Name of Unit:	Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry
Course/Subject Name:	Advanced Instrumentation Techniques
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Learning Outcome of Module-I

LO	**Learning Outcome (LO)**	**Course Outcome Code**
LO1	To understand the basic concept of NMR	BP811.1
LO2	To know about the components of a NMR instrument, FT-NMR spectrometer and modern NMR instruments	BP811.2
LO3	To understand the measurement of chemical shift value and shielding and deshielding effect and the factors affecting the value of chemical shift	BP811.3
LO4	To understand the basic concept and principle of mass spectrometry and types of mass spectrometers	BP811.1
LO5	Understand molecular ion, metastable ion and base peak and fragmentation rule	BP811.3 BP811.6

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Topic

INTRODUCTION TO NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

This technique is based on the fact that nuclei of atoms have magnetic properties that can be utilized to yield chemical information. Hence it is important to introspect and visualize the conditions as to when a nucleus will feel the magnetic field. For that, a nucleus is identified being active or non-active by determining its spin. It is also crucial to understand the mechanism and chemistry surrounding those nuclei and to know how shielded and deshielded a 100008888nucleus is. Nuclear magnetic resonance (NMR) spectroscopy is the most powerful and broadly applicable technique for structure determination used by the chemists. NMR allows characterization of a very small amount of sample (≈ 2 mg), and does not destroy the sample (non-destructive technique). It can provide vast information about a molecular structure, and in some cases, the structure of a compound can be determined using only NMR spectroscopy. In practice, the structures of complicated molecules are determined through a combination of techniques that include NMR and IR spectroscopy and mass spectrometry. Since organic compounds are primarily composed of carbon (C) and hydrogen (H), NMR identifies the various types of H and C atoms in organic molecules.

The H-nucleus is the most commonly studied by NMR spectroscopy because of its high natural abundance of 99.985% and its presence in the majority of organic compounds. NMR studying ^{1}H atom is called Hydrogen or proton NMR spectrum. The proton NMR spectrum gives the information about the number of different types of protons and also the chemical environment around it.

Carbon forms the backbone of the organic molecules therefore C NMR is also important and gives valuable information about the molecule. The ^{12}C do not absorb radio frequency energy but the other isotope *i.e.* ^{13}C absorbs the energy but the ^{13}C has a natural abundance of only about 1%. Therefore the sensitivity of ^{13}C is less than the ^{1}H NMR and requires longer time to record. ^{19}F and ^{31}P are also frequently examined to get crucial information about the structure of the compound containing these elements.

NMR Active Nuclei:

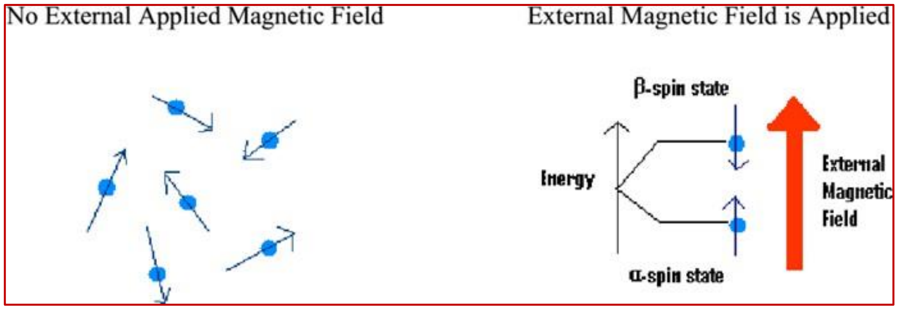
The nuclei are built up of protons and neutrons each possessing an angular momentum due to its motion about the centre of the nucleus. The total angular momentum of a particular nucleus is the vector sum of the individual momentum of the constituent protons and neutrons. The total angular momentum of a nucleus in its ground state is called as nuclear spin. This is characterized by a spin quantum number I, which may be integral, half-integral or 0.

There are some empirical rules which determine the net spin of a nucleus given as below. Only those nuclei which have non-zero spin number can absorb/emit radiofrequency (RF) radiation andhence are NMR active nuclei.

1. If the number of neutrons and the number of protons are both even, then the nucleus has NO spin (I = 0). Example: For ^{12}C, ^{16}O, ^{32}S No NMR signal will be obtained.
2. If the number of neutrons and the number of protons are both odd, then the nucleus has an integer spin (I = 1, 2, 3 and so on). Example: ^{2}H, ^{10}B, ^{14}N are NMR active.
3. If the number of neutrons plus the number of protons is odd (odd mass number or we can say odd number of neutrons/protons and even number of protons/neutrons), then the nucleus has a half-integer spin (I = n/2, where n is an odd integer; I = 1/2, 3/2, 5/2 and so on) Example: **^{1}H, ^{13}C, ^{15}N, ^{31}P** are NMR active.

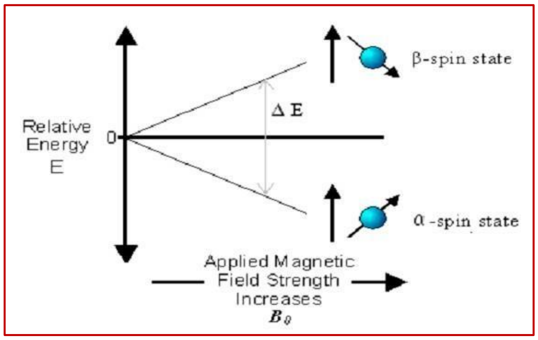
It is very well known that the moving charged particle generates a magnetic field. Since all the atomic nuclei are charged (+ve) particles and hence spinning nuclei or spin active nuclei will generate a magnetic field along the axis of rotation. Thus we can say that the spinning nuclei behave like a tiny bar magnet with a magnetic moment μ.

In the absence of external magnetic field, all the magnetic nuclei (nuclear magnetic dipoles) are randomly oriented. But, when an external magnetic field is applied, the nuclei align themselves either with or against the field of the external magnet. Quantum mechanics explains when a magnetic active nuclei with nuclear spin I is placed in a uniform magnetic field, its magnetic dipole or magnetic moment will align in only 2I+1 ways. Thus a nucleus with spin I = 1/2 will have 2 possible orientations. In the presence of an external magnetic field, some of the nuclei arrange themselves in the direction of the external magnetic field while others in the opposite direction. More energy is needed for a nucleus to align against the external magnetic field than to align it with the external magnetic field. Therefore, nuclei which align with the field are in the lower energy state called as α-spin state and those which align against the field are in the higher energy state called as β-spin state



The total number of nuclei in the α-spin state is always higher than in the β-spin state. The difference in the populations is very small (≈ 10 out of a million), but is sufficient to form the basis of NMR spectroscopy.

The difference in energy between the two spin states depends upon the external magnetic field strength. The greater the strength of the external magnetic field, greater is the difference in energy between the α- and β-spin states. The following diagram illustrates that the energy between the two spin states increases as the field strength increases.
The energy difference between two spin states is given by



$$\Delta E = h\frac{\gamma}{2\pi}B_0$$

Where h is the planck‟s constant, γ is the gyromagnetic ratio (also called magnetogyric ratio). It is a constant that depends on the particular type of nucleus. Thus it is clear that for a given nucleus the value of ΔE depends on the applied magnetic field strength B_0 (operating frequency of the spectrometer).

When an appropriate energy in the form of RF radiation is supplied to a nucleus occupying in the α-spin state, a transition takes place between the two spin states and the nucleus is said to be in **resonance** with the external magnetic field. The following fundamental equation correlates the applied radiofrequency with the magnetic field strength B_0

$$\nu = \frac{\gamma}{2\pi} B_0$$

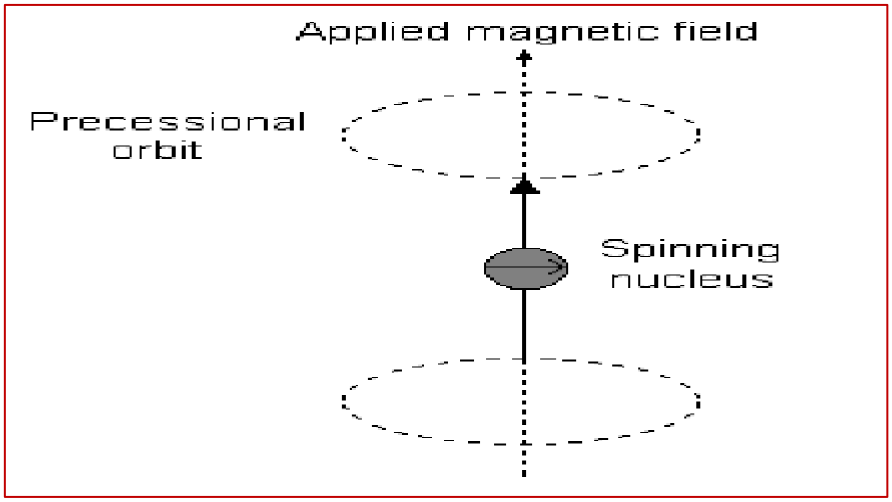
The value of γ depends on the particular type of nucleus. For proton the value of 2.675×10^{8} $T^{-1}s^{-1}$ whereas and in the case of ^{13}C nucleus, it is 6.688×10^{7} $T^{-1}s^{-1}$.

The introduced radiofrequency is given in megahertz (MHz). The following calculation shows that a frequency of 300 MHz is required at a magnetic field strength B_0 of 7.046 Tesla.

$$\begin{aligned}\nu &= \frac{\gamma}{2\pi} B_0 \\ &= \frac{2.675 \times 10^{8}}{2(3.1416)} \mathrm{T}^{-1}\,\mathrm{s}^{-1} \times 7.046\,\mathrm{T} \\ &= 300 \times 10^{6}\,\mathrm{Hz} = 300\,\mathrm{MHz}\end{aligned}$$

Now because each kind of nucleus has its own gyromagnetic ratio, different energies are required to bring different kinds of nuclei into resonance. For example, an NMR spectrometer with a magnet requiring a frequency of 300 MHz to flip the spin of a ^{1}H nucleus requires a frequency of 75 MHz to flip the spin of a ^{13}C nucleus (The value of gyromagnetic ratio, γ for ^{13}C is ≈ 1/4th of the value for ^{1}H nucleus). NMR spectrometers are equipped with radiation sources that can be tuned to different frequencies so that they can be used to obtain NMR spectra of different kinds ofnuclei.

In the presence of external magnetic field, the nucleus, besides its spin motion, also precesses about its own axis of rotation in a similar manner as the spinning top. To understand the precession motion let us consider the behavior of a spinning top. In addition to the spinning motion, top also perform a slower waltz-like motion (unless absolutely vertical) in which the spinning axis of the top moves slowly around the vertical. This type of motion is called as precessional motion and it arises due to the interaction of spin with the earth‟s gravitational field acting vertically downward.



It is important to note that the spinning frequency of the nucleus does not change, but the precessional frequency (νL) does. The precessional frequency is directly proportional to the applied magnetic field. Stronger the applied magnetic field, higher is the precession rate.

If the RF radiation of frequency equal to Larmor frequency is supplied to the precessing nucleus, the energy can be absorbed and the nucleus can be promoted to the higher energy state. This energy absorption is called resonance because the frequency of the applied radiation and the precession coincide or resonate.

Hence the basic NMR relationship can be written as follows

$$\nu_L = \nu = \frac{\gamma}{2\pi} B_0$$

It is important to note here that the energy difference between the two energy states is very small even with very large external magnetic field. Because the energy difference is so small that the population of nuclei in the two energy states are nearly equal. However the lower energy state has slight excess population than the higher energy state. The relative populations of the two spin states will change if energy of correct frequency is supplied to induce upward or downward transitions. Nuclei in the lower energy state absorb RF radiation and undergo transitions to the higher energy state. If this process keeps on going continuously, the excess nuclei in the lower state may diminish and hence no further absorption of energy will occur and so the observed signals may fade out or diminish entirely. This situation when the populations of the two energy states become equal is known as saturation. However, the populations of the two spin states do not become equal because nuclei in the higher energy state are constantly returning to the lower energy state.

Relaxation process

How do nuclei in the higher energy state return to the lower state? Emission of radiation is insignificant because the probability of re-emission of photons varies with the cube of the frequency. At radio frequencies, re-emission is negligible. In NMR, there are two radiationless processes which enable the high energy nuclei to lose energy. The radiationless transitions by which a nucleus in the high energy state returns to the lower energy state are called relaxation process. This process is very important in NMR as it maintain the excess of nuclei in the lower energy state, necessary condition for the NMR phenomenon.

There are two major relaxation processes;

- **Spin-lattice (longitudinal) relaxation**
- **Spin-spin (transverse) relaxation**

Spin - Lattice Relaxation

It involves the transfer of energy from the nucleus in its higher energy state to its environment, i.e. to the molecular lattice. The sample having all the nuclei is called the *lattice*. Nuclei in the lattice are in vibrational and rotational motion, which creates a complex magnetic field. The magnetic field caused by motion of nuclei within the lattice is called the *lattice field*. This lattice field has many components. Some of these components will be equal in frequency and phase to the Larmor frequency of the nuclei of interest. These components of the lattice field can interact with nuclei in the higher energy state, and cause them to lose energy (returning to the lower state). The energy that a nucleus loses increases the amount of vibration and rotation within the lattice resulting in a slight increase in the temperature of the sample. The total energy of the sample remains constant. This process maintains an excess of nuclei in the lower energy state required for the phenomenon of NMR.

The spin-lattice relaxation process contributes to the width of spectral line. In solids or viscous liquids, the molecular motions are greatly restricted. As the mobility increases, the vibrational and rotational frequencies increase, making it more likely for a component of the lattice field to be able to interact with excited nuclei. Thus solids and highly viscous liquids exhibit long spin- lattice relaxation time and hence broadening of the NMR signal. However, at extremely high mobility, the probability of a component of the lattice field being able to interact with excited nuclei decreases.

Spin - spin Relaxation

Spin-spin relaxation describes the interaction between neighbouring nuclei with identical precessional frequencies but differing magnetic quantum states. In this situation, the nuclei can exchange quantum states; a nucleus in the lower energy level will be excited, while the excited nucleus relaxes to the lower energy state. There is no net change in the populations of the energy states, but this relaxation process decreases the average lifetime of a nucleus in the excited state.

The spectral line width is inversely proportional to the lifetime of the higher energy state. The shorter the lifetime of the excited state, greater is the line width. An efficient relaxation process results the shorter time T_2 leading to the broadening of the NMR peak. Solids and viscous liquids usually have the properly oriented nuclei in the lower spin state which may exchange the spins with the nuclei in the higher spin state resulting low value of T_2 and hence peak broadening.

Continuous wave and Fourier transform are two general types of instrumentations in NMR spectrometers. Initially, experiments were conducted with the help of continuous wave (C.W.) instruments. However, in 1970 the first Fourier transform (F.T.) instruments were building. Since then, F.T –NMR spectrometers dominates the market. Initially, NMR spectrometers were expensive and less of private companies owned them. Nowadays, modern spectrometers contain a large, strong and expensive liquid helium-cooled superconducting magnet, so that resolution of the spectrometer can directly depend on its magnetic field strength. Less expensive instruments which use permanent magnets, hence show lower resolution are also available. These can still give acceptable performance for many applications such as in reaction monitoring and quick checking of samples.

Unlike in other types of spectroscopy, NMR requires high attention while preparing a sample because the quality of the sample has a profound effect on the performance of the corresponding spectrum. Therefore, simple but necessary steps should be followed to prepare an NMR test sample.

Instrumentation of NMR spectroscopy

There are two general types of NMR instruments namely continuous wave (CW) and Pulsed or Fourier transform (FT). Early experiments were conducted with continuous wave (CW) instruments and in 1970 the first Fourier transform (FT) instrument became available. In the present time CW-NMR spectrometers have almost been replaced with pulsed FT-NMR instruments. However due to their lower maintenance and operating cost, they are still commonly used for routine ^{1}H NMR spectroscopy at 60 MHz. (Low resolution CW instruments require only water cooled electromagnets instead of the liquid He cooled superconducting magnets found in higher field FT-NMR spectrometers). These two spectrometer designs can be described in the following way.

Continuous wave (CW) or field sweep instrument

These systems are currently obsolete except for a few wide line experiments that are performed in special solid-state NMR applications. A schematic representation of a 60 MHz CW-NMR spectrometer is shown below.

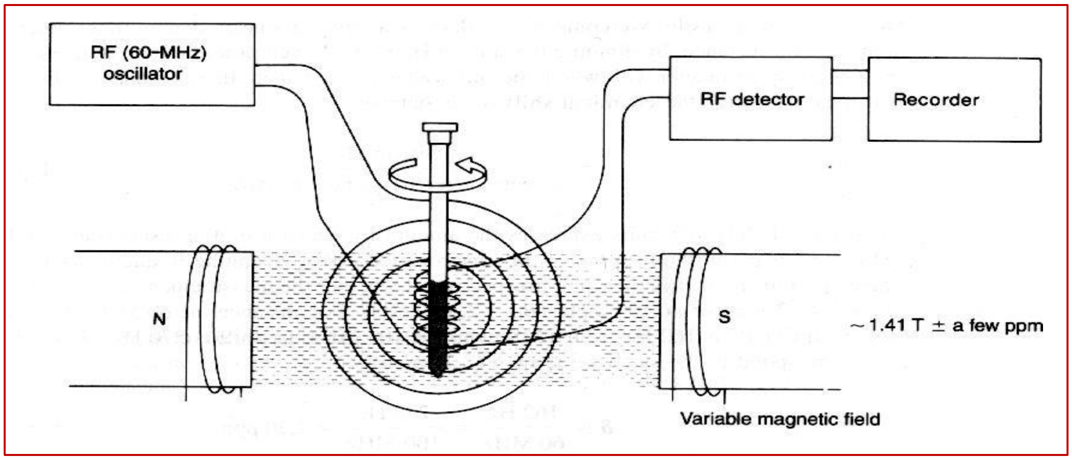


Figure 1: Schematic representation of a typical NMR spectrometer

The sample is held in a strong magnetic field, and the frequency of the source is slowly scanned. There are two orthogonal coils of wire that serve as antennas for radiofrequency (RF) radiation. One coil is attached to an RF generator and serves as a transmitter. The other coil is the RF pickup coil and is attached to the detection device. Since the two coils are orthogonal, the pickup coil cannot directly receive any radiation from the generator coil. One coil supplies a constant RF frequency and the second coil detects the RF emission from the excited nuclei as they get relaxed. When a nucleus absorbs RF radiation the reorientation of the nuclear spins takes place. The excited nucleus when comes to the ground state reemits the RF radiation in a direction that can be received by the pickup coil and the instrument responds by recording it as a signal or peak (Figure 1)

In some instruments, rather than changing the RF frequency, the magnet field is varied and RF frequency is kept constant. As the magnetic field strength increases the precessional frequency of all the protons also increases. When the precessional frequency of a given type of proton reaches 60 MHz, it is said to be in resonance. The two coils can be adjusted to vary the applied magnet field slightly by passing a current through them, causing each chemically different proton to come into resonance sequentially which can be recorded in the form of peaks/signals.

Varying the magnetic field is exactly equivalent to varying the RF frequency, and a change of 1 ppm in the magnetic field strength (increase) has the same effect as a 1 ppm change (decrease) in the RF frequency. Hence, changing the field strength instead of the RF frequency is only a matter of instrumental design. Instruments which vary the magnetic field in a continuous manner, scanning from the downfield end to the upfield end of the spectrum, are called continuous-wave instrument.

The peaks generated by a CW instrument have ringing; a decreasing series of oscillations that occurs after the instrument has scanned through the peak. Ringing occurs because the excited nuclei do not have time to relax back to their equilibrium state before the instrument scans another proton. The excited nuclei have a relaxation rate that is slower than the rate of scan. As a result, they are still emitting an oscillating, rapidly decaying signal which is recorded as ringing.

Fourier Transform (FT) NMR instrument

To record a complete spectrum using a CW NMR-instrument, it requires a long time (few minutes) because each transition is induced in succession by continuous scans from low field to high field. Therefore each different type of proton is excited individually and its resonance peak is observed and recorded independently of all the others. In FT-NMR instrument, an alternative approach is used where all the protons are excited simultaneously. Thus in FT-NMR, the sample is irradiated (at fixed field strength) with a strong pulse of radio frequency energy containing all the frequencies over the ^{1}H range. All protons are excited simultaneously and then begin to return (or decay) to their original spin states and reemit RF radiation at their respective resonance frequencies creating an interference pattern in the resulting RF emission versus time, known as free induction decay (FID). The frequencies are then extracted from the FID by a mathematical technique called a Fourier transform. An FTNMR spectrometer consists of a control console, magnet, and a coil of wire that serves as the antenna for transmitting and receiving the RF radiation. (Only one coil is necessary because signal reception does not begin until after the end of the excitation pulse.) Because the FID results from the emission due to nuclei in all environments, each pulse contains an interference pattern from which the complete spectrum can be obtained. Since each FID is acquired in 1–2 seconds, it is possible to acquire hundreds of FIDs in just a few minutes, and the FIDs can be summed and averaged to greatly improve the signal to noise ratio of the resulting FID (Figure 2).

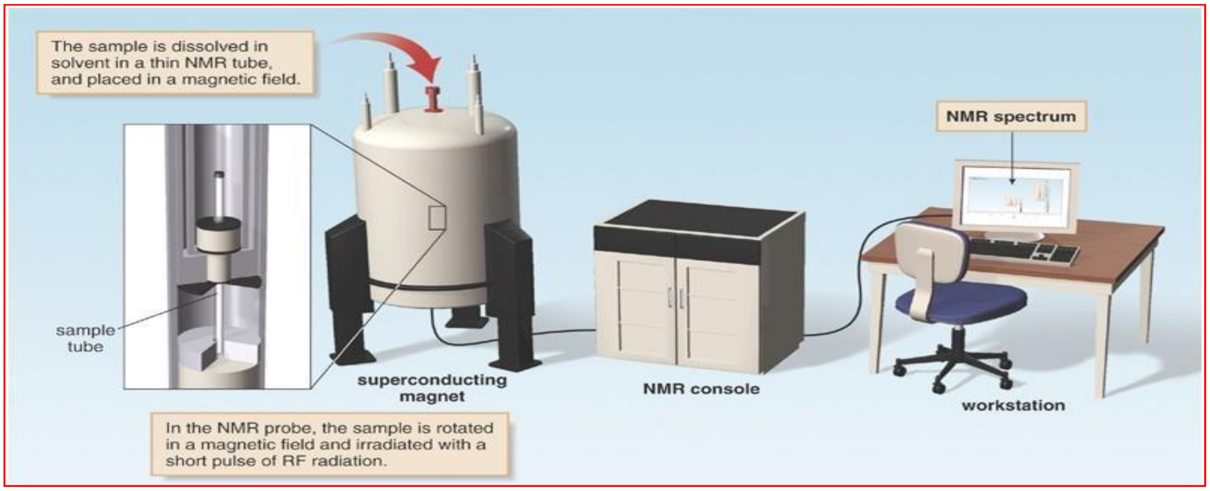


Figure 2: Schematic representation of a Modern NMR spectrometer

Sample handling

To obtain high-quality NMR spectra it is important to prepare sample correctly. There are some facts which we should be followed while making sample to record spectra.

NMR Tube selection

For recording NMR, we should use high quality NMR tube. The diameter of the sample tube should be same to the coil diameter of the NMR probe in the magnet. We should not use a 5 mm NMR tube in a 10 mm probe unless we have no choice. Bad quality NMR tubes contain regions where the tube wall thickness varies, and this variation makes our sample difficult to shim well.

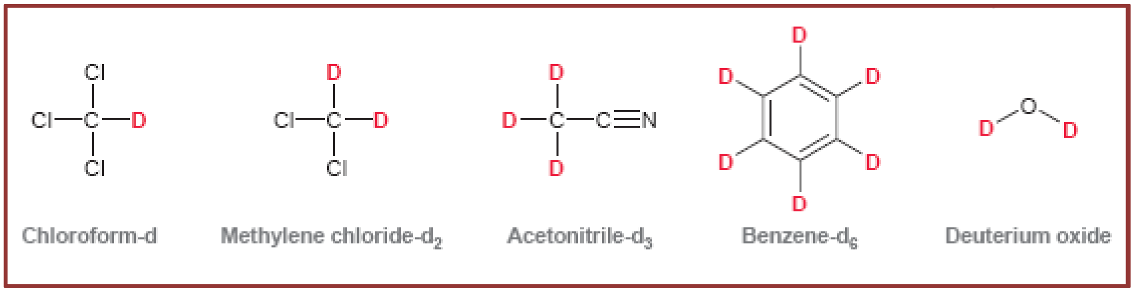
Sample Purity

The sample must be as pure as possible. Peaks due to impurities can make the spectrum unnecessarily complicated. One should ensure that the sample is free of magnetic impurities as these can distort the magnetic field and hence degrade the spectrometer resolution.

Solvent Selection

Samples must be prepared using high-quality deuterated solvents. We must try to use individual ampules rather taking solvent out of a bottle that originally contained 50 or 100 g of solvent. Deuterated chloroform more than six months old may be acidic enough to exchange with labile protons from our sample therefore we must take care if our compound contains labile hydrogen atoms or H‟s particularly susceptible to acid-catalyzed degradation. If the solvent itself has protons, the spectrum will be saturated with signals from the solvent, rendering it unreadable. As a result, solvents without protons must be used. Although there are several solvents that lack protons, such as CCl_4, these solvents do not dissolve all compounds. In practice, deuterated solvents are generally used.

The nuclei of deuterium also exhibit nuclear spin, and therefore also resonate, but they absorb RF radiation over a very different range of frequencies than the protons. In an NMR spectrometer, a very narrow range of frequencies is used, covering just the frequencies absorbed by protons. For example, a 300-MHz spectrometer will use a pulse that consists only of frequencies between 300,000,000 and 300,005,000 Hz. The frequencies required for deuterium resonance do not fall in this range, so the deuterium atoms are invisible to the NMR spectrometer. All of the solvents shown below are routinely used, and many other deuterated solvents are also available commercially, although quite expensive.



Chloroform-d Methylene chloride-d_2 Acetonitrile-d_3 Benzene-d_6 Deuterium oxide

Sample Concentration

Ideally, 1-10 mg of the sample (except polymers) is generally required to obtain 1H

NMR spectrum of an organic compound. Although, it is possible to obtain spectra from smaller quantities of compound (dilute concentration) much greater care needs to be taken with sample preparation, so that peaks from common contaminants such as water and grease do not overwhelm peaks from the sample and also it may take hours to acquire it. If the sample is too concentrated then broad signal are observed because of increased viscosity of the solution which slows molecular tumbling. Slow molecular tumbling only partially averages the dipolar and chemical shift tensors, depriving us of the full orientational averaging that occurs with rapid molecular tumbling; only complete orientational averaging allows us to observe narrow resonances. To get ^{13}C NMR or DEPT NMR spectrum, more amount of sample (10-50 mg) is required along with a longer period of time (10-30 minutes) due to reduced sensitivity of ^{13}C relative to ^{1}H.

Solubility: Sample should be completely soluble in the chosen solvent. We must avoid having solids present in the tube. The better is the solubility better is the sensitivity of the experiment. Solid particles distort the magnetic field homogeneity which causes broad lines and indistinct spectra that cannot be corrected. Water must also be removed by drying the sample prior to dissolution.

It is good in practice to filter the NMR solutions before putting it the NMR tube. Adding small amount of extra solvent to the solution may also help minimize the line broadening caused by the microscopic nucleation of colloidal or crystalline particles present in saturated solutions.

The solvent produces NMR signals which will obscure regions of interest in the spectrum. Example: Benzene (C_6D_6) and toluene is not suitable if the compound has several aromatic rings.

a. Temperature: The melting and boiling points of the solvent should also be considered especially when the NMR has to be taken above or below room temperature. Example: DMSO is not appropriate for measurements below 16°C.
b. Residual Water Peak: Almost all NMR solvents contain traces of water and most are hygroscopic and hence the longer they are stored the more water they contain. The presence of a HDO peak will only serve to degrade the quality of the NMR spectra.
c. Cost: Cost is also important. In general, prices increase with the number of deuterated atoms. Example: $CDCl_3$, D_2O are relatively cheap, whereas C_6D_6, toluene-

d_8, DMF-d_7 are expensive. Hence we must first check the solubility of our compounds in the cheap solvents.

Sample Volume

The samples must be prepared up to a certain height (4.5 cm and 5.5 cm) in the NMR tube. Less sample height in the tube is very difficult to shim, and cause considerable delay in recording the spectrum. Samples that have too much solvent (long height) are also difficult to shim and are a waste of costly solvent. After preparation of the sample we should ensure that the cap is tightly kept onto the tube to minimize solvent loss through evaporation. In a 5 mm diameter NMR tube,a volume of between 0.6 and 0.7 ml is normally optimal.

Cleaning NMR tubes

Before using, NMR tube should be washed properly and dried. The NMR tubes should be first washed with detergent solution and rinsed several times with distilled water and then again several times with acetone or some other suitable solvent. We never use high boiling solvent such as dimethyl sulfoxide (DMSO) for the final rinse because of difficult evaporation. After complete wash, NMR tube should be kept in a “cool” oven (say 50 °C) for several hours to ensure the removal of residual acetone. An alternative method is to blow nitrogen or air through the tube while warming it **gently** for a few minutes with a heat gun. Do NOT dry NMR tubes in a hot oven (100 °C and above) as the high temperature may result in deforming of the NMR tubes.

The most proper way to keep NMR tubes in oven is by putting them flat on a paper towel or clean cloth. We never store NMR tubes upright inside a beaker or an Erlenmeyer flask. If we lean our tubes in a beaker in the drying oven, gravity will bend the tube and make it out of uniformity. If we lay our tubes flat for too long, though, they will develop an oval cross section and thus will nolonger be concentric.

Chemical Shift Measurement

In general there are four different features of a NMR spectrum which provide important information about the structure of a compound.

- **Number of signals**: The number of signals gives the information about how many different types of hydrogens are present.
- **Position of signals (Chemical shift):** The value of chemical shift is determined by the nearby environment of each hydrogen and thus we get information about the

local electronic surroundings for each hydrogen.

- **Intensity of signals**: The intensities of the signals tell us the number of identical hydrogens.
- **Spin-spin splitting of signals**: The splitting of each signal tells about the other groupspresent in the proximity to that particular hydrogen.

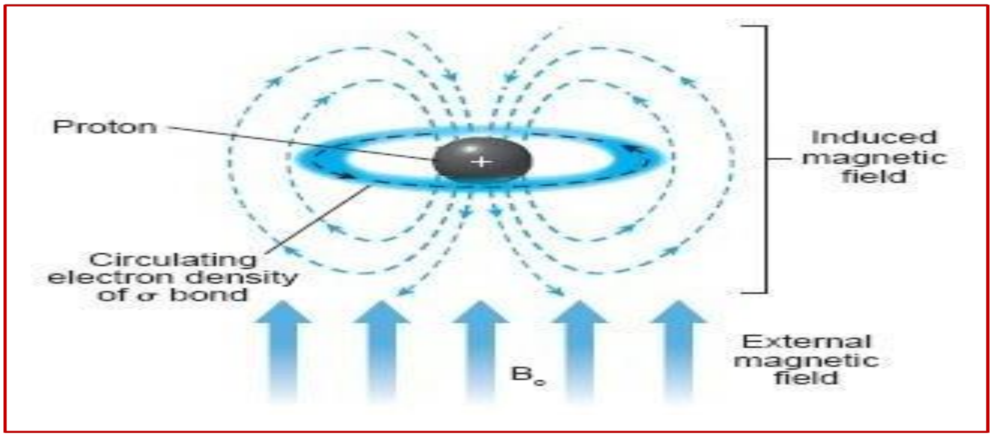
An NMR spectrum is a plot of the intensity of a peak against chemical shift, measured in parts per million (ppm). Chemical shifts are reported on the horizontal axis of the spectrum. Most protons absorb between 0-12 ppm. The terms “upfield” and “downfield” describe the relative location of peaks. Upfield means the low value of chemical shift (right side) and downfield means the high value of chemical shift (left side). In a molecule each type of proton has a different electronic environment and shows signal at different value of chemical shift. All these values are measured relative to the position of a reference peak at 0 ppm on the δ scale due to tetramethylsilane (TMS). TMS is a volatile inert compound that gives a single peak at 0 ppm.

Shielding and Deshielding

At a particular magnetic field strength, we might expect all nuclei to absorb the same frequency of RF radiation. But this is not true because the nuclei are surrounded by electrons and these electrons shield the nucleus. In the presence of an external magnetic field, the electrons circulate which generate a local (induced) magnetic field (IMF). The direction of IMF is opposite to that of the external magnetic field. This effect is called **diamagnetism**. More the electron density around the nucleus more is the shielding of the nucleus. This effect is particularly important for NMR spectroscopy. Without this effect, all protons would absorb the same frequency and hence would not provide any useful information about the molecule.

The magnitude of induced magnetic field is small but has important effect on the nucleus. Now the proton experiences two magnetic fields i.e. the strong, external magnetic field and the weak induced magnetic field produced by the circulating electron density. Hence the net magnetic field (B_{eff}) feel by the proton is slightly smaller than the external magnetic field and the proton is said to be **shielded** by the electrons.

$B_{eff} = B_0 - \sigma \times B_0$ (where σ is screening or shielding constant)



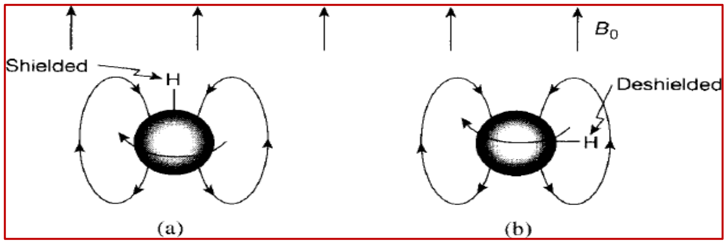
The induced magnetic field generated by the motion of electrons that are surrounding the proton.

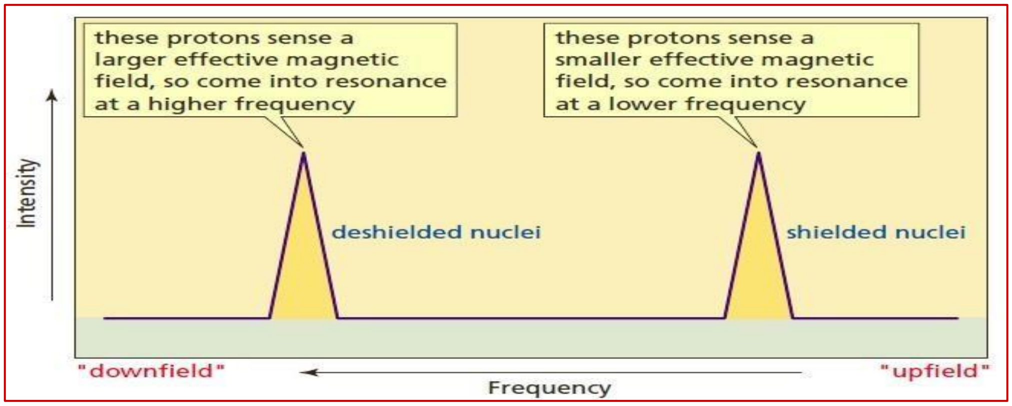
Not all protons occupy identical electronic environments. Some protons are surrounded by more electron density and are more shielded, while other protons are surrounded by less electron density and are less shielded or **deshielded**. As a result, protons in different electronic environments will exhibit a different energy gap between α and β spin states and will therefore absorb different frequencies of RF radiation. This phenomenon of shielding and deshielding allows in exploring the chemical environment of each hydrogen atom in a molecule.

When a molecule placed in external magnetic field, the electrons surrounding a nucleus start to circulate perpendicular to the applied magnetic field and they produce secondary magnetic field or local magnetic field called as induced magnetic field which opposes the applied magnetic field in the region of nucleus. Thus the nucleus experiences a weaker magnetic field than the applied magnetic field and is said to be shielded. The difference between the applied magnetic field and the induced magnetic field is termed the *nuclear shielding*.

The magnitude of the induced magnetic field is directly proportional to the magnitude of the applied magnetic field. The higher the electron density around the proton the higher is the diamagnetic shielding. Circulation of electrons about nearby nuclei generates an induced magnetic field that can either oppose or reinforce the applied magnetic at the proton, depending upon its location in the induced magnetic field. If the induced magnetic field generated by electron around the proton opposes the applied magnetic field, the nucleus experiences a weaker magnetic field than the applied magnetic field and the proton is said to be **shielded**. This type of shielding is called as diamagnetic shielding.

One the other hand if the induced magnetic field reinforces the applied magnetic field, the proton experiences the higher field strength. Such type of proton is said to be **deshielded.**





This type of shielding is called as *paramagnetic shielding*.

Compared to a naked proton, a shielded proton requires a higher applied field strength, whereas a deshielded proton requires lower field strength for transition. Thus shielding shifts the absorption upfield and deshielding shifts the absorption downfield. Such shifting in the NMR absorption positions is called as chemical shift as it arises from the circulation of electrons in chemical bonds. The induced magnetic field is not uniform over the entire molecule and hence each nucleus experiences a slight different local magnetic field strength, therefore their absorption signals appear at different values. Thus signals in proton NMR spectra give information about the different kinds of protons and their chemical environment in the molecule.

Each proton in a molecule is in a slightly different chemical environment and hence they do not have much difference in their resonance frequency. This difference is very little in comparison to the instrument field strength (60 MHz). Therefore it is very difficult to measure the exact frequency values of each proton accurately. However the relative frequencies can be determined with more accuracy. For this reason a reference compound is added to the sample and the resonance frequeny of each proton is measured relative to the resonance frequency of the reference compound. Tetramethylsilane, $(CH_3)_4Si$ is the most commonly used compound as a reference. The position of various signals can be measured in terms of hertz by putting TMS peakat zero hertz.

This compound is chosen because the following properties.

1. It is chemically inert and miscible with a wide range of solvents.
2. It gives a single sharp peak as it has twelve chemically and magnetically equivalent protons.
3. It is highly volatile and hence can be easily removed if the sample has to be removed.
4. The protons of its methyl groups are more shielded than the protons of most organic compounds due to the low electronegativity of the silicon. Hence the NMR signal of particular proton in a molecule will appear at different field strength compared to a signal from TMS. The resonance position of the protons of TMS is assigned as zero on the δ-scale and the other protons are measured with respect to this.

Chemical shift values

Chemical shift when measured in Hz is directly proportional to the strength of the applied magnetic field and therefore these values will vary with the strength of applied magnetic

$$\boxed{\delta = \frac{\text{observed shift from TMS in hertz} \times 10^6}{\text{operating frequency of the instrument in hertz}}}$$

field. Hence if the chemical shift is given in hertz, the applied frequency should also be mentioned. The NMR instruments of different field strength are available hence It is advisable that the chemical shift should be expressed in the terms which are independent of field strength. One such unit is δ (delta). The chemical shifts are generally expressed in δ values whose units are ppm and it is independent of the field strength. The chemical shift of a particular proton is defined as the difference (in hertz) between the resonance frequency of the proton under observation and that of TMS, divided by the operating frequency of the spectrometer.

For example, when benzene is analyzed using an NMR spectrometer operating at 300 MHz, the protons of benzene absorb at a frequency that is 2181 Hz larger than the frequency of absorption of TMS. The chemical shift of these protons is then calculated in the following way:

$$\delta = \frac{2181\,\text{Hz} \times 10^6}{300 \times 10^6\ \text{Hz}} = 7.27$$

If a 60 MHz spectrometer is used instead, the protons of benzene absorb a frequency of rf radiation that is 436 Hz larger than the frequency of absorption of TMS. The chemical shift of these protons is then calculated in the following way:

$$\delta = \frac{436\ \text{Hz} \times 10^6}{60 \times 10^6\ \text{Hz}} = 7.27$$

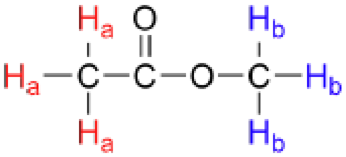
Thus the chemical shift value does not depend on the operating frequency and it is a dimension less. That is why chemical shifts have been defined in relative terms (δ-scale), rather than absolute terms (hertz). If signals were reported in hertz (the precise frequency of radiation absorbed), then the frequency of absorption would be dependent on the strength of the magnetic field and would not be a constant. For most organic compounds, the signals produced will fall in a range between 0 and 12 ppm. In rare cases, it is possible to observe a signal occurring at a chemical shift below 0 ppm, which results from a proton that absorbs a lower frequency than TMS. Most protons in organic compounds absorb a higher frequency than TMS, so most chemical shifts that we encounter will be positive numbers.

Chemical shift can also be measured on τ-scale in which TMS is given a value of 10 ppm. The values in δ can be converted into τ units as following

$$\tau = 10 - \delta$$

Number of Signals: Chemical Equivalence and Non-equivalence

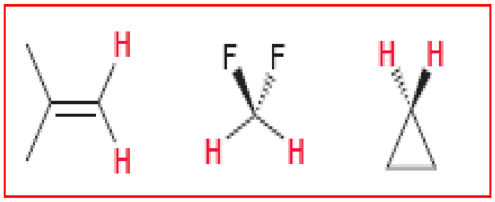
The number of signals in a ^{1}H NMR spectrum indicates that how many different kinds of protons are present in a molecule. This is due to the fact that the protons in different electronic environments absorb at different field strength and hence different chemical shift. Protons that have same chemical environment are called **chemically equivalent** and they



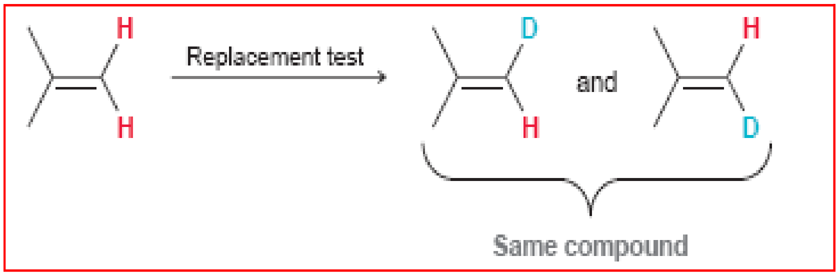
have same the same chemical shift. In methyl acetate, for example, there are two types of protons. The three protons labelled as H_a have a different chemical shift value than the three H_b protons, because the two sets of protons are in different environments i.e. they are chemically non-equivalent. On the other hand, the three H_a protons are all in the same electronic environment, and are chemically equivalent to one another. They have identical resonance frequencies. The same can be said for the three H_b protons.

Homotopic protons

Below are some examples of homotopic protons. **The homotopic nuclei are interchangeable by rotational axis of symmetry**. In each of the following examples, the two protons shown in red color are homotopic because as you draw



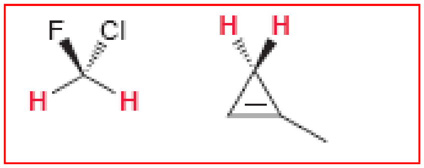
the two structures by replacing each one of these protons with deuterium, both the structures represent the same compound that means that these protons are homotopic.



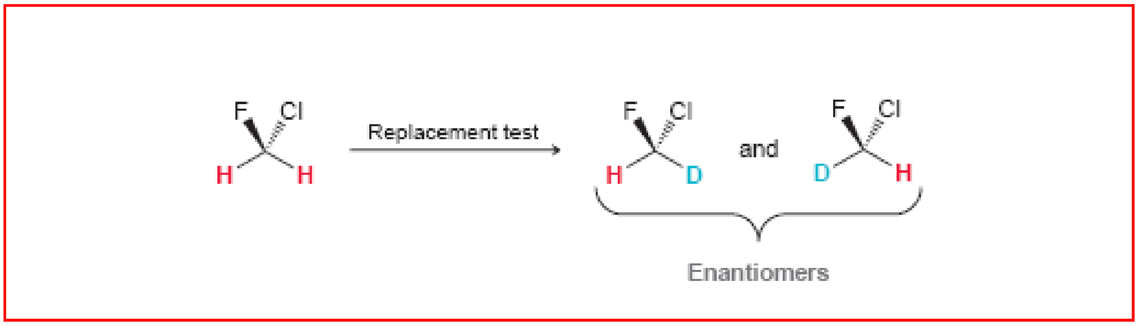
Enantiotopic protons

When a carbon is bonded to two hydrogens and to two different groups, the two hydrogens are called as enantiotopic hydrogens. The enantiotopic nuclei are interchangeable by reflection/plane of symmetry.

Here are some other examples of enantiotopic protons. In each of these examples the two

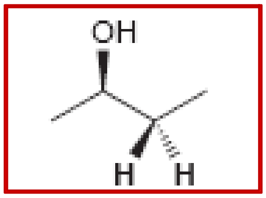


highlighted protons are enantiotopic because as you draw two compounds each time replacing one of the protons with deuterium. Then determine the relationship between the two drawings. If they are enantiomers, then the protons are enantiotopic.

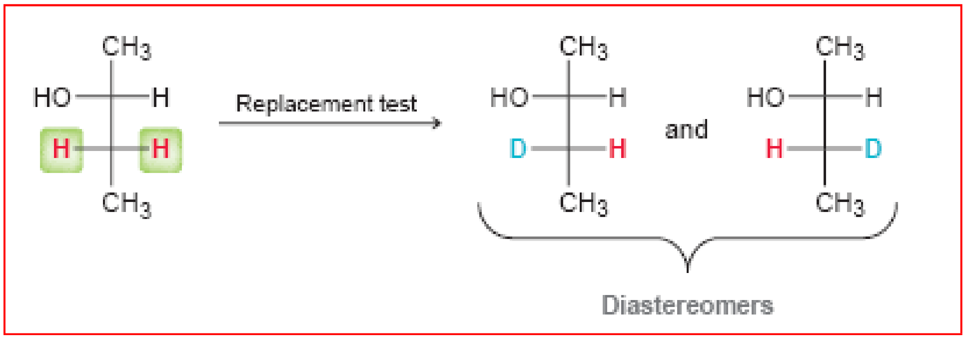


Diastereotropic protons

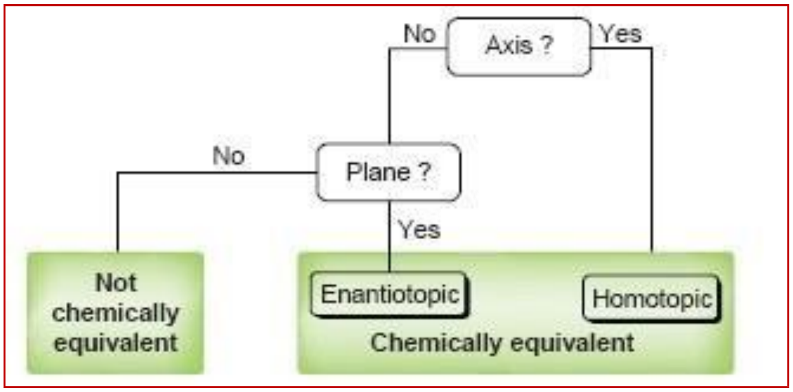
If two protons are neither homotopic nor enantiotopic, then they are not chemically equivalent. Consider the protons on C3 of (*R*)-2-butanol. These protons cannot be interchanged by either rotational symmetry or reflection/plane of symmetry. Therefore, these two protons are not chemically equivalent. If you replace these two hydrogens with



another atom/group say, deuterium each at one time and if it produces a pair of diastereomers, then these protons are called as diastereotopic protons which have different chemical shift and shows two signals in the NMR spectrum. Diastereotopic protons cannot be interchanged by symmetry.

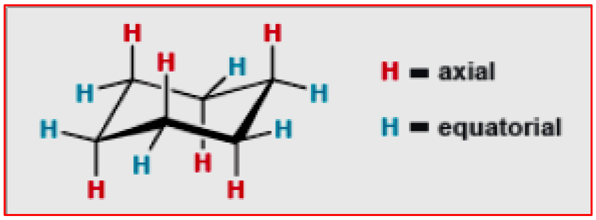


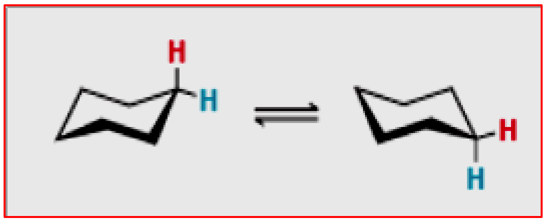
Note: While determining the relationship between two protons, first determine if there is an axis of symmetry that interchanges the protons. If there is, then the protons are homotopic, whether or not there is a plane of symmetry. If the protons cannot be interchanged by rotation, then look for reflectional symmetry. If there is a plane of symmetry, then the protons are enantiotopic.



If two or more protons are in an equivalent environment, then they will have the same chemical shift and appear as one signal. In a methyl group (CH_3) all three protons are always equivalent. In a methylene group (CH_2) the two protons are also equivalent, unless adjacent to a chiral center.

In the chair conformation of cyclohexane, axial and equatorial protons occupy a different electronic environment and hence they are not chemically equivalent. They cannot be interchanged by a symmetry operation. Consequently, we might expect two signals in the ^1H NMR spectrum; one for the six axial protons and the other for the six equatorial protons. But, the ^1H NMR spectrum of cyclohexane exhibits one signal. Why? The observation can be explained by considering the rapid rate at which ring flipping occurs at room temperature.



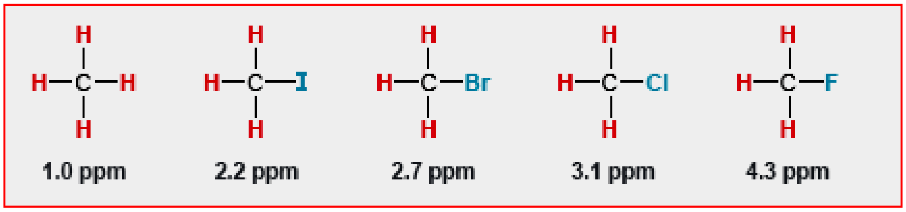


The NMR spectrometer is too slow to acquire a spectrum of a single chair conformation. While the spectrometer is acquiring the spectrum, the ring is flipping rapidly between the two chair conformations, producing a blurry picture. The spectrometer only "sees" the average electronic environment of the protons, and only one signal is observed. However, if the sample of cyclohexane is cooled inside the spectrometer, the ring-flipping process occurs at a slower rate. If the sample is cooled to −100°C, ring flipping occurs at a very slow rate, and separate signals are in fact observed for the axial and equatorial protons.

The chemical shift of a given proton is not an invariant property of a molecule (like a melting point or boiling point), but will change depending on the molecular environment. The chemical shift depends on several factors described below.

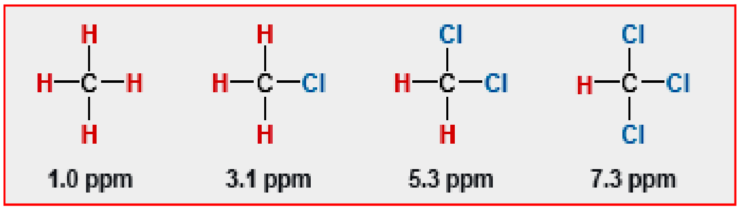
Electronegativity effects

A proton is said to be deshielded if it is attached to the electronegative atom or group. Greater is the electronegativity of the atom, greater is the deshielding caused to the proton. Larger is the deshielding of a proton, larger is the chemical shift value (δ). The chemical shift values of **CH_4, CH_3F, CH_3Cl, CH_3Br and CH_3I** protons are **0.33, 4.26, 3.05, 2.68 and 2.16** respectively. Since



„F" is the most electronegative atom and hence the chemical shift of CH3F protons is very high, whereas the Iodine being least electronegative among the halogens, show the lower value of chemical shift.

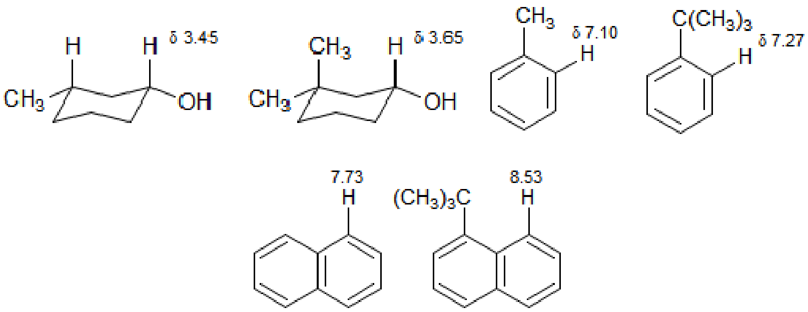
As the number of electronegative atoms or groups attached to a particular carbon containing the proton increases, the chemical shift value also increases because of the increasing deshielding of the concerned proton. The chemical shift values of **CH_4, CH_3Cl, CH_2Cl_2, $CHCl_3$** protons are **0.33, 3.05, 5.28 and 7.23** respectively.



The inductive effect weakens with increasing the distance. Therefore, as the distance of proton increase from the electronegative atom, the deshielding effect due to it also diminishes and hence the farther protons from the electronegative atom show lower value of chemical shift compared to the closer protons. The chemical shift values of methyl protons in **CH_3Cl and CH_3CH_2Cl** are **3.05 and 1.48** respectively.

Steric effects

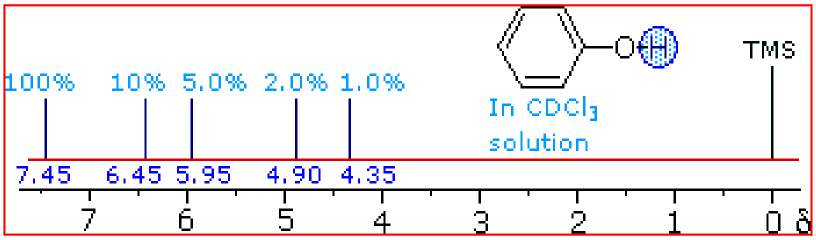
In overcrowded molecules, it is possible that some protons may occupy sterically hindered position resulting in van der Waal‟s repulsion. In such molecules the electron cloud of a bulky group (hindering group) will tend to repel the electron cloud surrounding the proton. Thus such a proton will be deshielded and will resonate at slightly higher value of chemical shift than the expected in the absence of this effect.



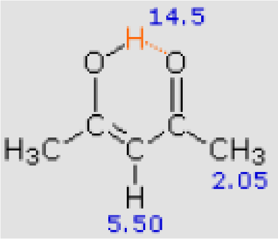
Hydrogen Bonding

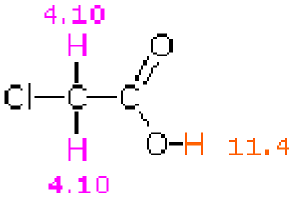
In general hydrogen bonded protons show higher value of chemical shift than the non-hydrogen bonded protons. Due to the high electro-negativity of the atom to which proton is hydrogen bonded, the electron cloud around the proton is decreased and thus causes deshielding of the proton. The downfield shift of the absorption depends on the strength of the hydrogen bonding. Greater is the degree of hydrogen bonding of proton, greater the downfield shift of a proton.

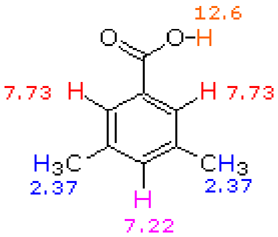
This is due to hydrogen bonding that the chemical shifts of OH, NH and SH protons vary over a wide range. It is unpredictable as the extent of hydrogen bonding depends on concentration, temperature, solvent and compound structure. The OH signals tend to move downfield at higher compound concentration because of increased hydrogen bonding. Also on increasing temperature, hydrogen bonding decreases and hence OH signals moves slightly upfield. Both OH and NH signals move downfield in H-bonding solvents like DMSO or acetone. The intermolecular and intramolecular hydrogen bonding can be distinguished by the use of 1H NMR spectroscopy. The intermolecular hydrogen bonding depends on the concentration of the sample and decreases on dilution with a non-polar solvent and also with increasing temperature. The pure ethanol show signals near 5 ppm but on dilution with non hydrogen-bonded solvents (CCl_4, $CDCl_3$, C_6D_6), the OH signal shift upfield due to the breaking of intermolecular hydrogen bonds. The more acidic hydroxyl group of phenol also shows similar concentration dependence to that of alcohols. The phenolic OH peak at different percent concentrations of phenol in $CDCl_3$ are shown in the following diagram (C-H signals are not shown).



The intramolecular hydrogen bonding does not depend on the concentration of the sample and hence show no change in their absorption position on dilution. The salicylates, enols of β-diketones and carboxylic acids are some classic examples of intramolecular hydrogen bonding. The hydroxyl proton of enol form of 2,4-pentanedione (4-hydroxypent-3-ene-2-one) shows a peak significantly down field (10-16 ppm) compared to the OH signal in other function groups due to strong intramolecular hydrogen bonding. Similarly the hydroxyl proton of carboxylic acid displays a very downfield peak at about 10-13 ppm which may be attributed to the favored hydrogen-bonded dimeric association.







Solvent Effects

Chloroform ($CDCl_3$) is the most common solvent for NMR measurements. Other deuterium labelled compounds, such as deuterium oxide (D_2O), benzene-*d6* (C_6D_6), acetone-d_6 (CD_3COCD_3) and DMSO-d_6 (CD_3SOCD_3) are also used as NMR solvents. All these solvents vary considerably in their polarity and magnetic susceptibility. Hence the NMR spectrum recorded in one solvent may be slightly different from that recorded in other solvent. Hence it is important to mention the solvent used. The NMR signals for protons attached to carbon are generally shifted slightly by changing solvent except where significant bonding or dipole-dipole interaction might arise.

The changes in chemical shifts due to solvents changes are minor being on the order of ±0.1 ppm. However, in some cases the changes are more. The aromatic solvents benzene and pyridine cause changes in chemical shifts as large as 0.5 to 0.8 ppm compared to less magnetically active solvents like chloroform or acetone. It is observed that the carbonyl groups form weak π–π collision complexes with benzene rings that persist long enough to exert a significant shielding influence on nearby groups.

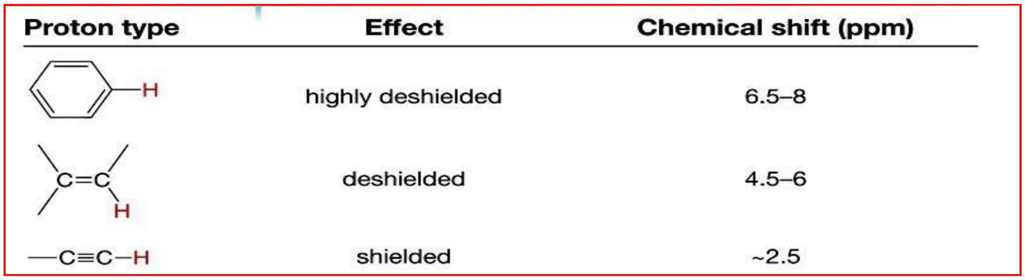
The second noteworthy change can be observed in the spectrum of tert-butanol in DMSO, where the hydroxyl proton is shifted 2.5 ppm downfield from where it is found in dilute chloroform solution. This is due to strong hydrogen bonding of the alcohol O–H to the sulfoxide oxygen, which not only deshields the hydroxyl proton, but secures it from very rapid exchange reactions that prevent the display of spin-spin splitting. Similar but weaker hydrogen bonds are formed to the carbonyl oxygen of acetone and the nitrogen of acetonitrile.

Shielding and deshielding in Aromatic Protons

Electron donating substituents cause shielding of the aromatic protons as they increase electron density on the benzene ring. The order of shielding is ortho > para > meta because the electron density at ortho and para positions are the higher due to the +M effect. On the other hand, the electron withdrawing substituents cause deshielding of all the aromatic protons. The order of deshielding is in the order of ortho > para > meta because the electron withdrawing effect is maximum at ortho and para positions due to the +M effect.

Anisotropic effect (space effect)

In some cases such as alkenes, alkynes, carbonyls and aromatic compounds the chemical shift cannot be explained only on the basis of the above facts. The carbon atom in acetylene is more electronegative (sp hybridized) than the carbon in ethylene (sp^2 hybridized) but the chemical shift value for actylenic protons are less (more shielded) than the ethylenic protons. Such anomalous behaviour can be explained by the anisotropic effect produced by circulation of π-electrons under the influence of applied magnetic field. Anisotropy means uneven magnetic field in space i.e. different regions of space are characterized by different magnetic field strengths. The anisotropic effect depends upon the orientation of the molecule with respect to the applied field.



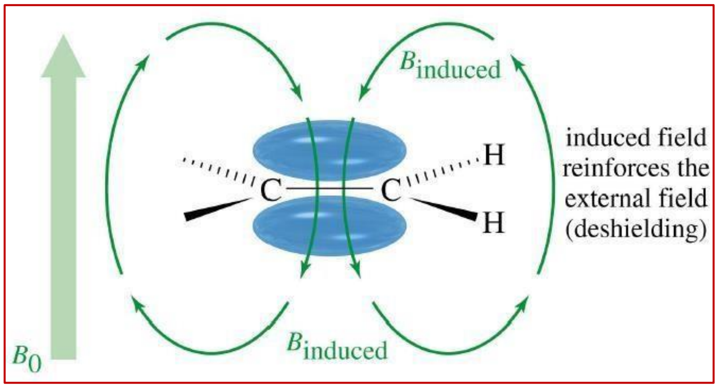
Proton type	Effect	Chemical shift (ppm)
—H	highly deshielded	6.5–8
C=C H	deshielded	4.5–6
—C≡C—H	shielded	~2.5

Effect of electrons on chemical shift values

In fact, a proton experiences three different fields, the applied field, the induced magnetic field generated by σ-electrons and the induced magnetic field generated by π-electrons. The π-electrons are more polarizable than the sigma bond electrons. Therefore, the induced magnetic field due to π-electrons movement is stronger (strong secondary fields that perturb nearby nuclei) than that generated by σ-electrons. Depending on the position of the proton in the induced magnetic field, it can be either shielded (smaller δ) or deshielded (larger δ).

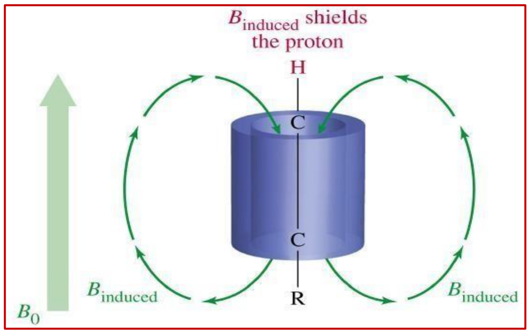
Alkene Protons

An alkene molecule in an external magnetic field is so oriented that the plane of the double bond is perpendicular to the applied magnetic field. Thus the circulation of π-electrons is perpendicular to the carbon-hydrogen framework of the molecule. As a result the induced magnetic field causedby the circulation of π-electrons is in the opposite direction of applied magnetic field (diamagnetic) around the carbon atoms and in the direction of applied magnetic field (paramagnetic) in the region of olefenic protons. Therefore olefenic protons experience greater field strength and consequently resonate at larger value of chemical shift.



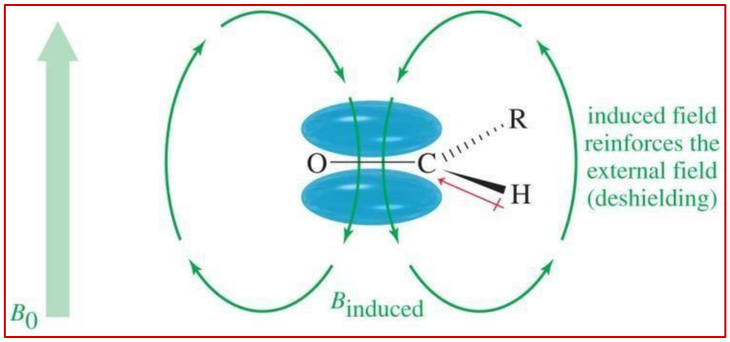
Alkyne protons

An alkyne molecule when placed in an external magnetic field is so oriented that the plane of the triple bond lies parallel to the direction of applied magnetic field. The induced magnetic field caused by the circulation of π-electrons of the triple bond is in the opposite direction of applied field (diamagnetic) in the region of acetylenic protons. Therefore acetylenic protons are shielded and experience lesser field strength and consequently resonate at smaller value of chemical shift.



Carbonyl protons

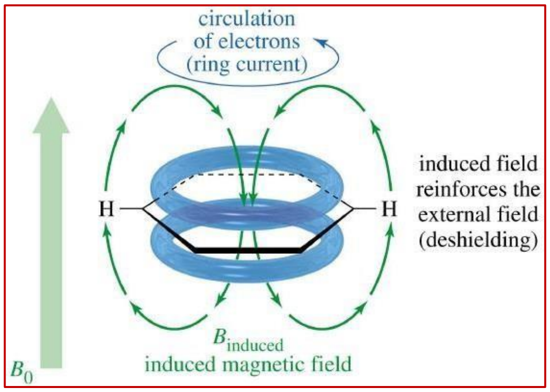
Carbonyl compound when placed in an external magnetic field is so oriented that the plane of the carbon-oxygen double bond is perpendicular to the applied field. The circulation of π-electrons generates an induced magnetic field which is in the direction of applied magnetic field at the

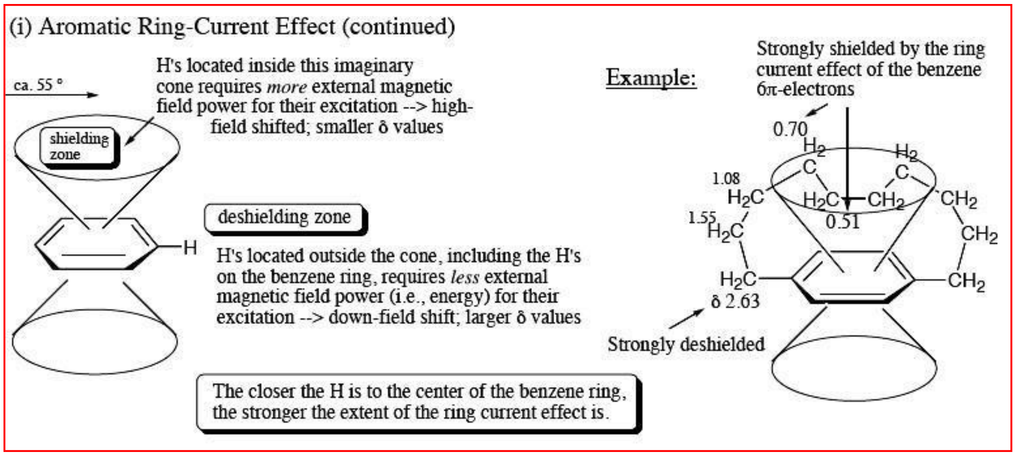


aldehydic protons Therefore, aldehydic protons experience greater field strength (diamagnetic effect) and consequently resonate at larger value of chemical shift (deshielding). In addition, the high electronegativity of oxygen atom also contributes to the higher δ value of aldehydic proton.

Aromatic protons

In benzene, the π-electrons are delocalized cylindrically over the aromatic ring (above and below the plane of the ring). When a magnetic field is applied perpendicular to the plane of the aromatic ring, the circulation of π-electrons produces a ring current which induces a magnetic field perpendicular to the plane of the ring. The induced magnetic field is in the opposite direction (diamagnetic effect) as the applied field at the centre of the ring, but outside the ring it is in the same direction (paramagnetic effect) of the applied field. Therefore, the protons at the periphery of the aromatic ring (Aromatic protons) experience larger magnetic field and resonate at higher δ values (deshielding). Protons which are present above or below to the plane of the aromatic ring resonate at low δ values (Shielded).




(i) Aromatic Ring-Current Effect (continued)

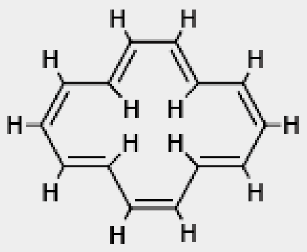
H's located inside this imaginary cone requires *more* external magnetic field power for their excitation --> high-field shifted; smaller δ values

H's located outside the cone, including the H's on the benzene ring, requires *less* external magnetic field power (i.e., energy) for their excitation --> down-field shift; larger δ values

The closer the H is to the center of the benzene ring, the stronger the extent of the ring current effect is.

Strongly shielded by the ring current effect of the benzene 6π-electrons

To make it more clear let us take an example of methylbenzene. Methylbenzene has two different types of protons. A multiplet near 7 ppm is observed due to the aromatic protons. The methyl group (CH_3) in methylbenzene produces a signal at 2.3 ppm, whereas a methyl group attached to alicyclic conjugated alkene (**CH_3**-CH=CH_2) appears at 1.95 ppm. This high value of chemical shift is due to the desheilding effect of ring current (cyclic system with delocalized electrons) in aromatic compound compared with the deshielding of conjugated alkene group. A similar effect is observed in [14] annulene. The protons outside the ring are strongly deshielded and produce signals at ≈8 ppm, whereas inside the ring protons are strongly shielded (≈-1.8 ppm).



[14] Annulene

Alkanes

The sigma bonding electrons also produce anisotropic effects but it is less pronounced than that produced by circulating π-electrons. The axis of the C-C bond is the axis of the deshielding. The single bond anisotropy can be observed occurs in the alkanes of type R-CH_3, R_2CH_2, R_3CH as well as the equatorial versus axial protons in a fixed cyclohexane ring. The equatorial protons in a conformationally rigid cyclohexane ring appear more downfield by nearly 0.5 ppm than the axial protons on the same carbon. This effect can be explained on the basis of anisotropy effect shown in the figure, where the equatorial protons reside in the deshielding (+δ) region of the C-C anisotropy, and the axial in the shielding (-δ) region. The axial and equitorial protons on C1 are equivalenty positioned with respect to the C1-C2 and C1-C6 bonds and these do not produce any differential effect. However the C1 axial proton is within the shielding region of the C2-C3 and C5-C6 bonds, whereas C1 equitorial proton is within their deshielding region. An alternative explanation, or additional contributing effect, is based on the supposition that a C-H bond is a stronger σ donor than a C-C bond, which leads to increased electron density in the axial protons (anti to two C-H bonds), hence shielding effect (-δ region).

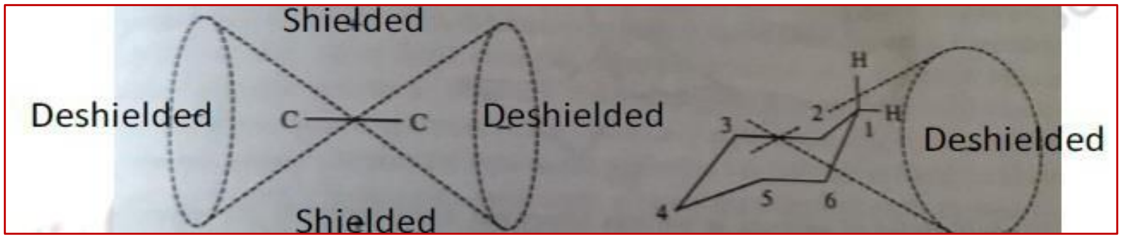


Figure: Shielding and deshielding region around a carbon-carbon single bond and a rigid six membered ring system

The **methyl** protons (CH_3) appear near 0.9 ppm, a **methylene** protons (CH_2) shows signal near 1.2 ppm, and a **methine** proton (CH) shows near 1.7 ppm. The high frequency position of methine protons (R_3CH) compared with methylene protons (R_2CH_2) and that of compared with methyl protons $R-CH_3$) may be attributed to the anisotropy of the additional C-C bonds. The tertiary proton falls in the deshielding region of three C-C bonds, secondary protons in the deshielding region of two C-C bonds and the primary proton falls in the deshielding region ofonly one C-C bond.

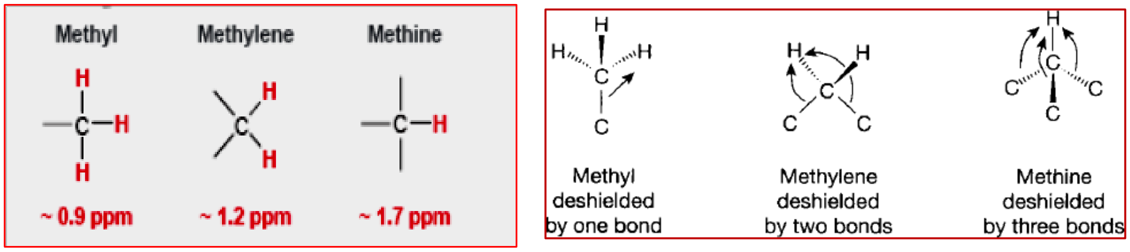


Figure: Chemical shift values and shielding properties of methyl, methylene and methine groups

MASS SPECTROMETRY

Introduction

Mass Spectrometry (MS) is an analytic technique used to determine the relative masses of molecular ions and fragments by calculating the degree of deflection of charged particles in a magnetic field. It provides a great deal of information with very small amount of samples. Mass spectrometry is used to:

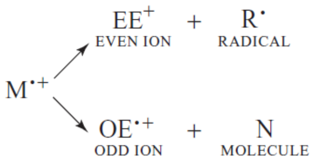
- Determine molecular mass
- Determine molecular formula of a compound
- Determine structural features of the compound
- Find out the structure of an unknown substance
- Provide data on isotopic abundance
- Verify the identity and purity of a known substance

Mass spectrometry is an instrumental technique that does not involve electromagnetic radiation. Thus, it is called spectrometry, whereas the others such as UV, NMR, IR are called spectroscopy. The principle of mass spectrometry involves the generation of ions from either inorganic or organic compounds, to separate the ions by their mass-to-charge ratio (m/z) and to detect them qualitatively and quantitatively by their m/z value and relative abundance.

In the first step a beam of energetic electron produce gas phase ions of the compound.

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

Removal of one electron from the molecule (M) results into generation of parent ion (M $^{+}$ or molecular ion). The molecular mass of the compound is equal to the m/z value of the parent ion. This parent ion or molecular ion normally undergoes fragmentations (fragment ions ordaughter ions). The parent ion (M $^{+}$) is a radical cation with an odd number of electrons, it can fragment to give either a radical (R) and an ion with an even number of electrons (EE^{+}), or a molecule (N) and a new radical cation (OE +).



These two types of ions derived from the molecular ion can, further undergo fragmentation, and so on. All these ions formed are then separated by the mass spectrometer according to their mass- to-charge ratio. Only positively charged species reach the collector. The charge (*z*) on all the fragments is +1, therefore *m/z* is equal to the molecular mass (*m*) of the fragment. A graph of the relative abundance of each fragment plotted against its *m/z* value is called a mass spectrum.

For example, in the case of methane (CH4), the impact of high energy electrons causes the molecule to lose an electron and form a radical cation with m/z 16. A species, with a positive charge and one unpaired electron is called radical cation.

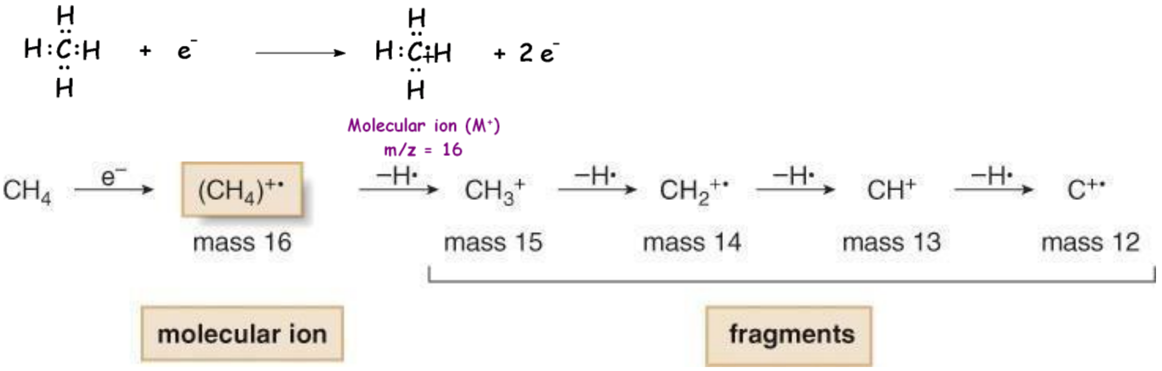
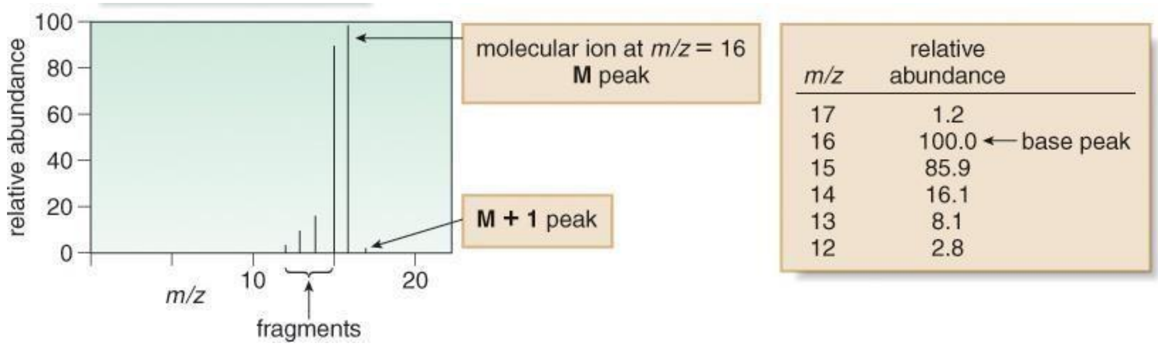


Figure: Fragmentation in methane



m/z	relative abundance
17	1.2
16	100.0 ← base peak
15	85.9
14	16.1
13	8.1
12	2.8

Figure: Mass spectrum of methane

Instrumentation: Mass Spectrometer

The mass spectrometer consists of:

- **Ion source**
- **Mass analyzer**
- **Ion detector**

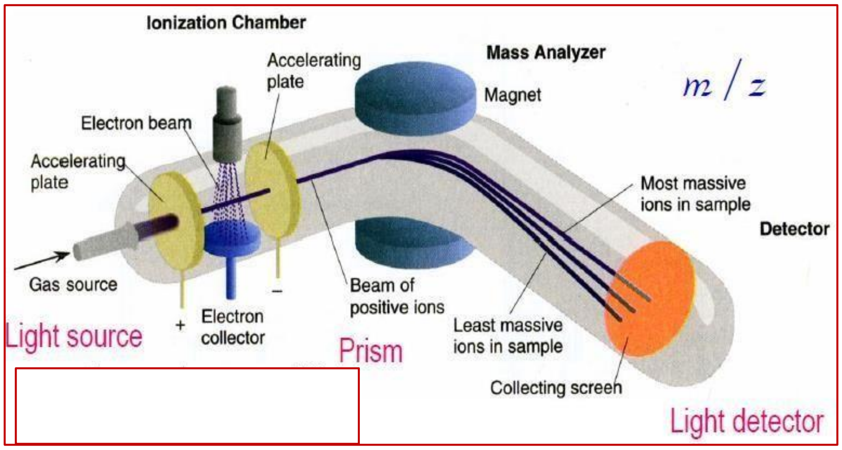


Figure: Diagram of mass spectrometer

Ion source (Ionisation):

In the first step a little amount of a compound is evaporated. The vaporized sample is, then ionized by bombardment with a beam of high energy electrons (usually 70 eV). The electron beam knocks out an electron from the molecule of the injected sample, creating a molecular ion (a radical cation). The molecular ion further breaks into fragments as it travels through the mass spectrometer as loss of electron weakens its bonds and collision gives it extra kinetic energy.

There is a pair of oppositely charged plates in the ionization chamber. The positively charged onecauses the positively charged radical cation to accelerate into an analyzer tube.

The energy required for removing one electron from the neutral parent molecule is usually 10 eV. With this much energy, no fragmentation of the parent ion takes place. But the energy of the bombarding electron is around 70 eV, the additional energy is consumed in fragmenting the parent ion. This results in the formation of fragment ions of the daughter ions.

Mass analyzser:

The separation of ions takes place in the analyzer at a pressure of about 10-8 mbar. The analyzer tube is surrounded by a curved magnetic field, which causes the path of the radical cation to be deflected in proportion to its mass-to-charge ratio (m/z). The flight path of the ion depends on its molecular mass, its charge, and the strength of the magnetic field. Thus, at a given magnetic field strength, ions of only one specific mass collide with the detector and are recorded.

Ion detector:

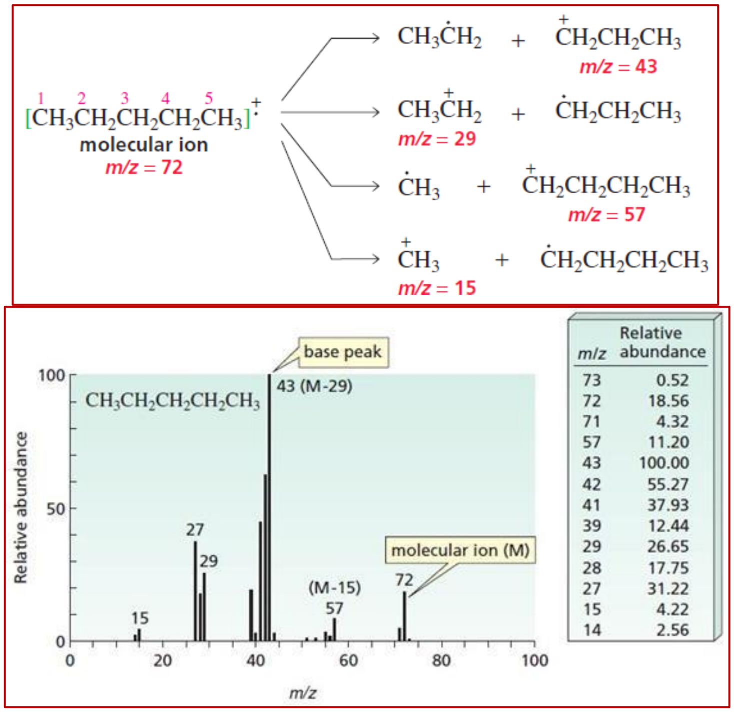
The ions which are separated by the analyser are detected and measured electrically or photographically. After the ions have passed the exit-slit, they collide on a collector-electrode. The resulting current is amplified and registered as a function of the magnetic-field force or the accelerating voltage.

The strength of the magnetic field is varied in increments to produce a mass spectrum, which is a plot of m/z (on the x axis) against relative abundance (on the y axis). If we assume that all ions have a charge of +1, then the peaks give the mass ratios and their heights give the proportions of ions of different masses.

Fragmentation in pentane

The *m/z* value is the **nominal molecular mass** of the fragment and it is the molecular mass to the nearest whole number.

For example, pentane has a molecular mass of 72.0939 and a nominal molecular *mass* of 72. Pentane undergoes fragmentation, between carbon-carbon bonds to form various radical cations with different m/z value. Weak bonds break in preference to strong bonds, and bonds that break to form more stable fragments break in preference to those that form less stable fragments.



m/z	Relative abundance
73	0.52
72	18.56
71	4.32
57	11.20
43	100.00
42	55.27
41	37.93
39	12.44
29	26.65
28	17.75
27	31.22
15	4.22
14	2.56

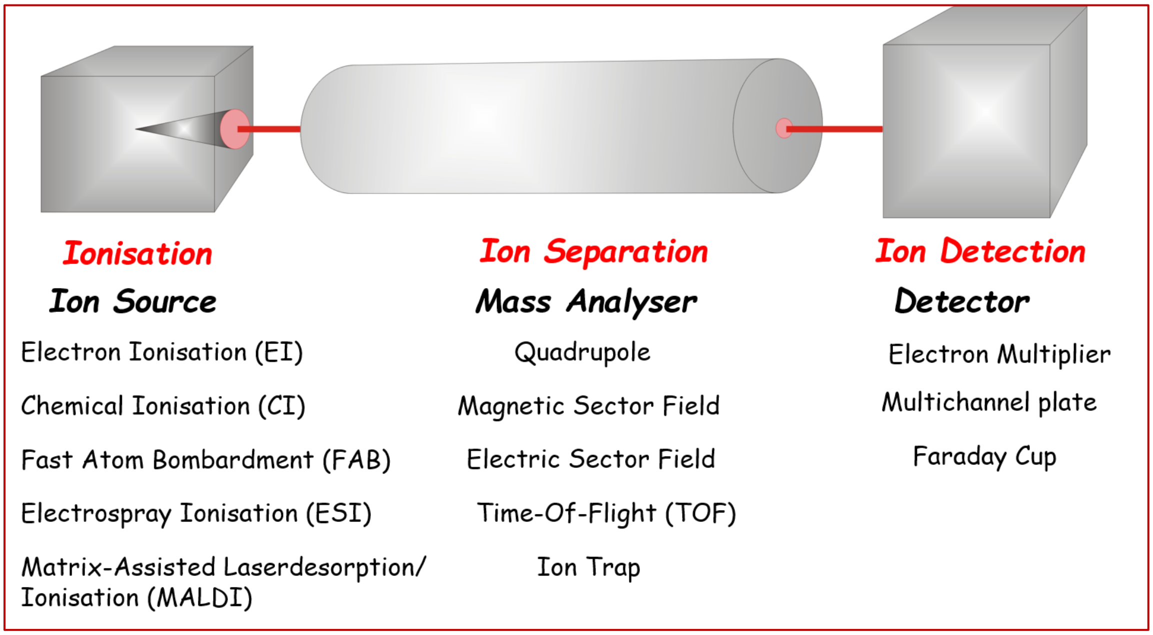
Figure: Mass spectrum of pentane (bar graph and tabular form)

Mass spectra can be shown either as bar graphs or in tabular form.

The peak with the highest *m/z* 72 value in the spectrum of pentane is due to the fragment that results when an electron is knocked out of a molecule (figure 4). It is called **molecular ion** peak and it is equal to molecular mass of pentane.

Peaks with *m/ z* values less than 72 are called fragment ion peaks. The peak at m/z 43 with the greatestintensity is called **base peak**.

Types of Mass Spectrometer



Ionisation	**Ion Separation**	**Ion Detection**
Ion Source	**Mass Analyser**	**Detector**
Electron Ionisation (EI)	Quadrupole	Electron Multiplier
Chemical Ionisation (CI)	Magnetic Sector Field	Multichannel plate
Fast Atom Bombardment (FAB)	Electric Sector Field	Faraday Cup
Electrospray Ionisation (ESI)	Time-Of-Flight (TOF)	
Matrix-Assisted Laserdesorption/ Ionisation (MALDI)	Ion Trap	

Depending upon the methods of ionization, ion separation and detection, there are various types of mass spectrometers as shown.

Ionization Methods in Organic Mass Spectrometry

There are many types of ionisation methods available with their own, advantage and disadvantage**.** The ionisation method to be employed depends on the type of sample under investigation and the mass spectrometer available.

The various ionization methods are following:

- Electron Impact Ionisation (EI)
- Chemical Ionisation (CI)
- Fast Atom Bombardment (FAB)
- Electrospray Ionisation (ESI)
- Matrix Assisted Laser Desorption Ionisation (MALDI)

Most of the ionisation methods crwate both positively and negatively charged sample ions, depending on the proton affinity of the sample. In **positive ionisation** mode the **protonated molecular ions ($M+H^+$)** are generated. In **negative ionisation** mode the **deprotonated molecular ions ($M-H^-$)** are usually generated.

Analysis and Separation of Sample Ions

The main function of the mass analyser is to separate**,** or resolve**,** the ions formed in the ionisation source of the mass spectrometer according to their mass-to-charge (m/z**)** ratios. There are a number of mass analysers currently available, the better known of which include quadrupoles**,** magnetic sectors**,** electric sectors, time-of-flight (TOF) analysers, and both Fourier transform and quadrupole ion traps**.**

Tandem mass spectrometers are instruments that have more than one analyser and so can be used for structural and sequencing studies. Two, three and four analysers have all been incorporated into commercially available tandem instruments, and the analysers do not necessarily have to be of the same type, in which case the instrument is a hybrid one. More popular tandem mass spectrometers include those of the quadrupole-quadrupole, magnetic sector-quadrupole**,** and more recently, the quadrupole-time-of-flight geometries.

Detection and recording of sample ions

The detector monitors the ion current, amplifies it and the signal is then transmitted to the data system where it is recorded in the form of mass spectra**.** The m/z values of the ions are plotted against their intensities to show the number of components in the sample, the molecular mass of each component, and the relative abundance of the various components in the sample.

The type of detector is supplied to suit the type of analyser; the more common ones are the electron multiplier**,** the multi-channel plate and Faraday cup detectors.

A vertical bar graph generally depicts mass spectrum, each bar represents an ion with a specific mass-to-charge ratio (m/z) and the height of the bar depicts the relative abundance of the ion. If the molecule gets devoid of an electron in the process of ionization then a molecular ion is observed that yields its molecular weight and it is indicated as M^+ in the spectrum. As z is more than often +1, m/z actually amounts upto the mass (m) of the individual ions.

For example, the mass spectrum of dopamine has a peak at m/z 153, which is equal to its mass (figure 1).

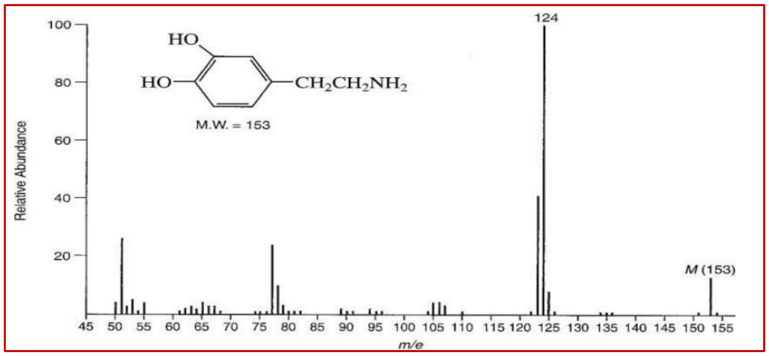


Figure: Mass spectrum of dopamine

Radical cation: In the process of ionization of a molecule, the species so formed is known as a radical cation, and is depicted as $M^{+\cdot}$.

Molecular ion or parent ion: The former species mentioned above is also called the molecular ion or the parent ion.

The mass of $M^{+\bullet}$ represents the molecular weight of the molecule. $M^{+\cdot}$should be the highest m/z inthe spectrum, apart from weak satellite peaks that result from other isotopes.

Base peak: The ion formed most abundantly during the process of ionization gives rise to the tallest peak in the mass spectrum which is also known as the base peak. All other peak intensities are obtained are appear as relative to the peak known as the base peak as a percentage.

Fragment ions: When a molecule loses a valence electron, further bonds are broken, and new species are formed called fragment ions.

Index of hydrogen deficiency or Degree of unsaturaion or Double Bond Equivalent (DBE): The Index of hydrogen deficiency is the number of pi bonds and/or rings a molecule contains. One Double Bond Equivalent (DBE) (also known as a degree of unsaturation) is one pi bond or one ring. A triple bond counts as 2 DBE. Having 4 DBE shows the possibility of a benzene ring, since benzene has three pi bonds plus one ring. The formula for DBE is the following:

Metastable ions: The ion appearing at an m/z ratio that depends on its mass as well as the mass of the original ion from which it is formed is called metastable ion. These peaks are usually broad and appear at non integral values of m/z. The presence of metastable ion peak links two

$$m_1^+ \longrightarrow m_2^+ + \text{fragment}$$

$$m^* = \frac{(m_2)^2}{m_1}$$

peaks together.

m* is the apparent mass of the metastable ionm1 is the mass of original ion

e.g. $C_5H_9^+$ (m/z = 69) → $C_3H_5^+$ (41) + C_2H_4 (28)

Calculated m* = $(41)^2/69$ = 24.36, observed m* = 24.4

m2 is the mass of new fragment ion

Metastable ion arise because of fragmentation after the ion has left the ionization chamber. The kinetic energy of such a m $^{+2}$ is smaller than when it would have been formed in the ionization chamber and accelerated there, and it will be detected at a lower m/z than expected.

The Nitrogen Rule

Most of the hydrocarbons and other compounds which contain C,H and O atoms, often contains a molecular ion with an even mass. A molecular ion which is odd indicates that that a particular compound has an odd number of nitrogen atoms.

The Nitrogen Rule

The effect of N atoms gen rule a compound that contains an odd number of N atoms has an odd molecular ion. A compon the mass of the molecular ion in a mass spectrum is called the nitrogen rule. According to nitroound that contains an even number of N atoms (including zero) has an even molecular ion.

The 4-methyl-3-pentene-2-one, has no nitrogen so the mass of the molecular ion (m/z = 98) is an even number (figure 2). Most of the fragment ions have odd-numbered masses, and therefore are even-electron cations.

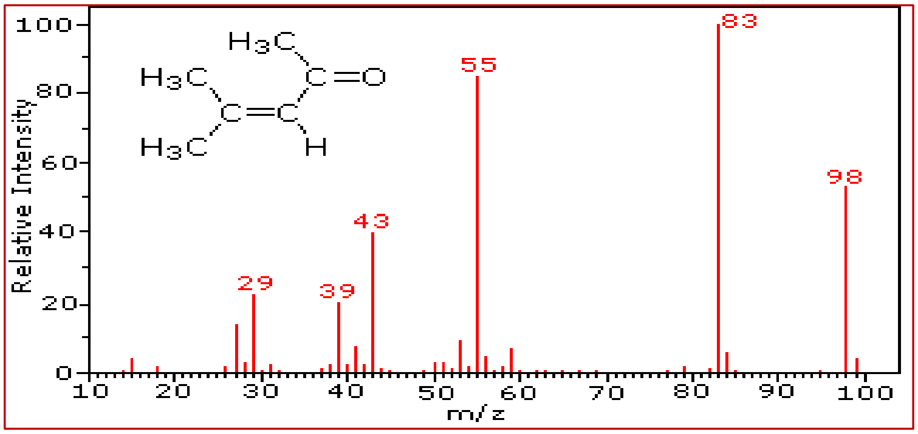


Figure: Mass spectrum of 4-methyl-3-pentene-2-one

Diethylmethylamine, has one nitrogen and its molecular mass (m/z = 87) is an odd number. A majority of the fragment ions have even-numbered masses and are even-electron nitrogen cations.

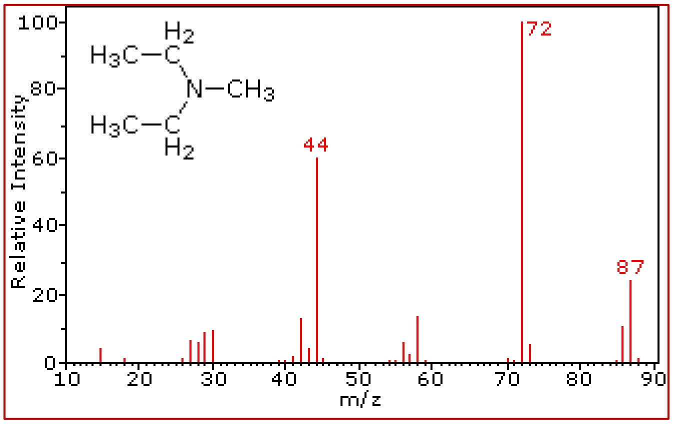


Figure: Mass spectrum of N,N-diethylmethylamine

The Rule of Thirteen

The Rule of Thirteen: Rule of thirteen is a useful method for determining the possible molecularformula of a compound from its Molecular Mass.

In the Rule of Thirteen first, a base formula is generated which consists of only hydrogen and carbon atoms. This base formula is calculated by dividing the molecular mass by 13 (C + H: 12+1 =13).

When a molecular mass, M^+, is known, a base formula can be generated from the following equation:

$$\frac{M}{13} = n + \frac{r}{13}$$

The base formula will be: C_nH_{n+r}

The index of hydrogen deficiency (IHD) or ***Double Bond Equivalent*** DBE will be:DBE = (n – r + 2)/2

Isotopes in Mass Spectrometry

A mass spectrometer separates and detects ions of slightly different masses and it easily distinguishes different isotopes of a given element.

The molecular ions of 2-methylbutane has *m/ z* values of 72, the spectrum shows a very small peak at m/z = 73 (figure 4). This peak is called M +1 peak because the ion responsible for it is one unit heavier than the molecular ion. The M+1 peak owes its presence to the fact that there are two naturally occurring isotopes of carbon: 98.89% of natural carbon is ^{12}C and 1.11% is ^{13}C. So 1.11% of the molecular ions contain a ^{13}C instead of a ^{12}C and therefore appear at M+1.

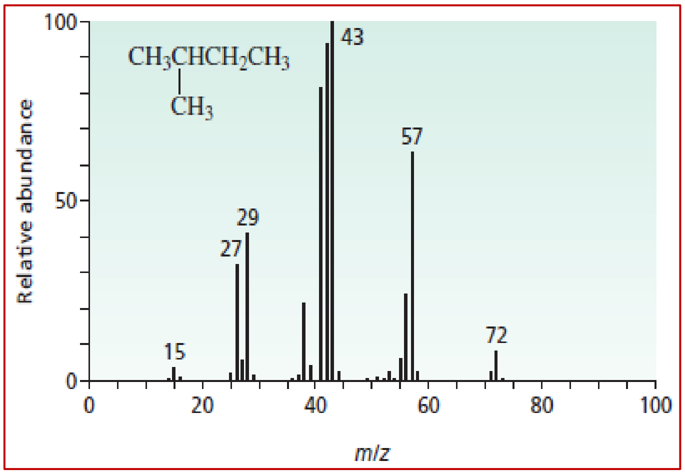
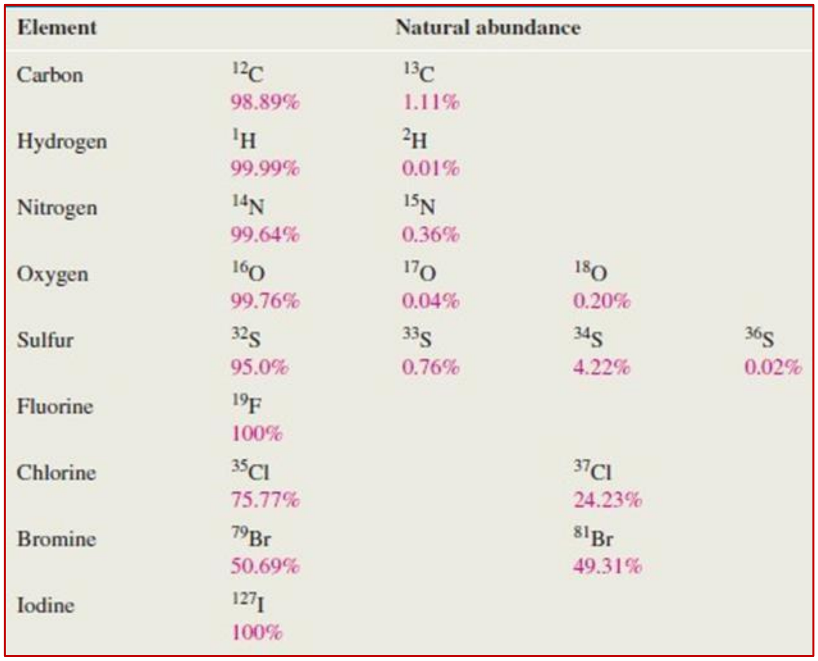


Figure: Mass spectrum of 2-methylbutane

$$\text{number of carbon atoms} = \frac{\text{relative intensity of M} + 1 \text{ peak}}{.011 \times (\text{relative intensity of M peak})}$$



Element	Natural abundance			
Carbon	^{12}C 98.89%	^{13}C 1.11%		
Hydrogen	^{1}H 99.99%	^{2}H 0.01%		
Nitrogen	^{14}N 99.64%	^{15}N 0.36%		
Oxygen	^{16}O 99.76%	^{17}O 0.04%	^{18}O 0.20%	
Sulfur	^{32}S 95.0%	^{33}S 0.76%	^{34}S 4.22%	^{36}S 0.02%
Fluorine	^{19}F 100%			
Chlorine	^{35}Cl 75.77%		^{37}Cl 24.23%	
Bromine	^{79}Br 50.69%		^{81}Br 49.31%	
Iodine	^{127}I 100%			

Table: The isotopic distributions of several elements

Peaks that are attributable to isotopes can help identify the compound responsible for a mass spectrum. For example, if a compound contains five carbon atoms, the relative intensity of the M+1 ion should be 5(1.1%) = 5 (.011), multiplied by the relative intensity of the molecular ion. This means that the number of carbon atoms in a compound can be calculated if the relative intensities of both the M and M+1 peaks are known.

From the isotopic distributions, we see why the M+ 1 peak can be used to determine the number of carbon atoms in a compound: It is because the contributions to the M +1 peak by isotopes of H, O, and the halogens are very small or nonexistent. This formula does not work as well in predicting the number of carbon atoms in a nitrogen-containing compound because the natural abundance of ^{15}N is relatively high. Mass spectra can show M +2 peaks as a result of a contribution from ^{18}O, or from having two heavy isotopes in the same molecule (say,^{13}C and ^{2}H or two ^{13}Cs). Most of the time, the M+2 peak is very small. The presence of a large M+2 peak is evidence of a compound containing either chlorine or bromine, because each of these elements has a high percentage of a naturally occurring isotope that is two units heavier than the most abundant isotope. This is majorly observed in compounds containing bromine and chlorine.

Mass spectrum of Br_2

It contains five peaks and has a nearly 50:50 mixture of isotopes having atomic masses of 79 and 81 amu respectively. Thus, the bromine molecule may be composed of two ^{79}Br atoms (mass 2 x 79 = 158 amu), two ^{81}Br atoms (mass 2 x 81 = 162 amu) or the more probable combination of ^{79}Br-^{81}Br (mass 79 + 81 =160 amu). Fragmentation of Br, to a bromine cation then gives rise to equal sized ion peaks at 79 and 81 amu.

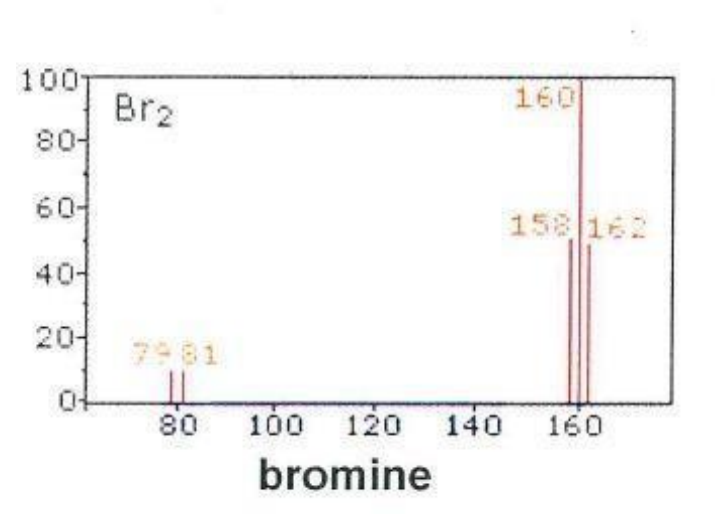


Figure: Mass spectrum of Bromine (Br_2)

Long answer type Questions (10 Marks)

1. Define chemical shift. Discuss the various factors affecting Chemical shift .
2. Give a detailed account of a Mass spectrometer.
3. What are the applications of Mass Spectroscopy?
4. Explain the various types of mass analyzers.

Short answer type questions (5Marks)

1. Write briefly about various types of ionization sources used in mass spectroscopy.
2. Draw a well labeled diagram of NMR spectrometer.
3. What is spin-spin splitting? Give its significance in NMR.
4. Give Pharmaceutical applications of NMR.
5. Write a short note on Nuclei relaxation.

Very Short answer type questions (2 Marks)

1. Name the standard used in NMR.
2. Give examples of Solvents used in NMR.
3. Define Processional Frequency.
4. What is Maclafferty rearrangement?
5. Define Molecular ion Peak and Base peak in MS.
6. Define Metastable ions.
7. Define coupling constant.
8. What is Pascal Traingle?
9. What is Nitrogen rule?
10. Draw the basic component of mass spectrometer.