 51 and 37. Appearance of all these peaks occur according to following fragmentation pattern.

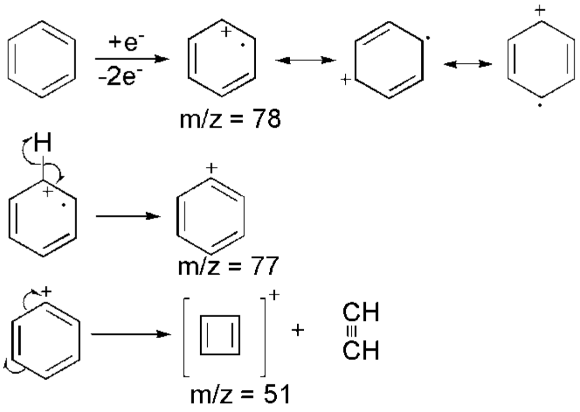


Figure8.7

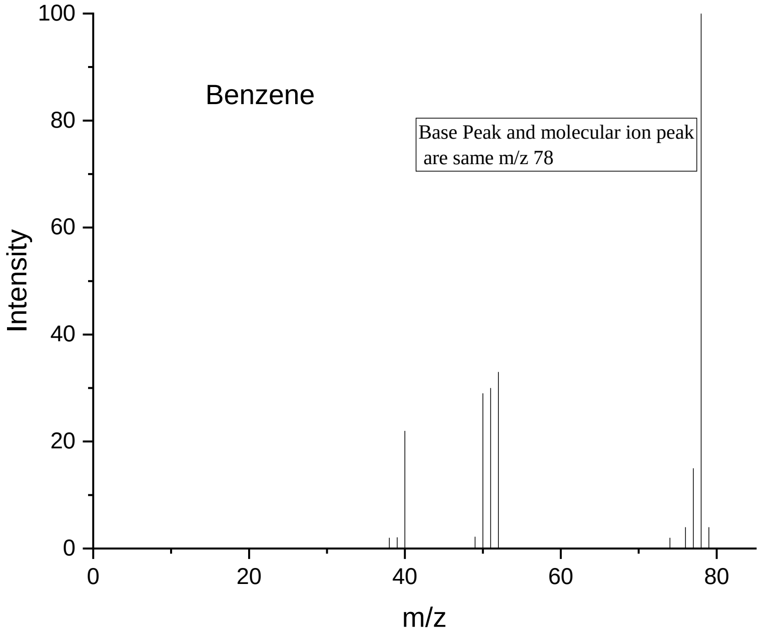


Figure8.8

In the case of alkyl benzene most intense peak occur due to tropylium ion after the cleavage of β-bond with respect to benzene ring in the molecular ion.

Example 2: Mass fragmentation of toluene involve the formation of highly intense peak of tropylium ion can be represented as:

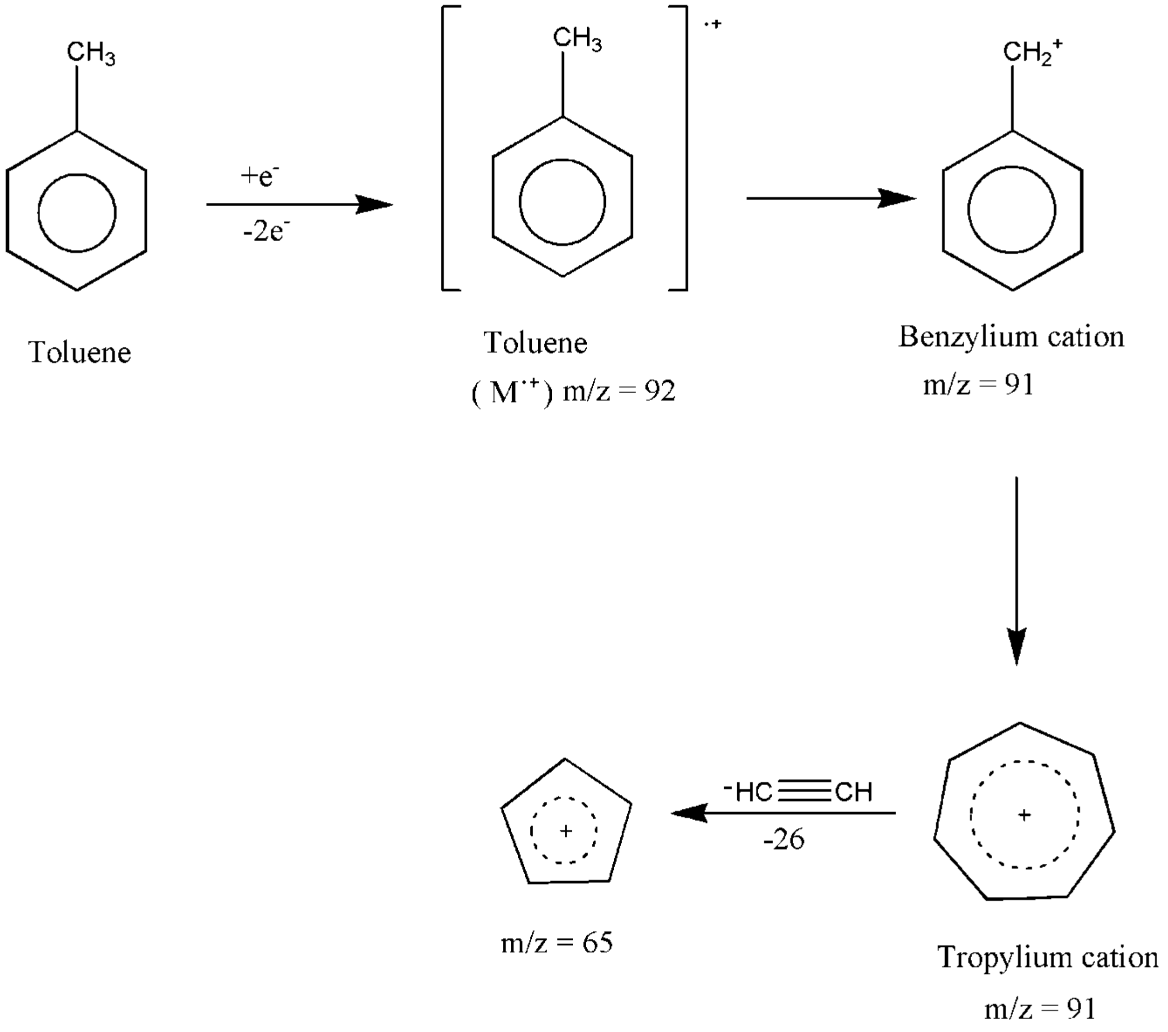


Figure8.9

Example 3: Mass fragmentation of ethyl benzene can be given as:

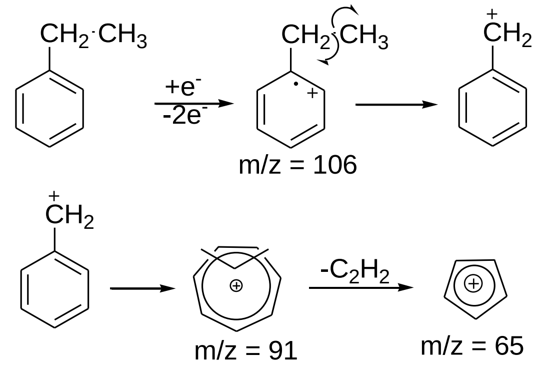


Figure8.10

8.2.6 Mass spectra of alkyl halides

1. Molecular ion peak of alkyl halide being not toomuch higher or too much lower.

2. Intensity of molecular ion peak in alkyl halide is increases with the decrease in the electro negativity of halogen. Intensity of molecular ion peak is also increases with the decrease in the extent of branching at α- position in alkyl halides.

3.In the case of alkyl chloride or alkyl bromide C-X bond is not favoured due to which elimination of other HX is favoured but in the case of alkyl bromide or iodide C-X can be broken easily.

4. In the case of alkyl chloride and alkyl bromide M and (M+2) peak in 3:1 intensity ratio indicate the presence of chlorine. Similarly M and (M+2) peak in 1:1 intensity ratio indicate the presence of bromine in the compound.

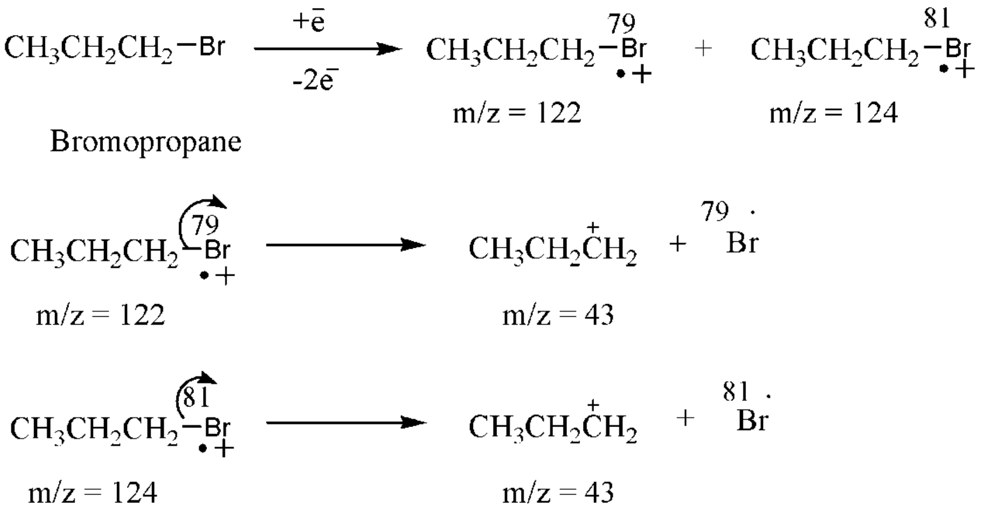


Figure 8.11

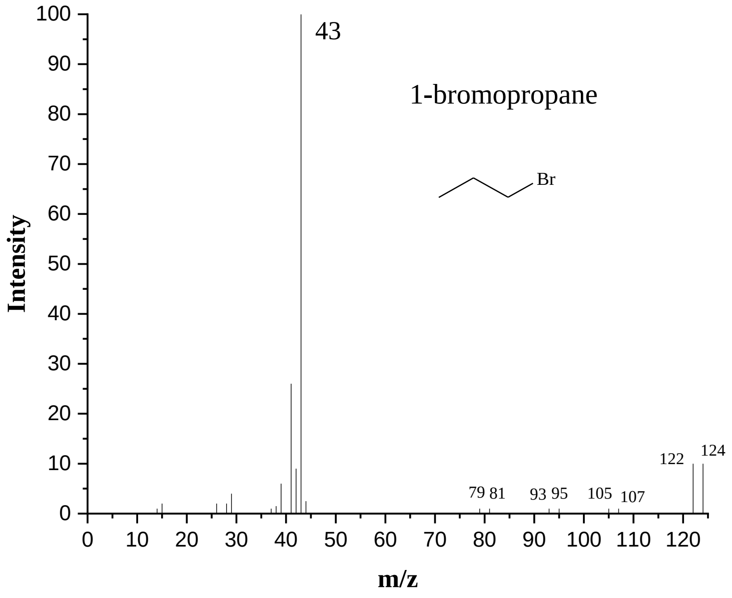


Figure 8.12

8.2.7Mass spectra of aryl halide

1. The molecular ion peak of aryl halides is quite intense.

2. The mode of fragmentation in aryl halide after the formation of molecular ion depends on the strength of C-X bond with respect to C-C bond.

3. In the case of aryl chloride, aryl bromide, and aryl iodide breaking of C-X bond is favoured to generate the phenyl cation but in the case of aryl fluoride elimination of acetylene is preferred over the C-Xbond breaking process.

4. In the case of benzylic halides formation of tropylium ion by the breaking of C-X bond is favoured.

Example 1: In the case of chloro benzene fragmentation pattern to obtain the peak at m/z value 112(molecular ion peak), 77 and 51 can be represented as:

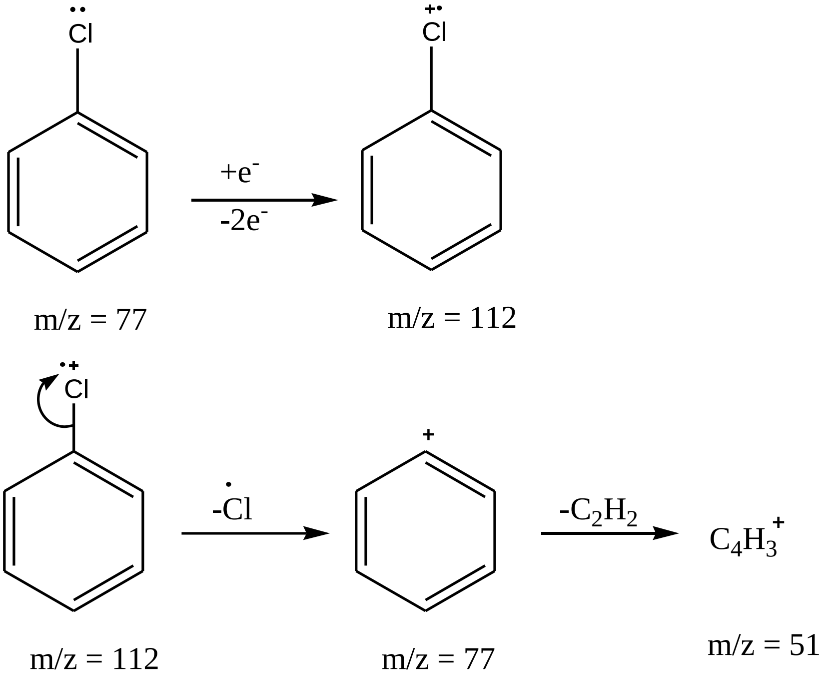


Figure 8.13

Example2: Mass fragmentation of benzylic chloride containing molecular ion peak at 136 and fragment ion peak at m/z 91, 65 can be represented as:

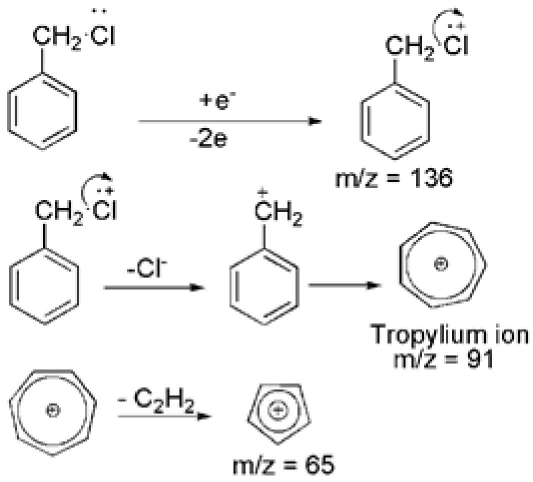


Figure8.14

8.2.8 Mass spectra of alcohols and phenol

1 Mass spectra of 1^0 and 2^0 alcohols containing very weak molecular ion peak and molecular ion peak of 3^0 alcohol are too much weak which cannot be detectable.

2 If the alcohol containing hydrogen at γ-position then they can exhibit M-18 peak by the elimination of H_2O molecule through 6 membered transition state.

3 In the mass spectra of phenol molecular ion peak being highly intense peak,it may be base peak.

4 In the mass spectra of phenol peak corresponding to M-28, due to the elimination of neutral CO molecule being highly intense peak.

5 In the case of allylic alcohol peak corresponding to m/z value equal to M-1 due to the removal of α- hydrogen being highly intense and may become base peak.

Example 1: Mass fragmentation patternof 1-butanol can be represented as:

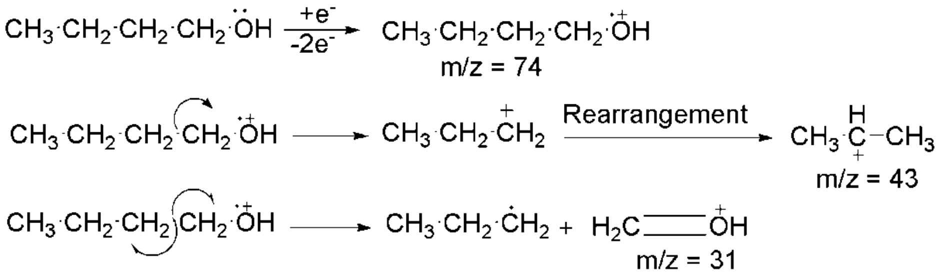


Figure 8.15

Example 2: Mass fragmentation of benzyl alcohol containing highly intense peak corresponding to hydroxyl tropylium ion can be given as:

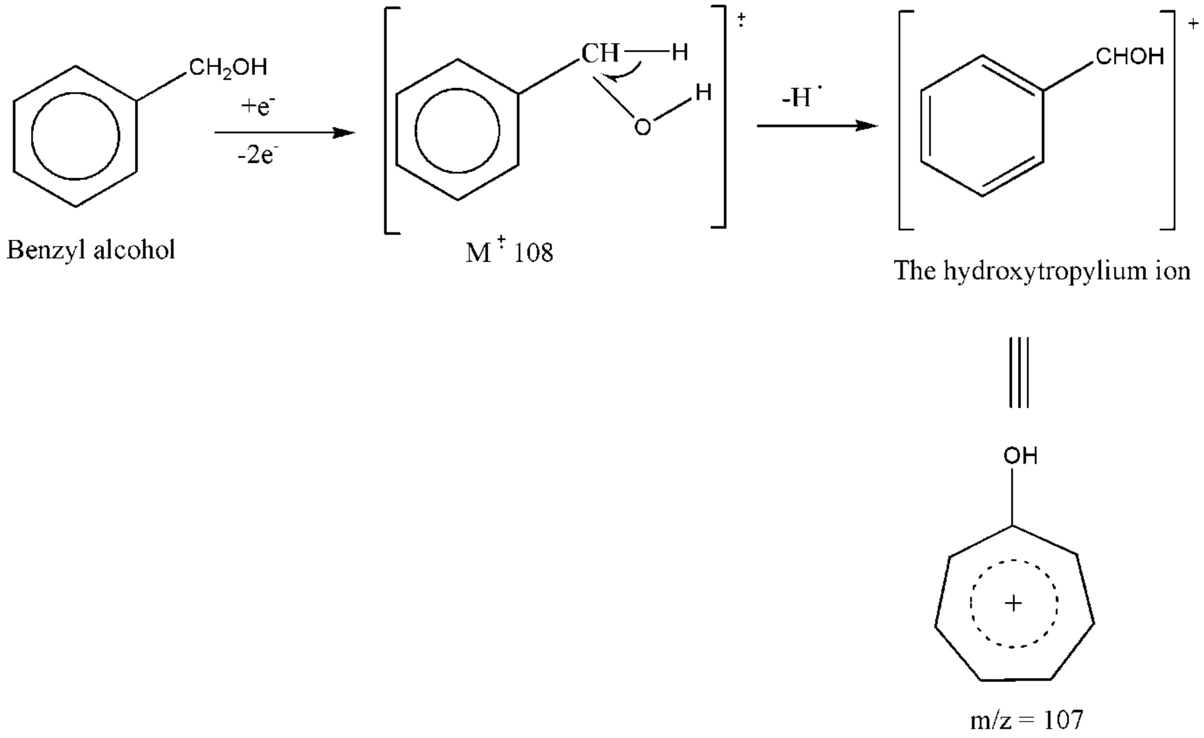


Figure8.16

Example 3: Mass fragmentation of phenol and p-cresol can be representation as:

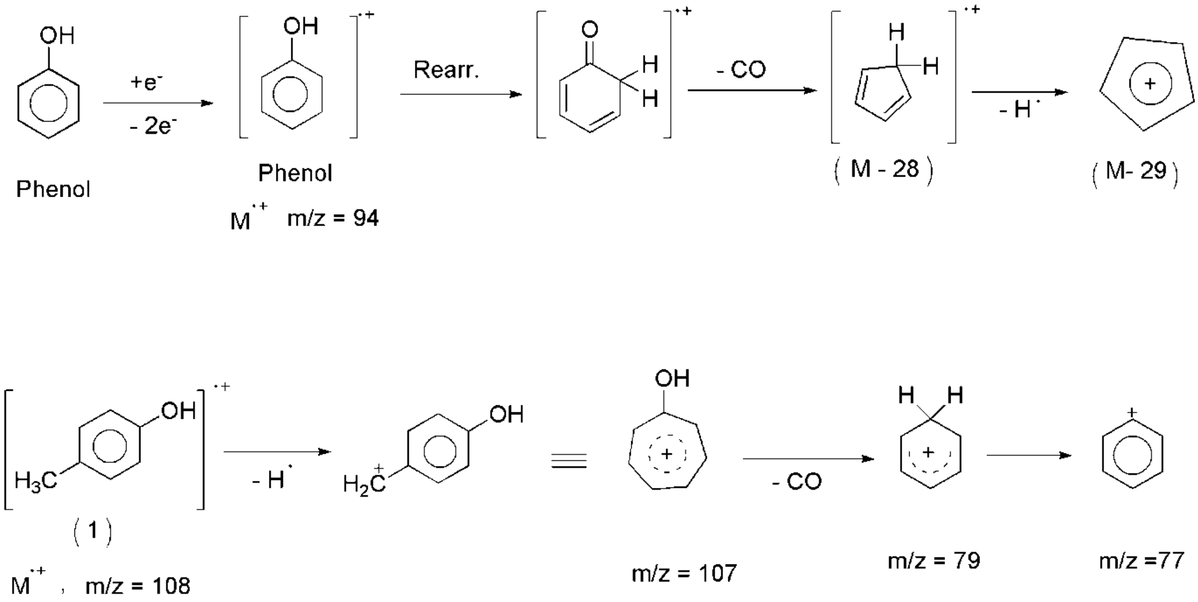


Figure8.17

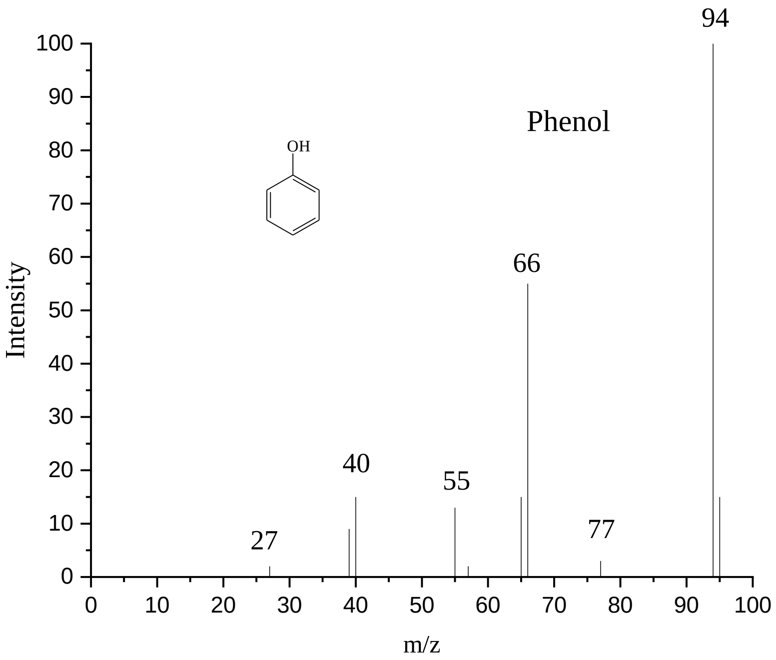


Figure8.18

Example 4: Mass fragmentation of 1-butanol by releasing H_2O through 6 membered transition state can be represented as:

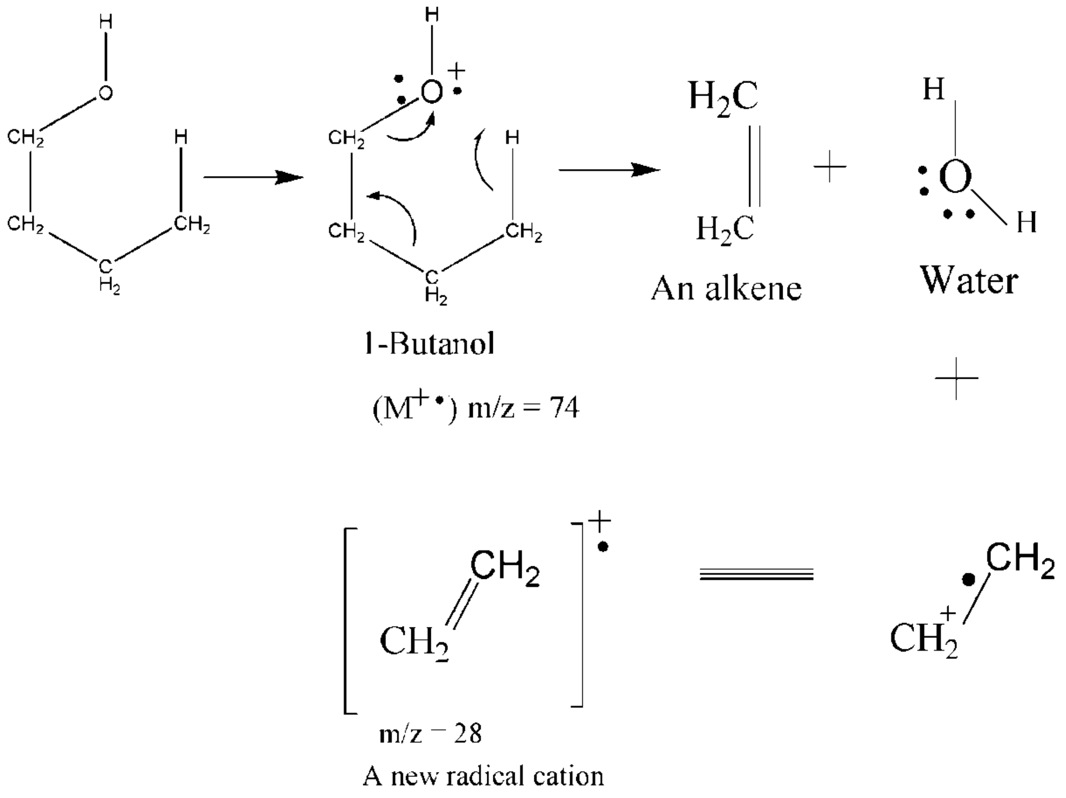


Figure8.19

8.2.9 Mass spectra of ethers

1 Molecular ion peak of the ethers having very low abundance while in the case of aryl ether the molecular ion peak being fairly abundant.

2 The more intense peak of ethers being due to RO^+ and $ROCH_2^+$ fragments at m/z value 31, 45, 59 etc.

3 In the case of benzyl alkyl ether most intense peak or base peak occur due to tropylium ion after the elimination of alkyl free radical.

Example 1: Mass fragmentation of anisol gives highly abundant molecular ion peak due to its resonance stabilised nature .Fragmet ion peak corresponding to m/z at 93, 77, 76, 65 being also good intensity peaks. Fragmentation of anisol can be given as:

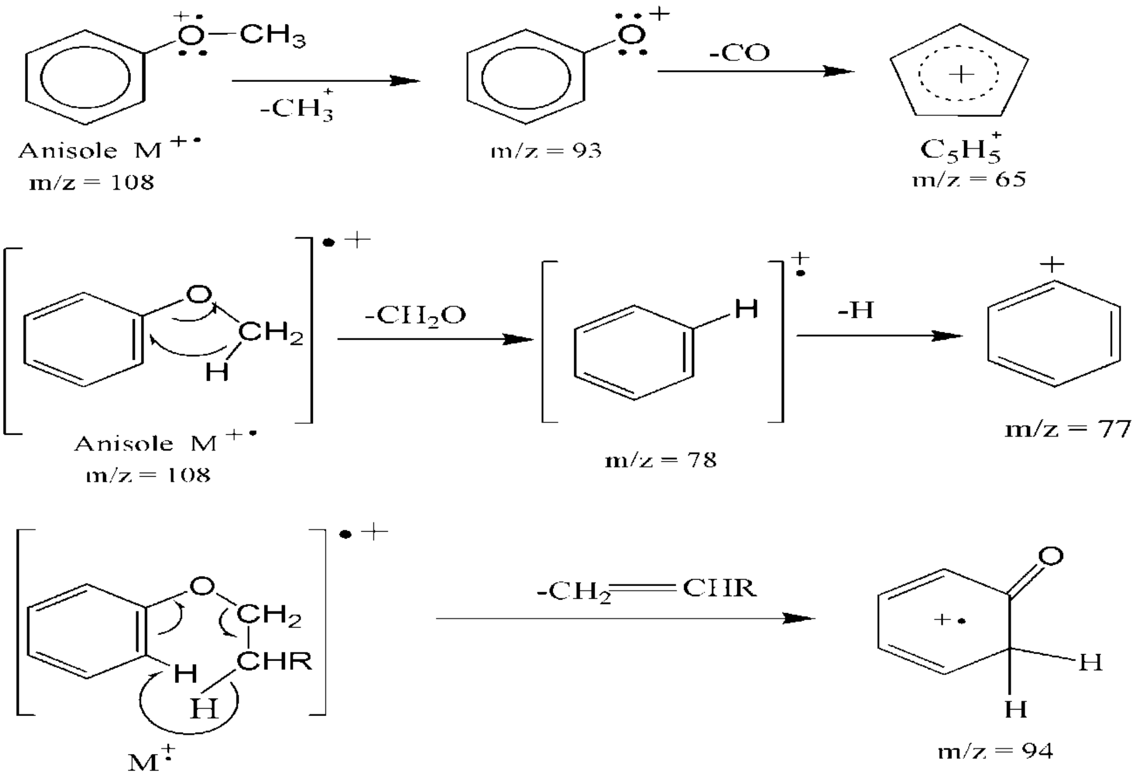


Figure 8.20

Example 2: Mass fragmentation of diethyl ether containing fragmen ion peak at m/z equal to 31 (base peak), 74, 59, 45, and 29 can be represented as

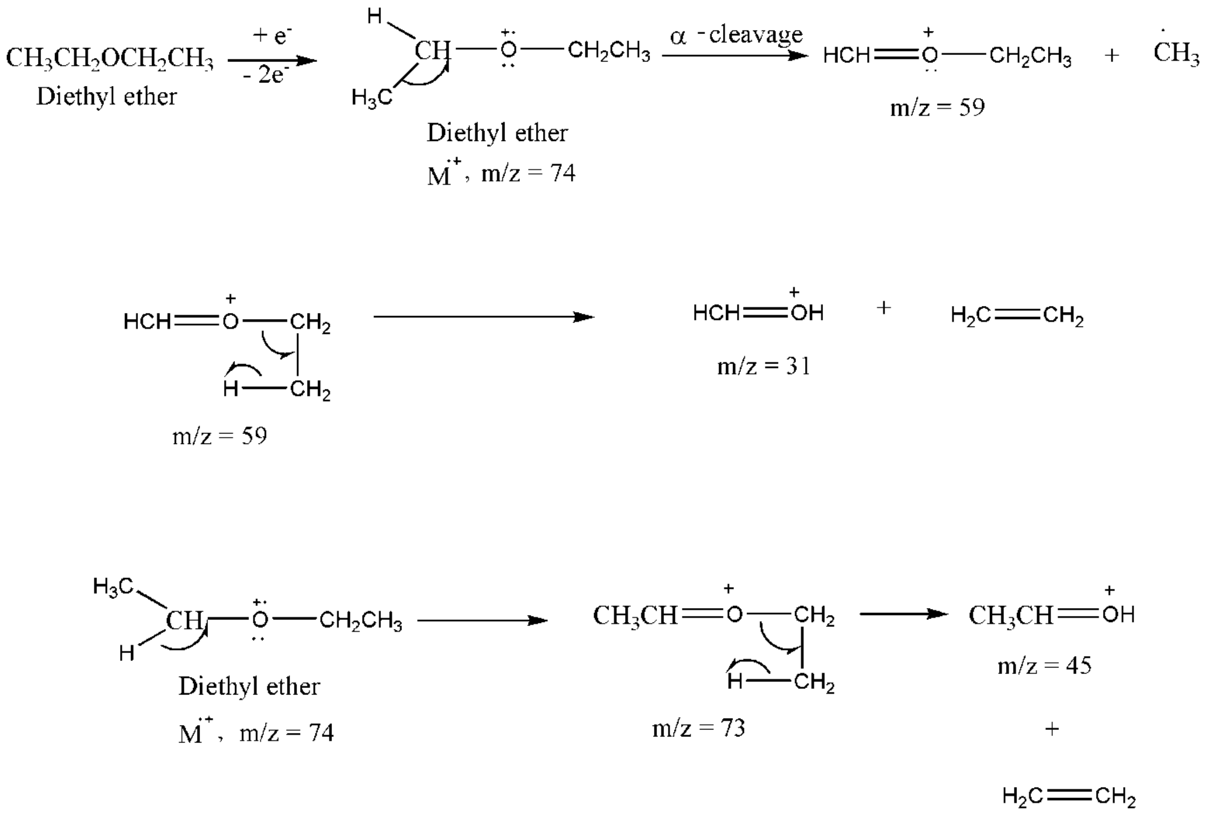


Figure8. 21

Example 3: Mass fragmentation of benzyl methyl ether exhibiting base peak corresponding to tropylium ion at m/z value 91 (base peak) can be represented as:

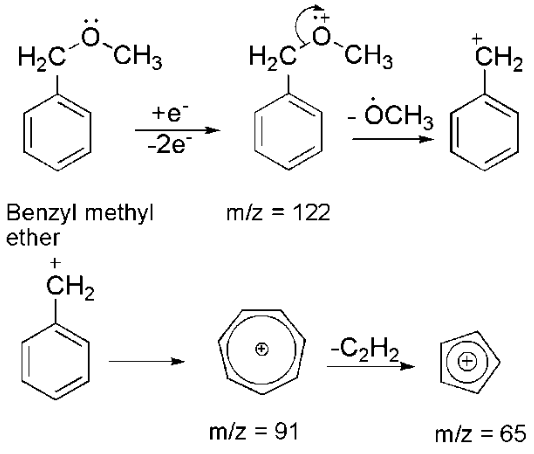


Figure 8.22

8.2.10 Mass spectra of carbonyl compounds

1. In the case of carbonyl compounds α- cleavage in the molecular ion gives the acylium ion . the m/z value of acylium ion at M-1 indicate aldehydic nature of carbonyl compounds while m/z value of acylium ion at M-15, M-29, M-43 etc indicate ketonic nature of carbonyl compounds.

2. Carbonyl compounds containing γ- hydrogen with respect to carbonyl group give the Mc Lafferty rearrangement reaction.

3. Aromatic aldehyde and ketone gives the good intense molecular ion peak.

4. Molecular ion peak of aliphatic ketone having good intensity in compare to aliphatic aldehydes.

5. In the aromatic aldehyde M-1 peak due to α – cleavage being highly favoured peak (base peak) . In the case of benzaldehyde M-1 peak due to α – cleavage at m/z 105 being base peak but peak at m/z 77 due to the lose of CO from acylium ion also being highly favoured.

Example 1: Mass fragmentation of 2-pentanone generating the highly intense acylium ion can be given as:

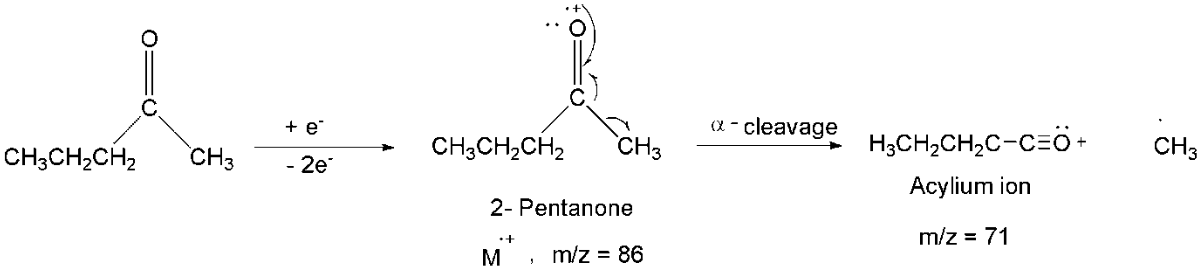


Figure8.23

Example 2: 3-pentanone and 2-methyl 2-butanone does not containing γ – hydrogen so they will not exhibit the Mc Lafferty rearrangement reaction.

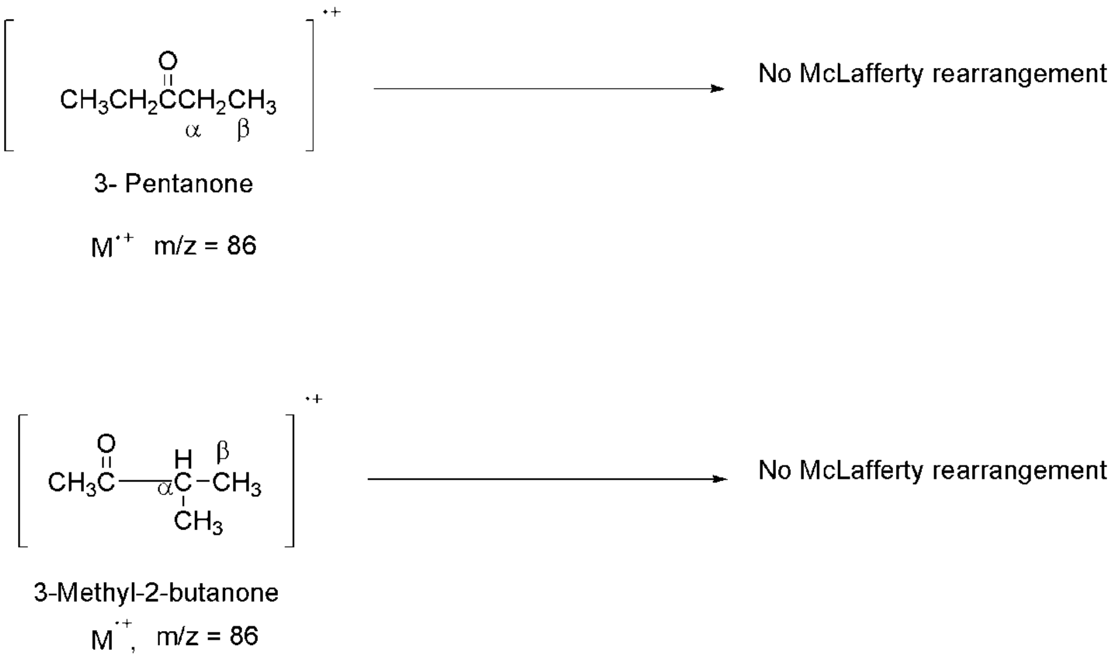


Figure8.24

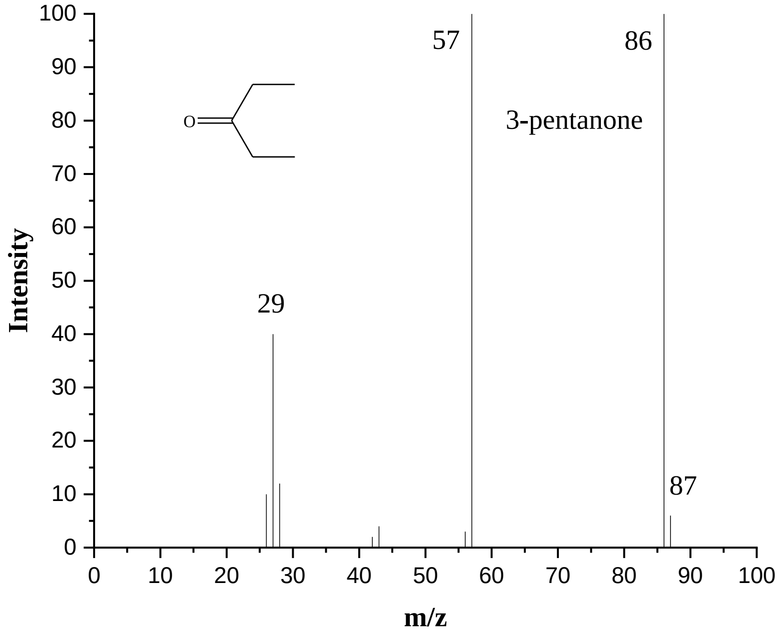


Figure8.25

Example 3: Mass fragmentation of benzaldehyde exhibiting highly intense M-1 peak and other intense peaks at m/z 77, 51 etc can be represented as.

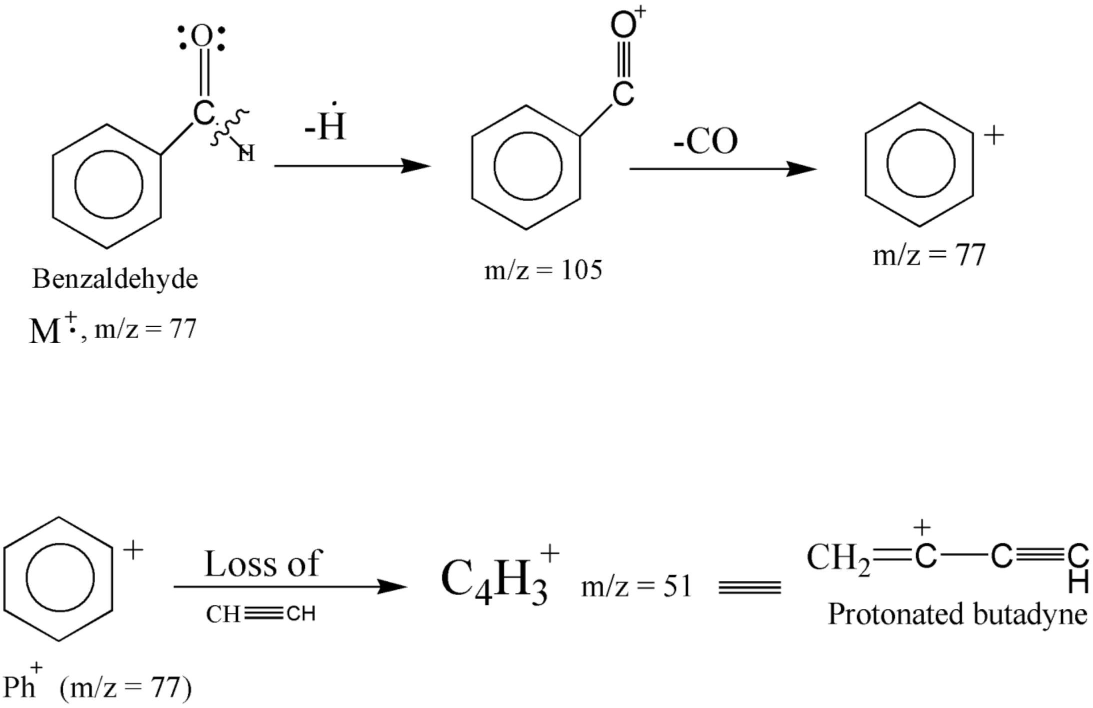


Figure8.26

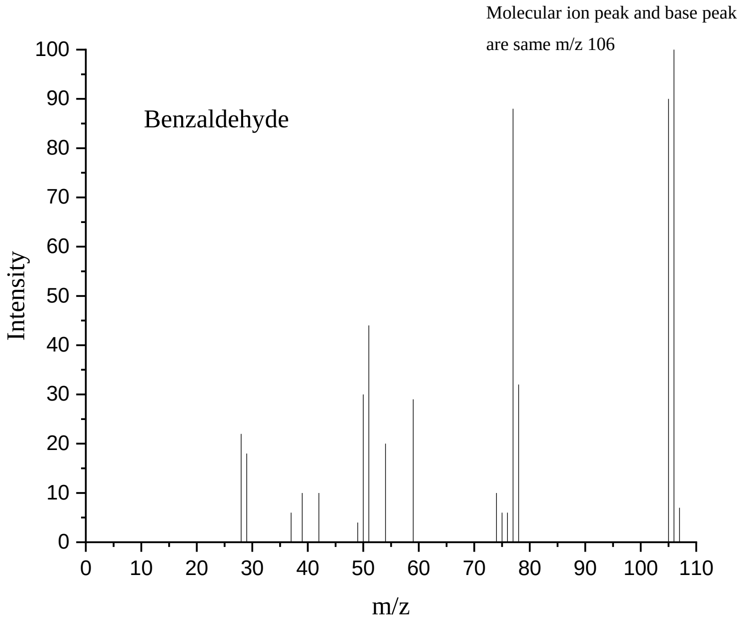


Figure8.27

Example 4: Significant peaks in the mass spectra of cyclohexanone occurs due to following fragmentation modes:

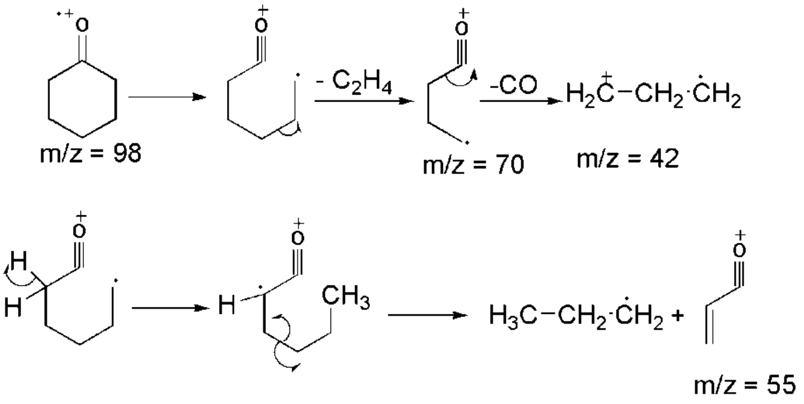


Figure8.28

Example 5: Mass fragmentation of cyclopentanone with base peak at m/z 55 can be represented as:

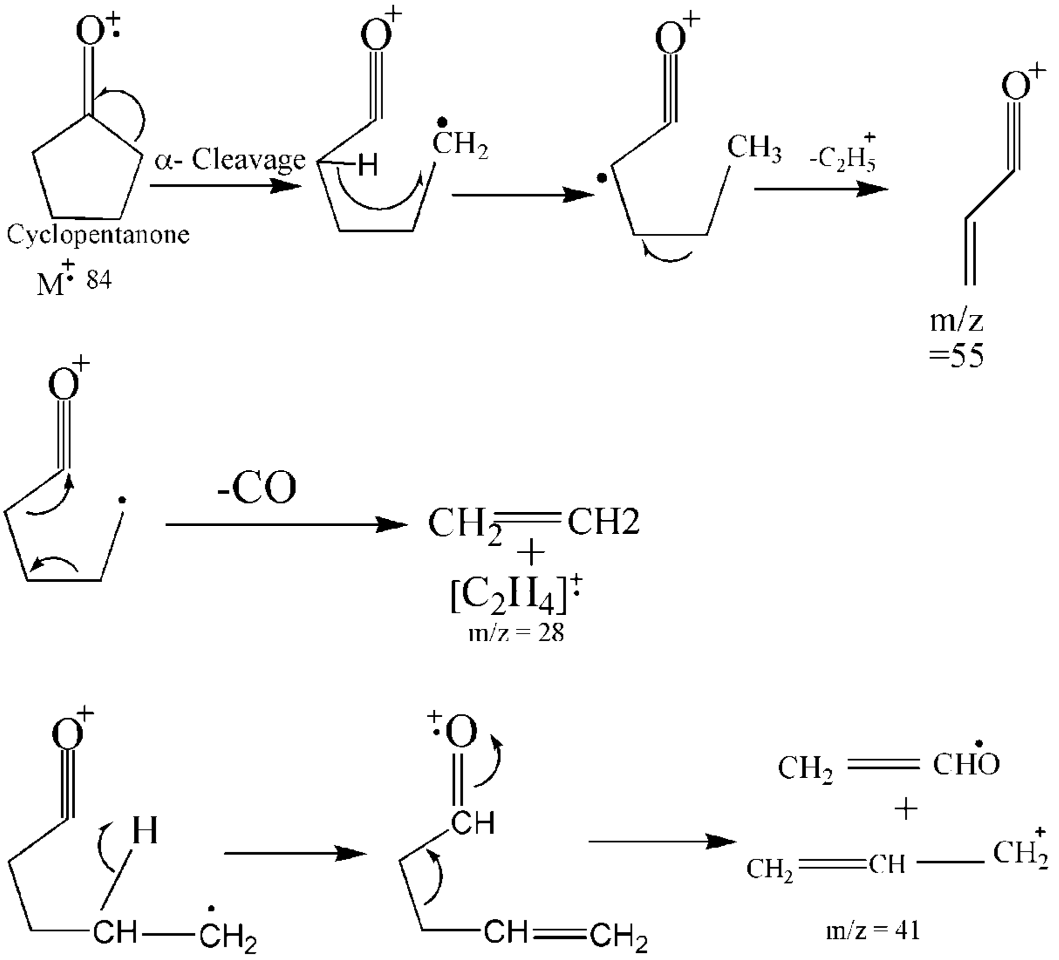


Figure8.29

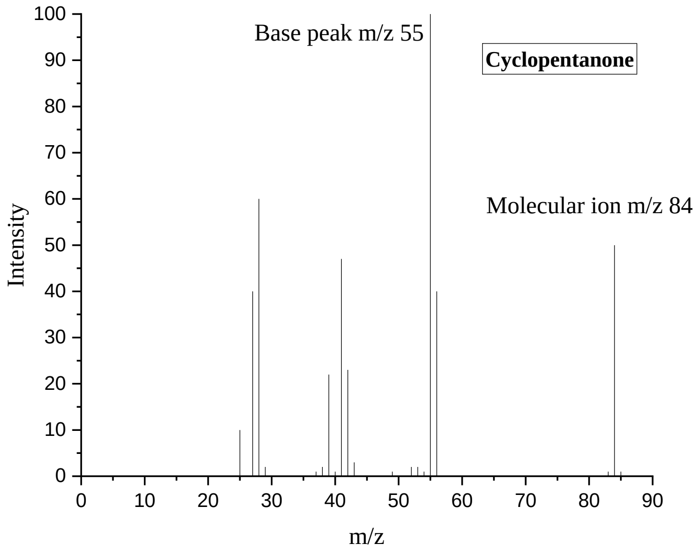


Figure 8.30

8.2.11 Mass spectra of carboxylic acids

1 The molecular ion peak in the mass spectra of aliphatic carboxylic acids having less intensity in compare to the molecular ion peak of aromatic carboxylic acids.

2 Carboxylic acids exhibit the peak at m/z 45 corresponding to COOH cation after α-cleavage.

3 If the carboxylic acid containing γ – hydrogen then the peak occur at m/z 60 by the Mc Lafferty type rearrangement reaction.The peak at m/z 60 is often base peak.

4 In the case of aromatic carboxylic acids peak can also occur at (M-17) and (M-45) due to the elimination of OH group and COOH group respectively.

5 If aromatic carboxylic acid containing the alkyl group or any other hydrogen containing group at ortho position with respect to COOH group then (M-18) peak can also observed.

Example 1: In the molecule of butanoic acid peak at m/z at 45 corresponding to –COOH group can be observed according to following fragmentation pattern (α – cleavage).

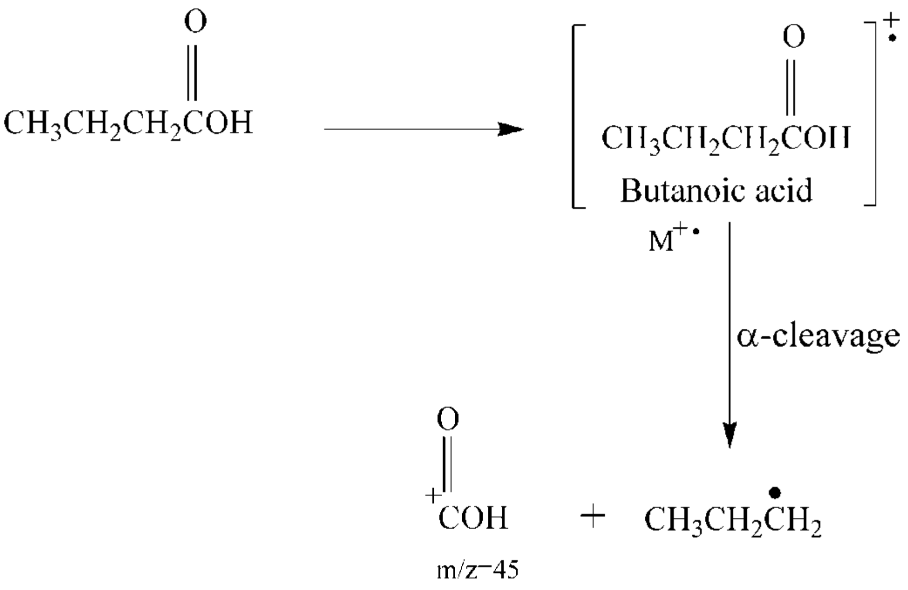


Figure 8. 31

Example 2: Mass fragmentation of anisic acid with base peak at m/z 135 by the α – cleavage can be represented as:

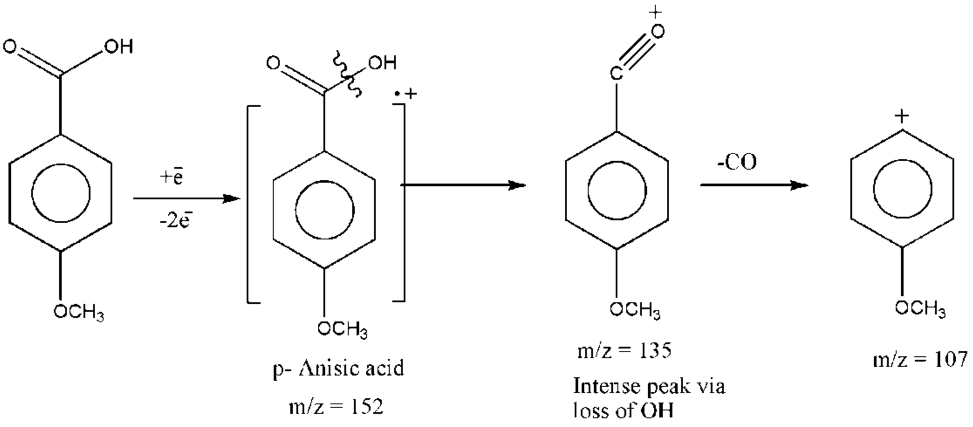


Figure 8. 32

Example 3: Mass fragmentation pattern of pentanoic acid containing base peak at m/z 60 can be represented as:

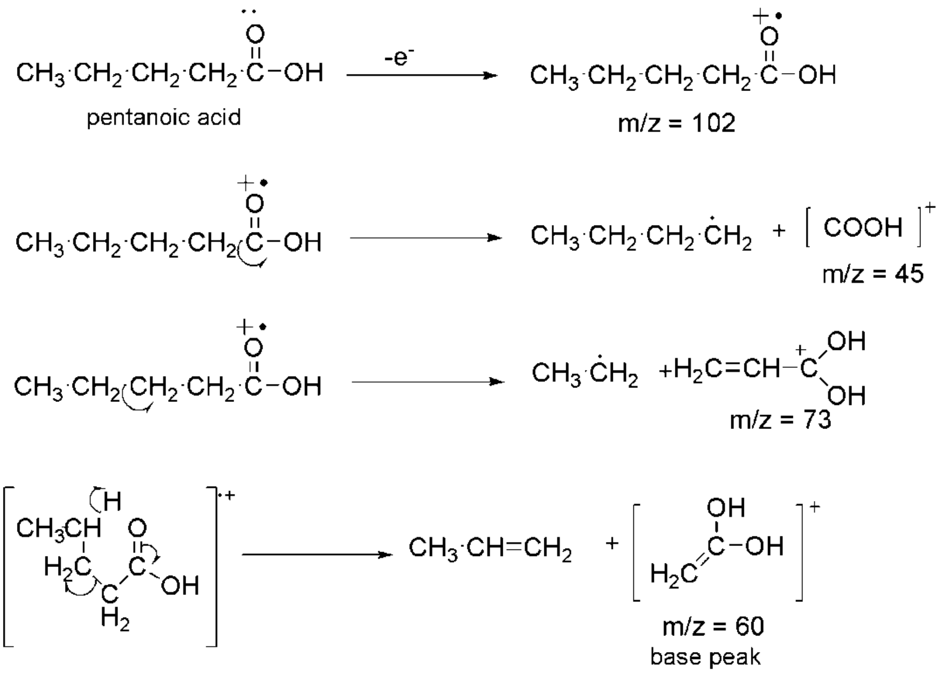


Figure 8.33

8.2.12 Mass spectra of esters

1. Molecular ion peak of ester is being very low intense.

2. Fragment ion peak due to α – cleavage occur as the good intensity peak.

3. If ester molecule containing γ – hydrogen then Mc Lafferty rearrangement can also observed in ester.

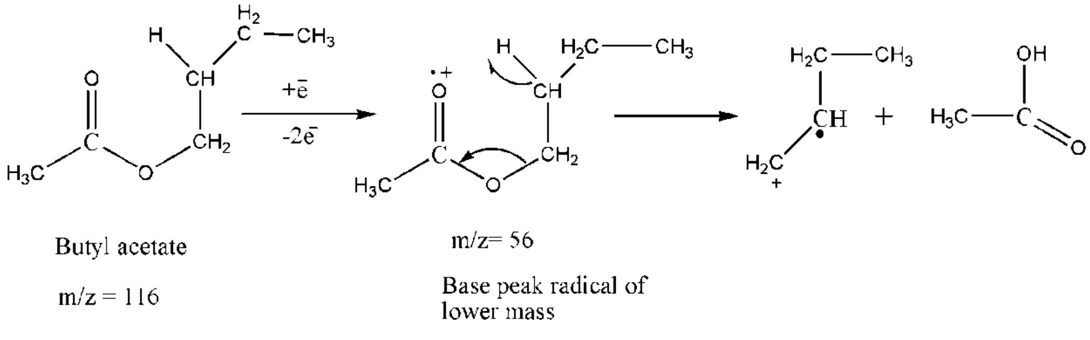


Figure 8.34

4 . In the mass spectra of methyl formate the base peak is at m/z 31 due to the elimination of CO from molecular ion in the following fashion.

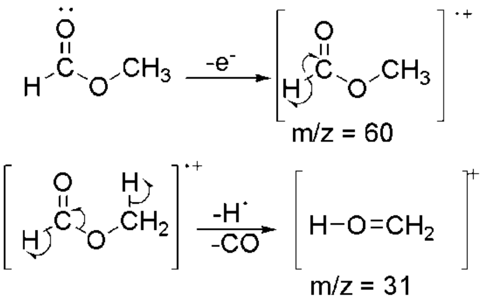


Figure8.35

5.In the case of esters α – cleavage can also give the four different types of ion in following way.

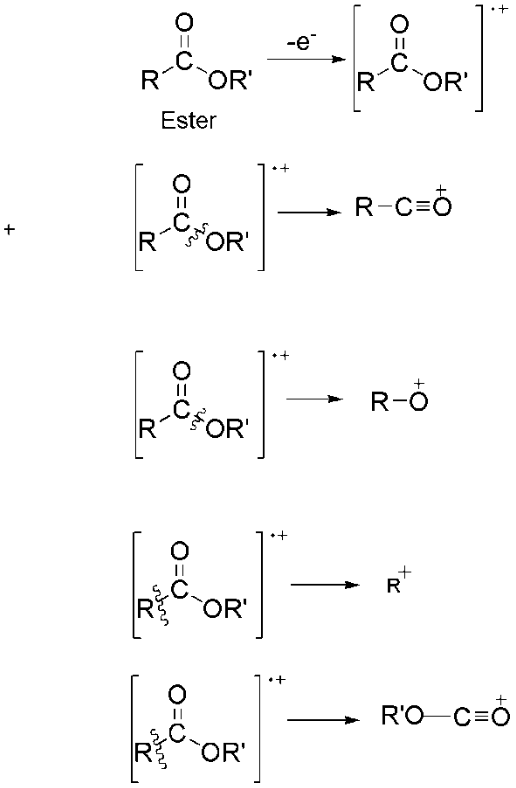


Figure8.36

6 . In the case of aromatic ester base peak occur due to the elimination of RO$^{\cdot}$,but peak due to the elimination of COOR radical being also good intense peak.

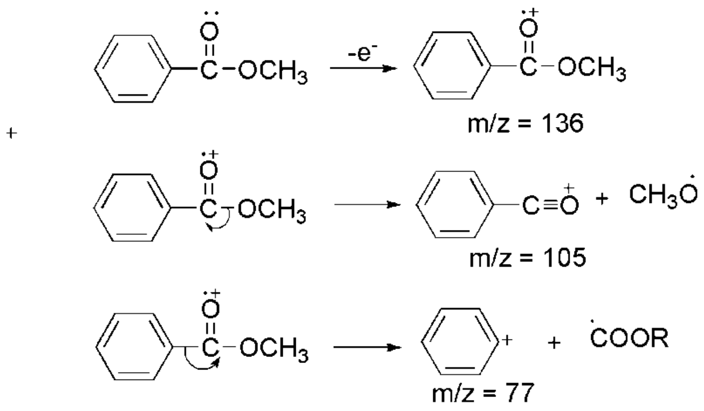


Figure8.37

8.2.13 Mass spectra of amines

1. Molecular ion peak in aliphatic amines are being very weak or some time invisible but aromatic amine exhibit intense molecular ion peak.

2. Most important fragmentation in 1^0 aliphatic amine being β – cleavage to give most intense peak at m/z equal to 30, 44, 58, 72 etc.

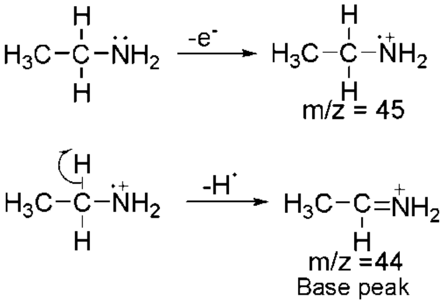


Figure8.38

3. Fragmentation of amine do not occur with the lose of NH_3 as in the case of alcohol which having the tendency to lose H_2O on fragmentation.

4 .Long chain 1^0 amine undergo fragmentation to form the cyclic ammonium ion derivative of the different ring size.

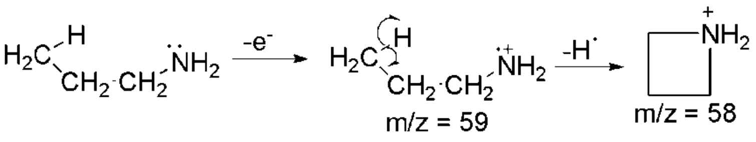


Figure8.39

5. In the case of 2^0 and 3^0 amine most intense peak occur due to β – cleavage that is observed from following fragmentation pattern:

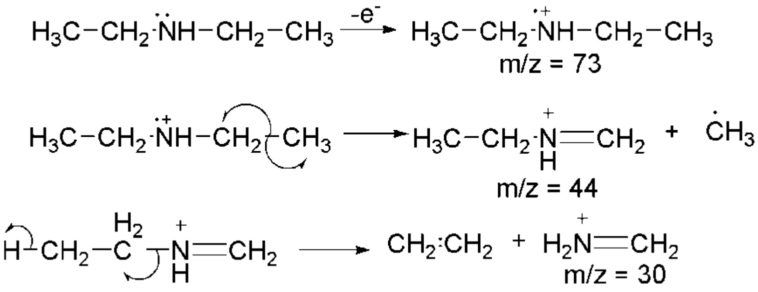


Figure 8.40

Example : Mass fragmentation of aromatic amine like aniline can be represented as:

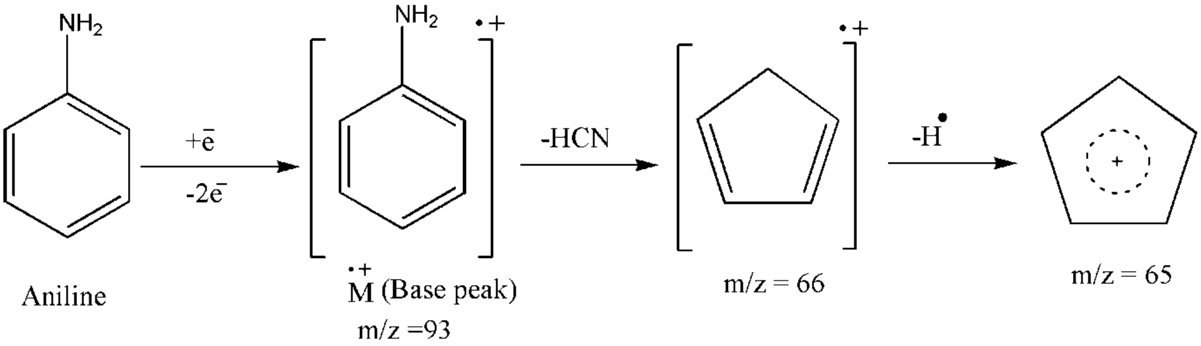


Figure 8.41

Example : mass fragmentation pattern in N-alkyl substituted aryl amine can be represented as:

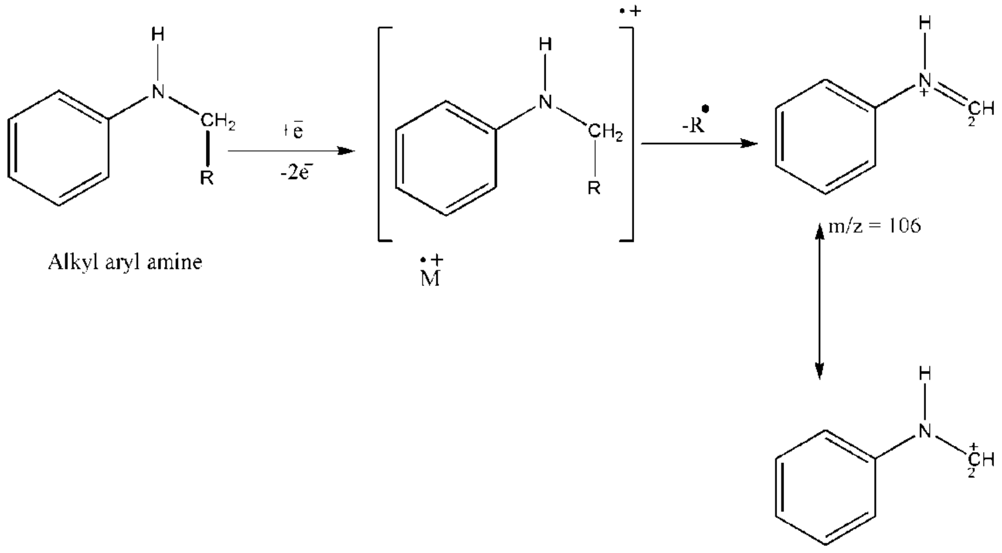


Figure 8.42

8.2.14 Mass Fragmentation of nitro compounds

1. Parent ion peak of aliphatic amine having very low intensity.

2. Nitro compouns undergo fragmentation to give the hydrocarbon fragment cation.

Example :

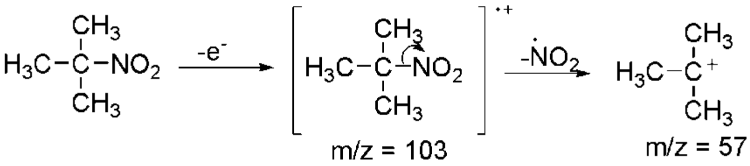


Figure8.43

3. Low intensity peak corresponding to lose of water molecule or lose of hydroxyl radical can also observed in the spectra of nitro compounds.

4 .Molecular ion peak of aromatic amine being quite intense.

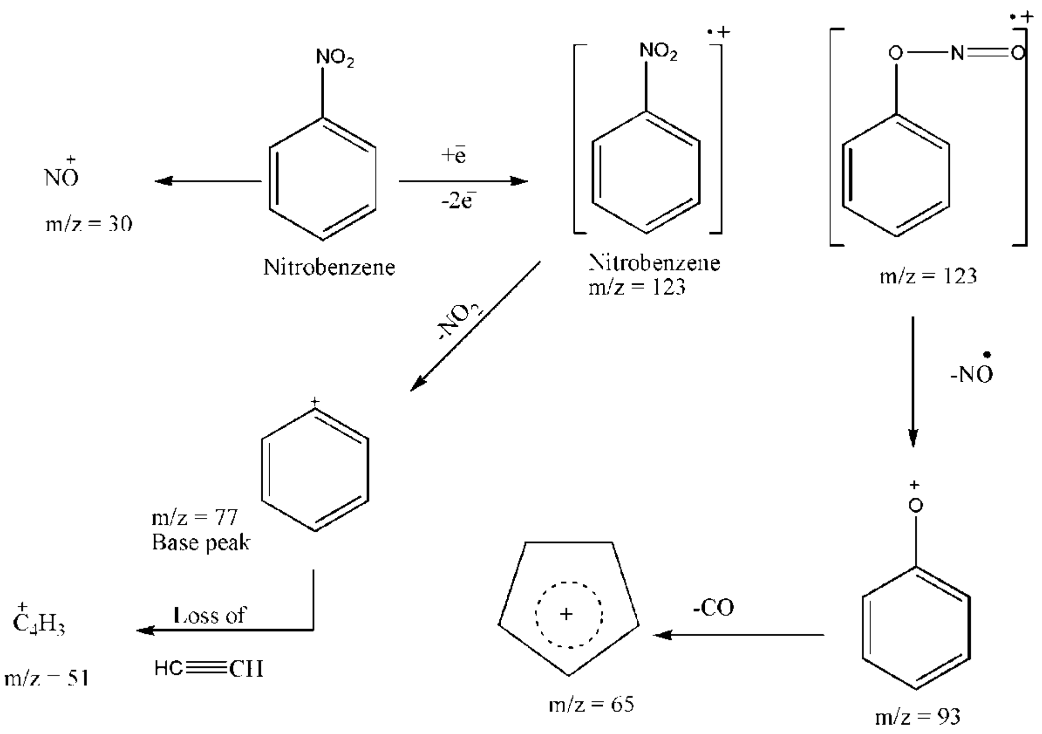


Figure8.44

5. Ortho effect can also observed in the mass spectra of ortho substituted aromatic nitro compounds.

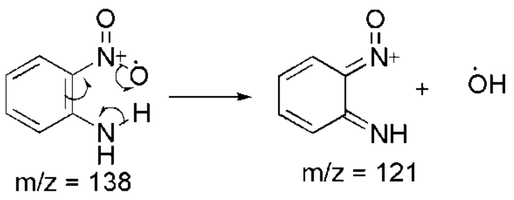


Figure8.45

8.2.15 Mass spectra of thiols and thiophenols

1. Thio alcohol exhibit more intense molecular ion peak in compare to alcohols but the molecular ion peak of thi alcohols being very weak in compare to thiophenols.

2 .As like to alcohols thio alcohols exhibit α – cleavage and give the peak corresponding to m/z 47, 61, 75 ….etc.

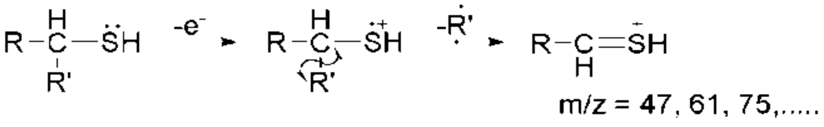


Figure8.46

3. Elimination of the H_2S having less probability in thio alcohols during fragmentation process.

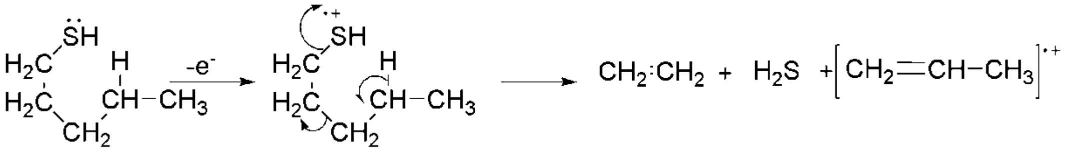


Figure8.47

8.2.16 Mass spectra of thioether

1 .Mass spectra of aliphatic thioether are being too much similar to ethers.

2. Parent ion peak of thioether are being more intense in compare to the ethers.

3. Both α – cleavage by C-S bond breaking as well as β – cleavage are favoured during the fragmentation of thio ethers.

4 .Number of sulphur atom in thio ether is determined by the contribution of S^{34} to the M+2 peak.

Example: α – cleavage and β – cleavage in 2-thio ethoxi propane can be represented as:

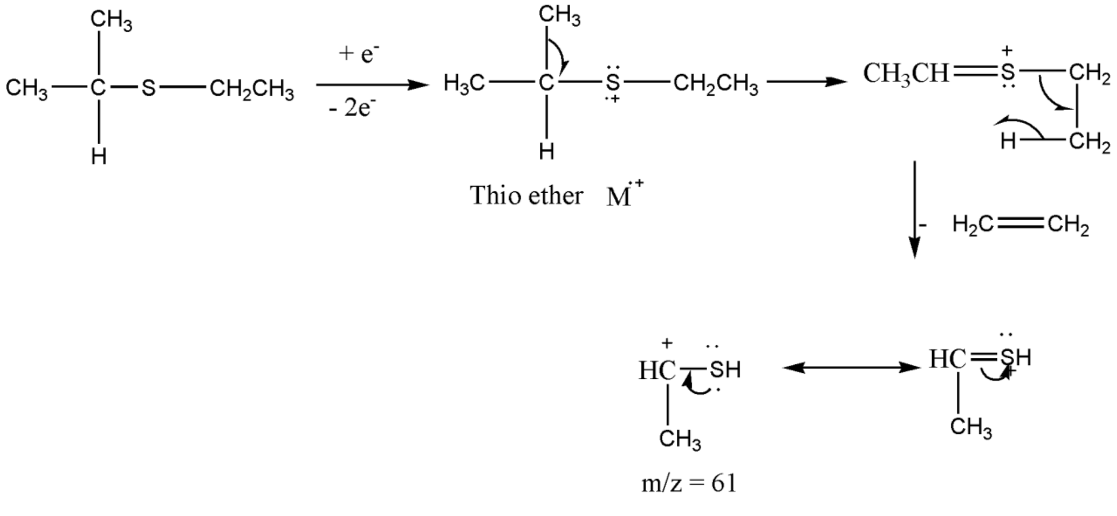


Figure8.48

8.3 REFERENCES / SUGGESTED BOOKS:

1- Organic spectroscopy (Principles and applications) by-Jag Mohan.
2- Introduction to spectroscopy by- Donald L. Pavia, Gary M. Lampman and George S. Vyvyan.
3- Spectroscopy of Organic Compounds by- P. S. Kalsi.
4- Elementry Organic Spectroscopy by- Y.R.Sharma

8.4 SELF ASSESSMENT QUESTION:

1. How will you differentiate the methyl butyrate and methyl isobutyrateon basis of mass spectrometric data?
2. Organic compound having the empirical formula C_3H_6O gives the peak corresponding to m/z 58 (molecular ion peak), 43 (Base peak) and 15. What organic compound can be predicted on the basis of these data?
3. A hydrocarbon with the molecular formula C_7H_{12} (Molecular ion peak at m/z=96) show large peak due to (M-15) and at m/z=54. What structure can be predicted for this compound.
4. How will you differentiate 2-pentanone and 3- pentanone on the basis of mass spectrometry.
5. Which type of organic compounds exhibit the high intense peak peak at m/z = 91.
6. What can be predicted if the organic compound exhibit M and M+2 peak in 1:1, 3:1?
7. Define the following in mass spectrometry

   (a)Base peak

   (b)Metastable ion and peak

   (c)Nitrogen rule

   (d)Fast atom bombardment (FAB)

   (e) Allylic cleavage

8. Predict the organic compound which gives mass spectrometric data at m/z 152 (Molecular ion peak), 121, 120, 92, 65 and 64.

9. What organic compound can be predicted if the compound exhibit mass spectrometric data at m/ z = 100, 85, 71, 57, 43 (base peak), 41, 29 and 27.
10. Explain why molecular ion peak of benzene being highly intense.