Module 9 Methods for Structure Determination

Lecture 24 UV-vis and Mass Spectrocopies

9.1 Introduction

The determination of structure of an organic compound requires specialized techniques. In the past, such techniques were laboriously time consuming and sometimes unreliable too. Modern technology has sorted out this difficulty with the advent of spectroscopic methods for determination of structure.

Spectroscopy is the study of interaction of electromagnetic radiation with matter. As the electromagnetic spectrum consists of radiations of different wavelengths, the responses of the molecules to these wavelengths differ. Thus, X-rays are scattered by the molecules, infrared radiation cause bonds in molecules to vibrate and radio waves make nuclei of atoms to resonate. Spectroscopy measures these interactions and a correlation is obtained between these interactions and molecular structure.

In this section, four major spectroscopic techniques are discussed.

- ***UV-visible spectroscopy***: It can provide information about the presence of unsaturation in the molecule as well as the presence of certain functional groups.
- ***Mass spectroscopy***: This can give information about the molecular weight of the molecule and its composition in terms of elements.
- ***NMR spectroscopy***: It can reveal a lot of information about a molecule and is by far the most widely used technique in organic chemistry. It can reveal information about the carbon skeleton, the number of protons attached to each carbon as well the number and position of certain other elements.
- ***Infrared spectroscopy***: It is used to determine the presence of certain functional groups in the molecule.

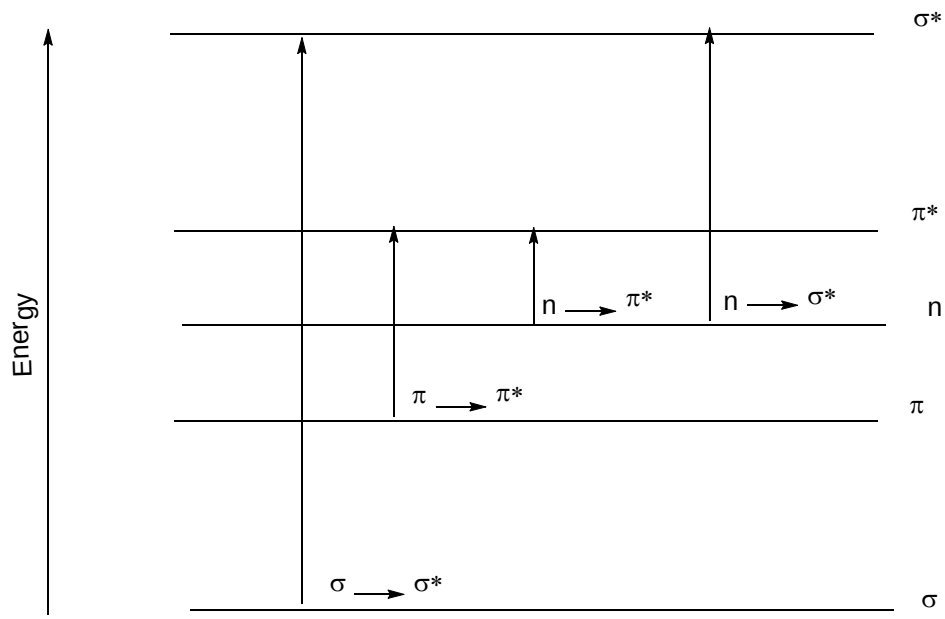
9.2 UV-Visible Spectroscopy

UV-Visible spectroscopy employs the UV (400-200 nm) and visible (800-400 nm) region of the electromagnetic spectrum. This energy associated with this region is quite high and thus irradiation of a molecule with such energy causes excitation of electrons from their Highest Occupied Molecular Orbital (HOMO) to Lowest Unoccupied Molecular Orbital (LUMO). For this reason it is sometimes referred as electronic spectroscopy. The UV spectrometer measures the wavelength (m_{ix}) at which the absorbance is maximum. The absorbance is defined the Beer-Lambert's law:

$$A = plc.$$

Where A is absorbance, l is the length of sample through which light traverses and c is the concentration of the sample. The ε is the constant of proportionality and has a particular value for a compound. The value of ε is the measure of transition probability of a transition from HOMO to LUMO. Transitions having large values ($<10^4$) are allowed absorptions while those having ($>10^3$) are forbidden transitions. The forbidden transitions are usually those transitions which are forbidden due to geometry of the participating orbitals.

- There are five kinds of orbitals that need to be considered: the bonding σ and π orbitals and their corresponding antibonding σ* and π* orbitals and the non-bonding n orbitals. Normally the bonding orbitals and the non-bonding orbitals are filled orbitals and function as HOMO, and the antibonding orbitals are vacant orbitals and function as LUMO. Of the various transitions possible among these orbitals, the σ to σ* orbital and π to π* orbitals are allowed while the transition from n to π* orbital is disallowed. The energy levels of these levels are given below.



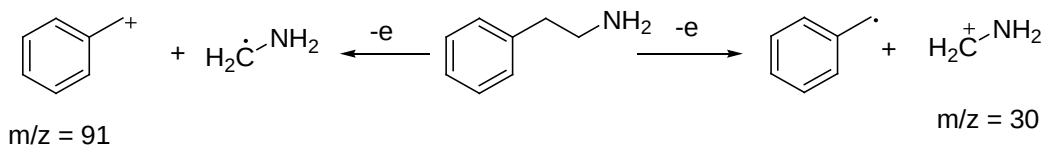
- It could be seen from this diagram that though disallowed by the Laporte selection rules, the n to π* is the one with the lowest energy and it is indeed observed in conjugated carbonyl compounds.
- The usefulness of UV-Visible spectrometry is in its simplicity. Some functional groups show a characteristic λ_{max} peak even when the rest of the structure differs.
- Simple conjugated chromaphores, i.e., dienes and α,β-unsaturated ketones have molar absorption coefficient values in the order of 10^4. The longer simple conjugated systems have principle maxima with correspondingly higher values. Low intensity absorption bands around 270-350 nm with ε values of 10-100, are the result of 10^3-10^4 always indicate the presence of an aromatic system.

9.3 Mass Spectroscopy

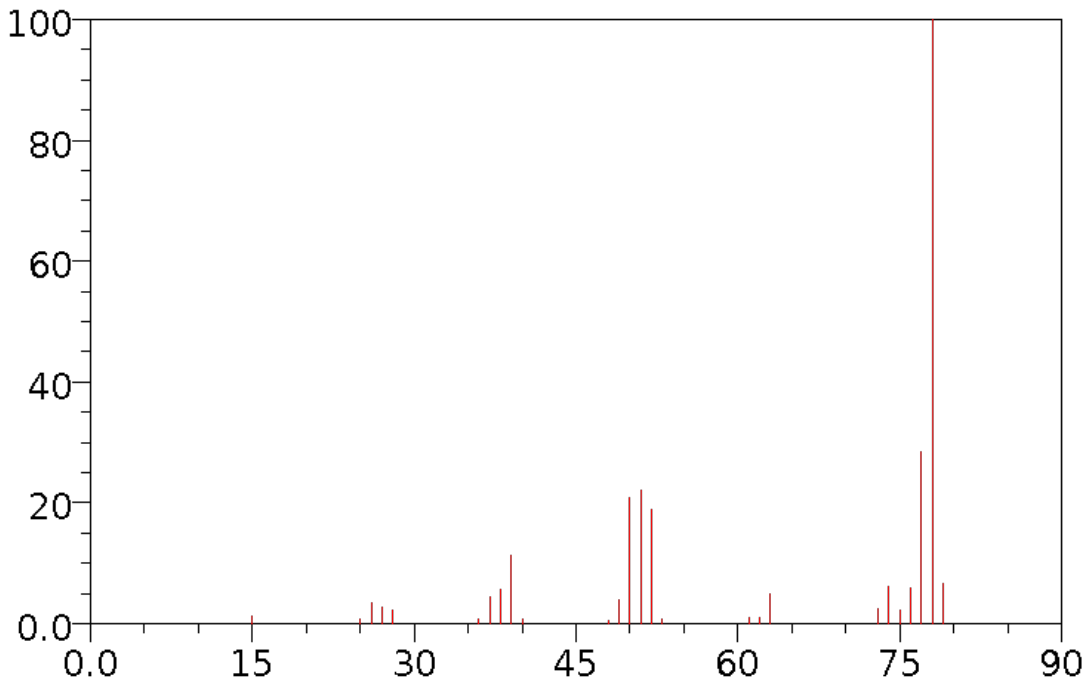
A mass spectrometer determines the molecular weight of a compound. It has three basic components: something to volatilize and ionize the molecule into a beam of charged particles; something to focus the beam so that particles of the same mass: charge ratio is separated from all others; and something to detect the particles.

- Two methods are usually used to ionize molecule: electron impact and chemical ionization. The former method called electron impact ionization (E.I.) mass spectrometry the molecule is bombarded with highly energetic electrons that knock a weakly bound electron out of the molecule. Losing a single electron leaves behind a radical cation: an unpaired electron and a positive charge. The electron that is lost will be one of relatively high energy, and this will typically be one not involved in bonding, for example, an electron from a lone pair. Thus ammonia gives $NH_3^{+\bullet}$and a ketone gives $R_2C{=}O^{+\bullet}$. The problem with this technique is that it is too "hard" i.e.; there is a lot of fragmentation which at times makes it difficult to assign the molecular ion peak. In some cases it may be totally absent. Also since the radical cations are extremely unstable they may decompose before reaching the detector. In the later method, chemical ionization, (C.I.) mass spectrometry the electron beam is used to ionize a simple molecule such as methane which in turn ionizes our molecule by collision and transfer of a proton. Under electron bombardment, methane loses a bonding electron to give $CH_4^{+\bullet}$ which reacts with an unionized methane molecule to give $CH_3^{\bullet}$ and CH_5^{+}. This unstable compound (CH_5^{+}) is a powerful acid, and can protonate just about any other molecule. When it protonates our sample, a proton has been added rather than an electron removed, so the resulting particles are simple cations, not radical cations, and are generally more stable than the radical cations produced by direct electron impact. Having more functional groups helps molecular ions to decompose. The aromatic amine 2-phenylethylamine is a brain active amine found in some foods such as chocolate, red wine, and cheese and possibly implicated in migraine. It gives a poor molecular ion by E.I., a base peak with a mass as low as 30 and the only peak at higher mass is a 15% peak at 91. The C.I. mass spectrum on the other hand has a good molecular ion: it is $[M + H]^+$.

Normally a fragmentation gives one cation and another radical, only the cation being detected. It is relatively unusual for one bond to be able to fragment in either direction, but here it does, which means that both fragments are seen in the spectrum.

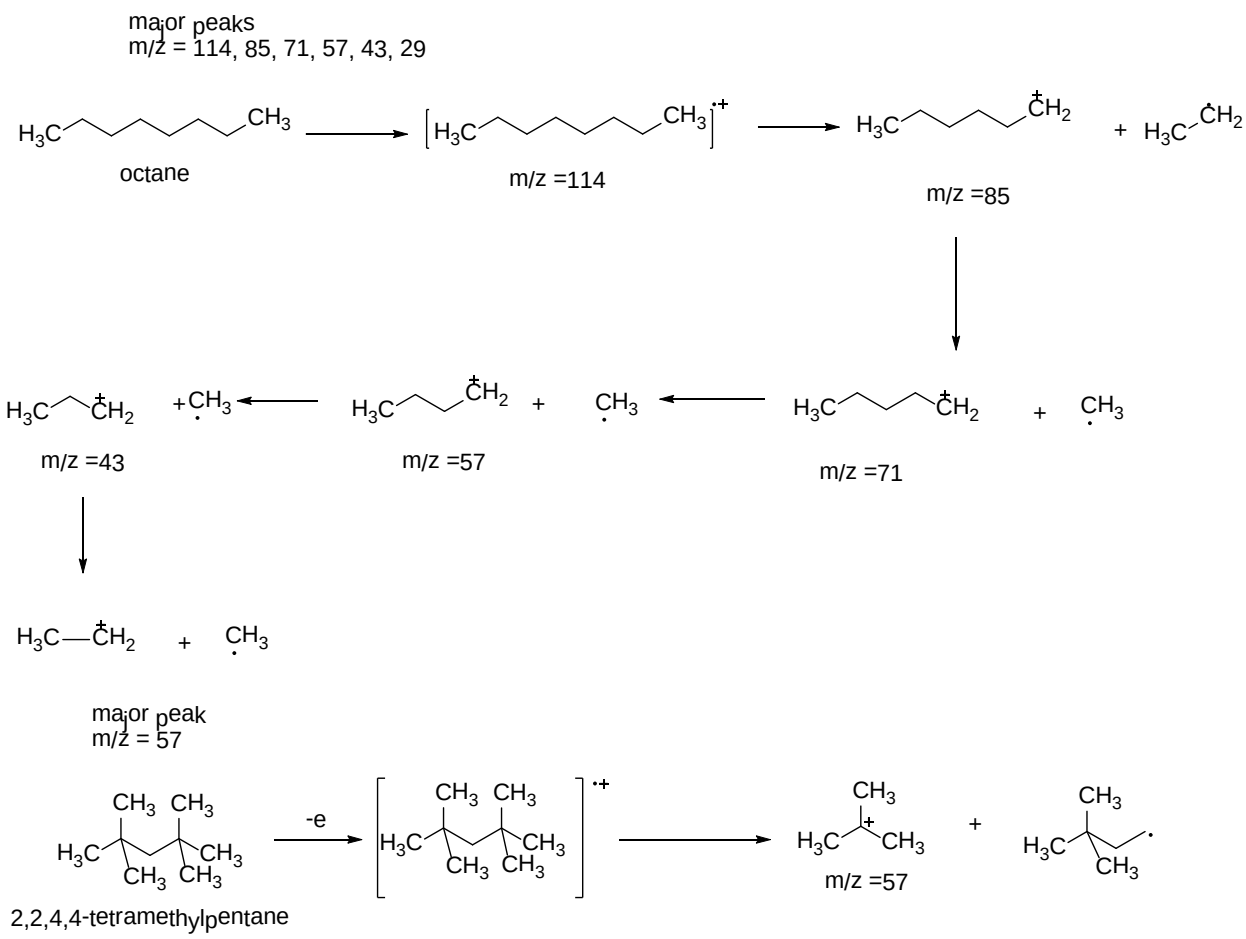


- The strongest peak is assigned an 'abundance' of 100% and called the ***base peak***. In the mass spectrum given below 78 is the base peak. The abundance of the other peaks is shown relative to the base peak. This spectrum is a plot of the abundance of the ions versus mass/ charge (m/z).



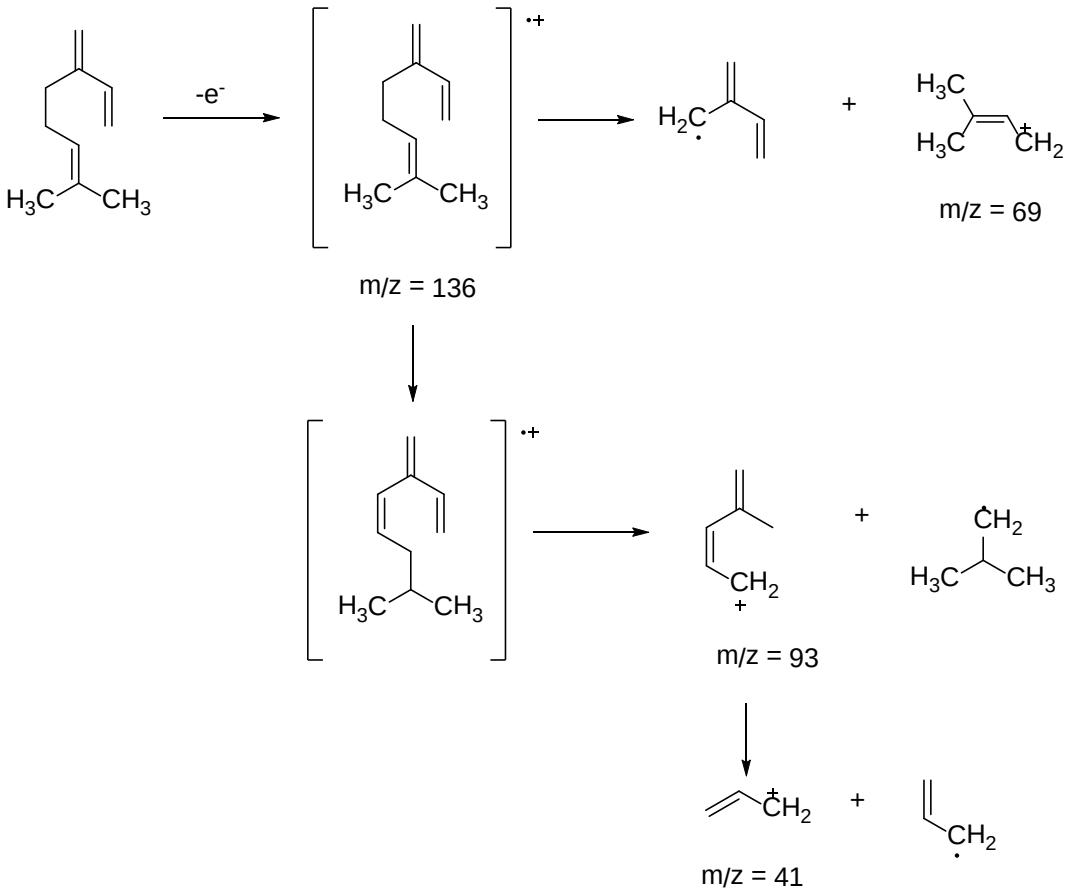
- Now it may argued that in the absence of a molecular ion peak it will be pain stakingly difficult to determine a particular mass is generated from the original molecule. Fortunately, however, molecules do show pattern in fragmentation depending on the stability of the fragments generated. Some common fragmentation patterns are given below.

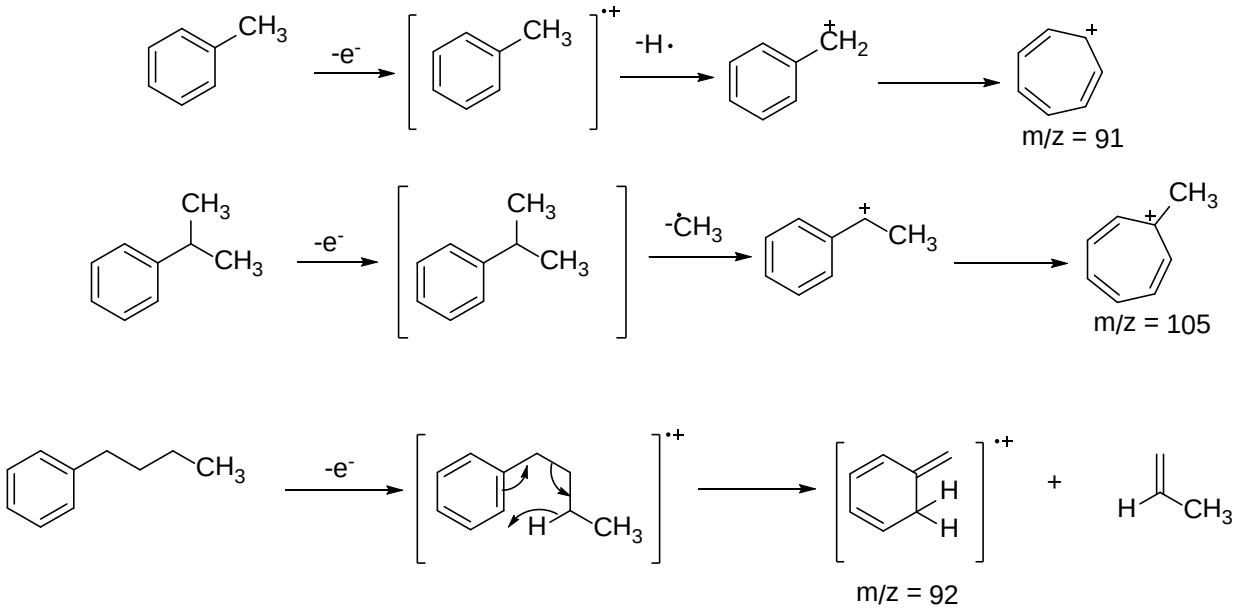
- ***Analysis of Hydrocarbons***: In molecules containing long saturated hydrocarbon chains, the corresponding molecular ion peak is observed along with peaks generated by loss of CH_2 groups i.e., M-14, M-28 and so on. With the increase in branching, however, the intensity of the molecular ion peak (M) decreases and instead that of a stabilized fragment becomes more visible. This can be understood from the comparison of mass spectrum of n-octane and 2,2,4-trimethylpentane (isooctane). In the former compound a peak corresponding to m/z = 43 is the base peak with m/z = 114 the molecular ion peak also visible whereas, in the later a peak corresponding to m/z = 57 is the base peak with no peak at m/z = 114.



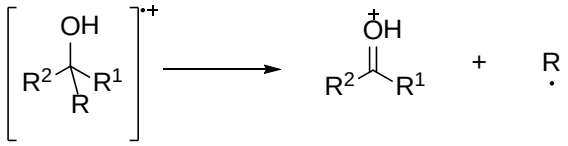
- ***Analysis of Alkenes***: In alkenes the molecular ion peak is usually distinct. Location of the double bond is however difficult in acyclic alkenes due to migration of the double bond. In cyclic alkenes, it may be possible to locate the double bond due to its strong tendency for allylic cleavage without much migration. Thus in the mass spectrum of β-myrcene, a molecular peak appears at 136 along with major peaks at 93 (base peak), 69, 55 and 41 (base peak) are observed.

- ***Aromatic Hydrocarbons***: These compounds show a strong molecular ion peak. When an alkyl chain is attached to benzene ring preferential fragmentation occurs at the benzylic position to form a fragment of the formula $C_7H_7^+$ (m/z =91). Thus, toluene exhibits a peak at 91. The peak at m/z =105 in isopropyl benzenes is also due to a similar fragmentation where one of the benzylic C-H is replaced by methyl group. Similarly, when an alkyl group attached to the benzene ring is a propyl group or larger, *Mclafferty rearrangement* occurs to give fragments. Using butyl benzene the effect of *McLafferty rearrangement* can be shown. The peak at m/z = 92 is obtained as a result of *McLafferty rearrangement*.

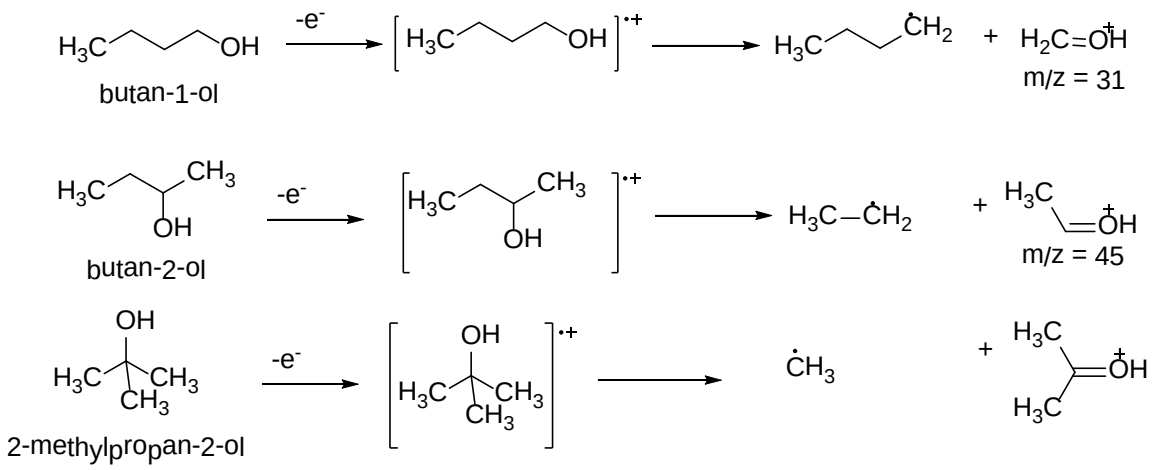




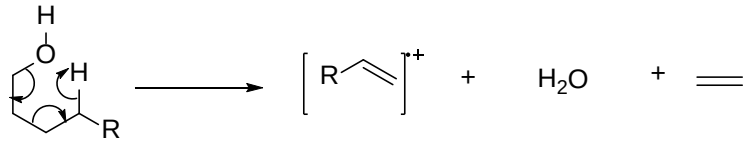
- ***Analysis of Alcohols***: In alcohols the molecular ion may be altogether absent. The predominant fragmentation involves loss of an alkyl group. In case of branched alcohols, the largest alkyl group is usually lost.



- In the spectrum of 1-butanol, the base peak is obtained at m/z =31 due to loss of a propyl group. Butan-2-ol loses an ethyl group to have base peak at m/z = 45. Similarly, 2-methylpropan-2-ol loses a methyl group to give a peak corresponding to m/z = 59.



- A second common mode of fragmentation is dehydration. Alcohols having four or more carbons may undergo simultaneous loss of ethylene and water.

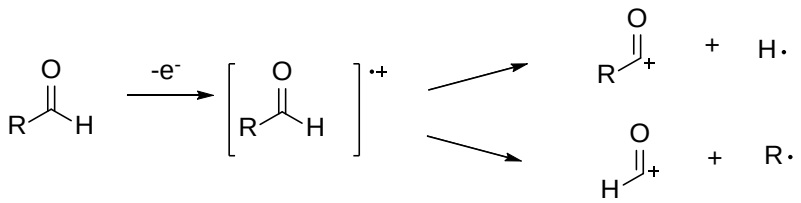


Module 9 Methods for Structure Determination

Lecture 25 Mass and Infrared Spectrocopies

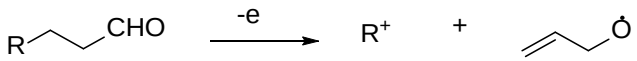
9.3 Mass Spectroscopy

- Analysis of Aldehydes: Aldehydes usually show weak molecular ion peak for aliphatic aldehydes but the peak is quite strong for aromatic aldehydes. Cleavage of one of the bonds to the carbonyl group, sometimes called α-cleavage occurs very often.

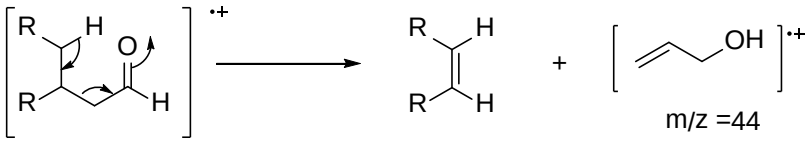


Thus, the peak corresponding to m/z =71 in butyraldehyde is due to loss of $H^{\cdot}$.

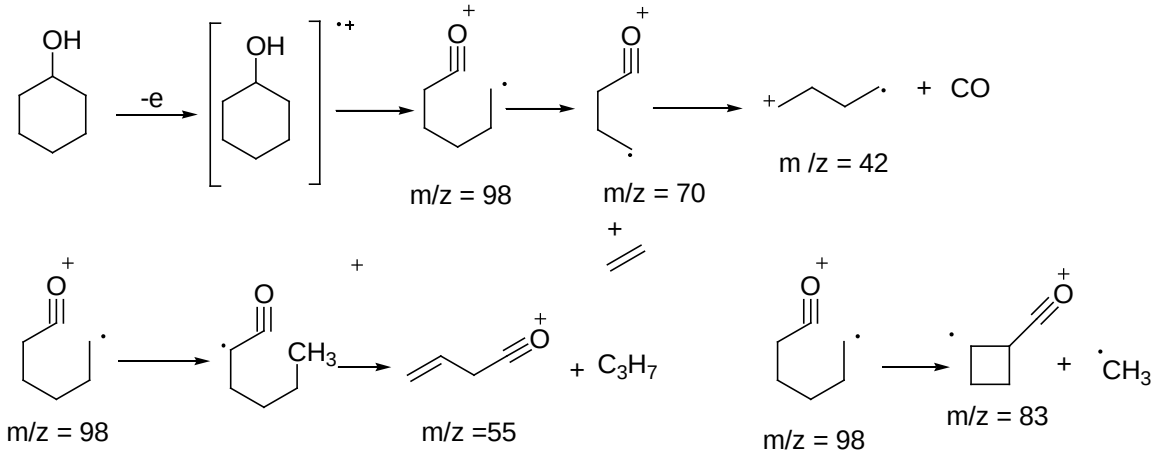
Similarly, they may undergo fragmentation of R group in what is called β-cleavage.This gives the peak at m/z = 29 for butyraldedyde.



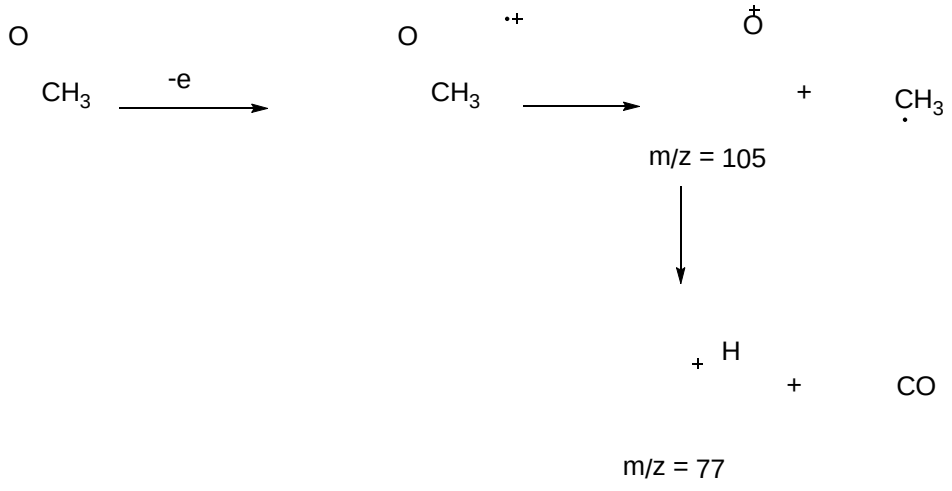
The third major fragmentation pathway for aldehydes is McLafferty rearrangement. The fragment has m/z =44.

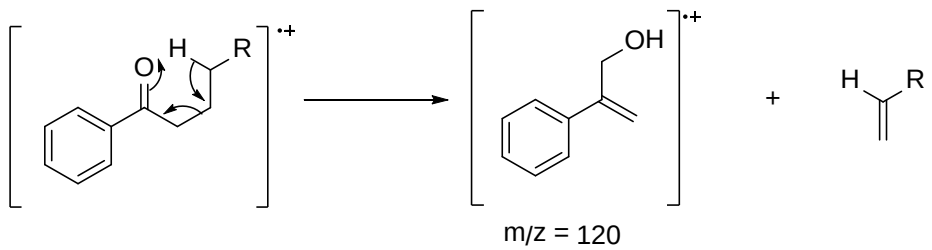


- Analysis of Ketones: In case of ketones, there is a strong molecular ion peak. For aliphatic ketones, fragment resulting from loss of a methyl, ethyl and so on groups occur in the mass spectrum. The *McLafferty rearrangement* also occurs to give its corresponding peak. Cyclic ketones may undergo loss of carbonyl group. Thus in cyclohexanone the following fragmentation occurs.



- Aromatic ketones undergo α-cleavage to lose the alkyl group and form $ArCO^+$. This ion loses CO to form Ar^+ ion. They also undergo McLafferty rearrangement. The fragmentation pattern of acetophenone is shown below.





- Several elements exist as isotopes in nature with their own relative natural abundance. Chlorine is normally a 3:1 mixture of ^{35}Cl and ^{37}Cl while bromine is an almost 1:1 mixture of ^{79}Br and ^{81}Br. Mass spectrometry separates these isotopes so that one gets true not average molecular weights. The molecular ion in the E.I. mass spectrum of the N-(4-bromophenyl)acetamide has two peaks at 213 and 215 of roughly equal intensity.

9.4 Infrared Spectroscopy

Some functional groups, for example, C=O or C=C, can be seen in the NMR spectrum because they contain carbon atoms, while the presence of others like OH can be inferred from the chemical shifts of the carbon atoms they are joined to. Others cannot be seen at all. These might include NH_2 and NO_2, as well as variations around a carbonyl group such as COCl, CO_2H, and $CONH_2$. Infrared (IR) spectroscopy provides a way of finding these functional groups because it detects the stretching and bending of bonds rather than any property of the atoms themselves. It is particularly good at detecting the stretching of unsymmetrical bonds of the kind found in functional groups such as OH, C=O, NH_2 and NO_2.

- ➢ The amount of energy needed for stretching and bending individual bonds, while still very small, corresponds to rather shorter wavelengths. These wavelengths lie in the infrared, that is, heat radiation just to the long wavelength side of visible light. When the carbon skeleton of a molecule vibrates, all the bonds stretch and relax in combination and these absorptions are unhelpful. However some bonds stretch essentially independently of the rest of the molecule.

  This occurs if the bond is either:

- much stronger or weaker than others nearby, or
- between atoms that are much heavier or lighter than their neighbours.

- The relationship between the frequency of the bond vibration, the mass of the atoms, and the strength of the bond is essentially the same as Hooke's law for a simple harmonic oscillator. The equation shows that the frequency of the vibration n is proportional to the (root of) a **force constant** *f*—more or less the bond strength—and inversely proportional to the (root of) a **reduced mass** m, that is, the product of the masses of the two atoms forming the bond divided by their sum.

$$\nu = \frac{1}{2\pi c}\left(\frac{f}{\mu}\right)^{1/2}$$

where

$$\mu = \frac{m_1 m_2}{m_1 + m_2}$$

- Infrared spectra are simple absorption spectra. The sample is exposed to infrared radiation and the wavelength scanned across the spectrum. Whenever energy corresponding to a specific wavelength is absorbed, the intensity of the radiation reaching a detector momentarily decreases, and this is recorded in the spectrum. Infrared spectra are usually recorded using a frequency measurement called **wavenumber** (cm^{-1}) which is the inverse of the true wavelength l in centimetres to give convenient numbers (500–4000 cm^{-1}). Higher numbers are to the left of the spectrum because it is really wavelength that is being scanned.

$$E = h\nu = hc/\lambda$$

The energy, *E*, required to excite a bond vibration can be expressed as the inverse of a wavelength *l* or as a frequency *v*. More energy is needed to stretch a strong bond. Larger *E* means higher wavenumbers (cm^{-1}) or smaller wavelength (cm).

- To run an IR spectrum, the sample is either dissolved in a solvent such as $CHCl_3$ (chloroform) that has few IR absorptions, pressed into a transparent disc with powdered solid KBr, or ground into an oily slurry called a mull with a hydrocarbon oil called 'Nujol'. Solutions in $CHCl_3$ cannot be used for looking at the regions of C–Cl bond stretching nor can Nujol mulls be used for the region of C–H stretching. KBr is however transparent to infrared radiation.
- An IR spectrum is usually recorded between 1000-4000 cm^{-1} and it may classify into broad regions- the functional group region and the fingerprint region. Typically, an IR spectrum is a plot of transmittance (%T) vs. wavenumber. Transmittance is related to absorbance (A) as follows:

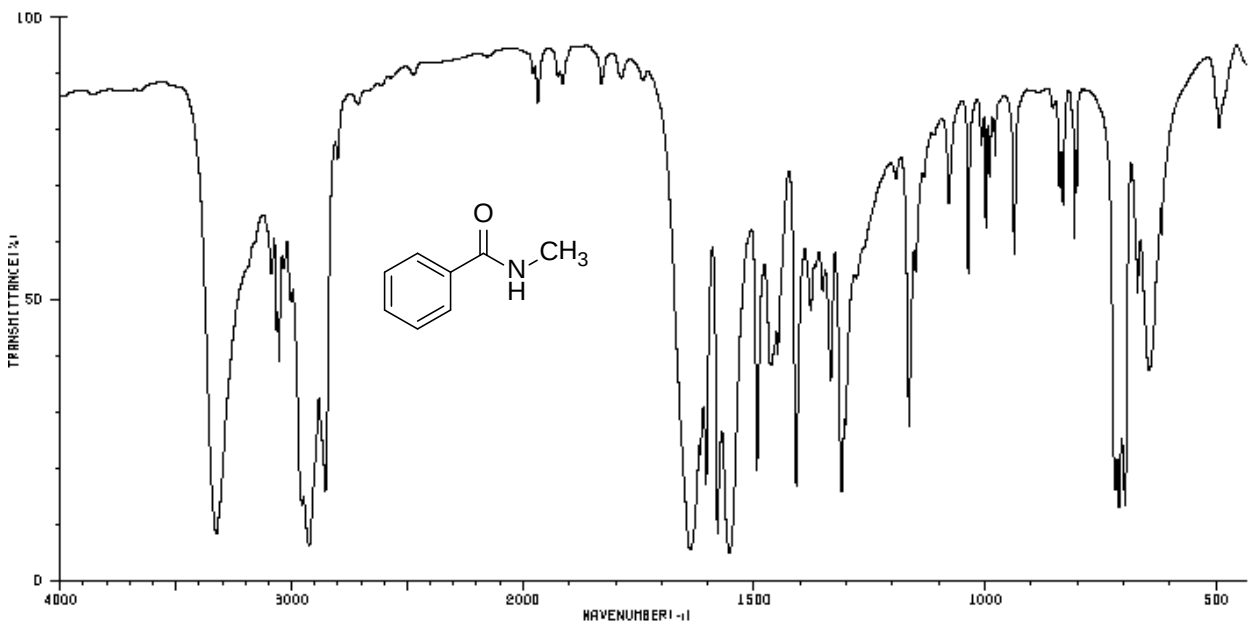
$$A = \log_{10} 1/T$$

- The functional group region (4000-1500 cm^{-1}) contains information about the different functional groups. The various functional groups and their regions are given the following diagram.

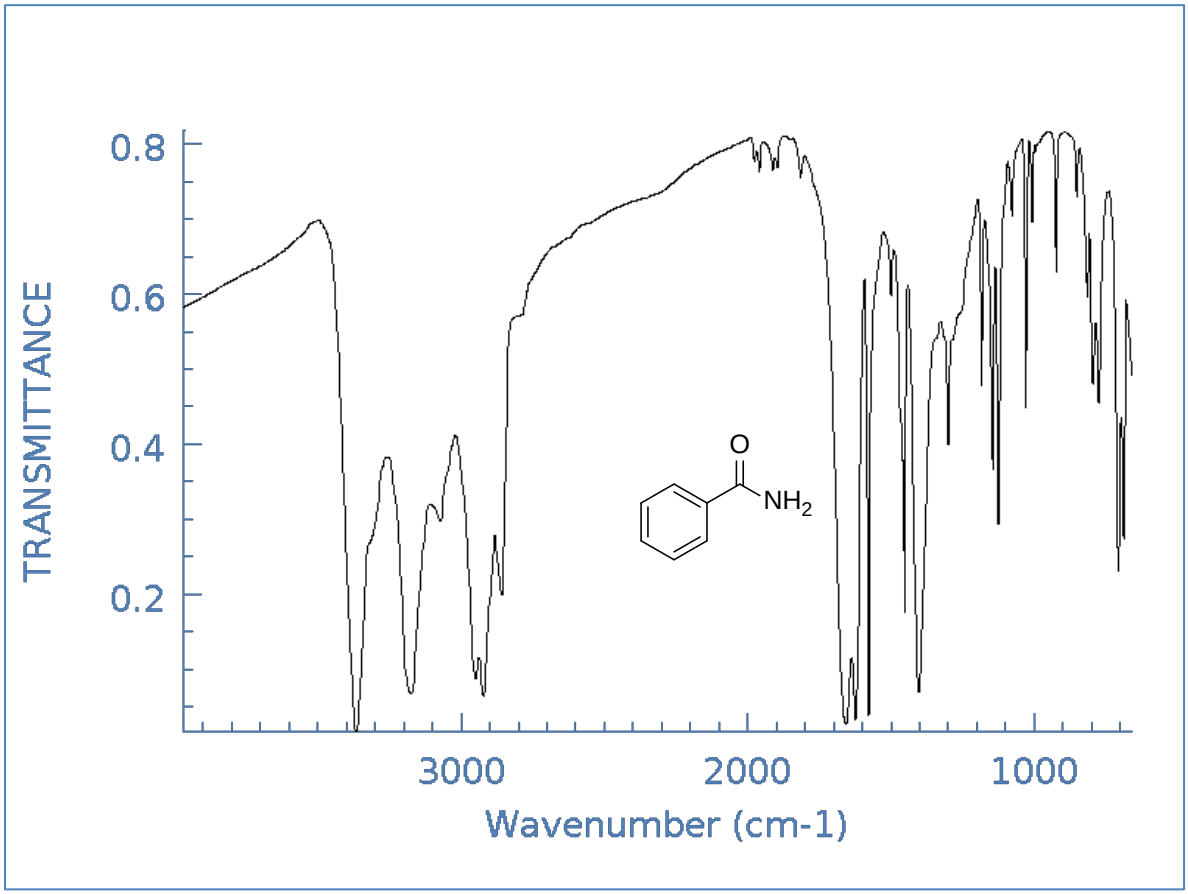
4000 3000 2000 1500

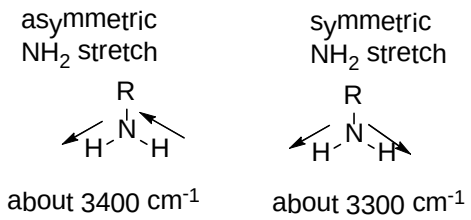
Bonds to hydrogen (X-H) O-H, N-H, C-H	Triple bonds C≡C, C≡N	Double bonds C=O, C=C

- In the X-H bond region, normally the signals that are looked for are those of $C_{(Ar)}$-H, $C_{(sp)}$-H, N-H, O-H. The $C_{(sp3)}$-H usually occurs at the borderline (2900-3000 cm^{-1}).
- A group gives an independent vibration only if both bond strength and reduced mass are different from those of neighbouring bonds. The IR peak of an NH group is fundamentally different from that of an NH_2 group. In the case of N–H, this is likely to be true and a sharp peak appears at about 3300 cm^{-1}, whether the NH group is part of a simple amine (R_2NH) or an amide (RCONHR). The NH_2 group is also independent of the rest of the molecule, but the two NH bonds inside the NH_2 group have identical force constants and reduced masses and so vibrate as a single unit. Two equally strong bands appear one for the two N–H bonds are vibrating in phase (symmetric) and one for the two N–H bonds vibrating in



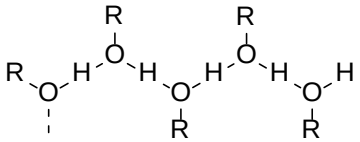
opposition (antisymmetric). The antisymmetric vibration requires more energy and is at slightly higher frequency.



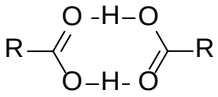


- The O–H bands occur at higher frequency, sometimes as a sharp absorption at about 3600 cm^{-1}. More often, you will see a broad absorption at anywhere from 3500 to 2900 cm^{-1}. This is because OH groups form strong hydrogen bonds that vary in length and strength. The sharp absorption at 3600 cm^{-1} is the non-hydrogen-bonded OH and the lower the absorption the stronger the H bond.
- Alcohols form hydrogen bonds between the hydroxyl oxygen of one molecule and the hydroxyl hydrogen of another. These bonds are variable in length (though they are usually rather longer than normal covalent O–H bonds) and they slightly weaken the true covalent O–H bonds by varying amounts. Alcohols thus give a

rounded absorption at about 3300 cm^{-1}. Carboxylic acids (RCO_2H) form hydrogen-bonded dimers with two strong H bonds between the carbonyl oxygen atom of one molecule and the acidic hydrogen of the other. These also vary considerably in length and strength and usually give very broad absorbances.



hydrogen bonding in alcohol



intramolecular hydrogen bonding in alcohol

- In the double bond region, there are three important absorptions, those of the carbonyl (C=O), alkene (C=C), and nitro (NO_2) groups. All give rise to sharp bands: C=O to one strong (i.e. intense) band anywhere between 1900 and 1500 cm^{-1}; C=C to one weak band at about 1640 cm–1; and NO_2 to two strong (intense) bands in the mid-1500s and mid-1300s cm^{-1}. Carbonyl and alkene clearly have one double bond each and gives rise to only one band each. In the nitro group absorptions for symmetrical and antisymmetrical stretching vibrations can be seen. As with NH_2, antisymmetrical vibration occurs at higher frequency (>1500 plus cm^{-1}).
- The region below 1500 cm^{-1} is where the single bond vibrations occur. The atoms C, N, and O all have about the same atomic weight and C–C, C–N, and C–O single bonds all have about the same strength. Hence here the characteristic vibrations for these bonds are difficult to detect. However, this region below 1500 cm^{-1} is called fingerprint region because it is the characteristic of a particular compound.

Module 9 Methods for Structure Determination
Lecture 26 NMR Spectroscopy

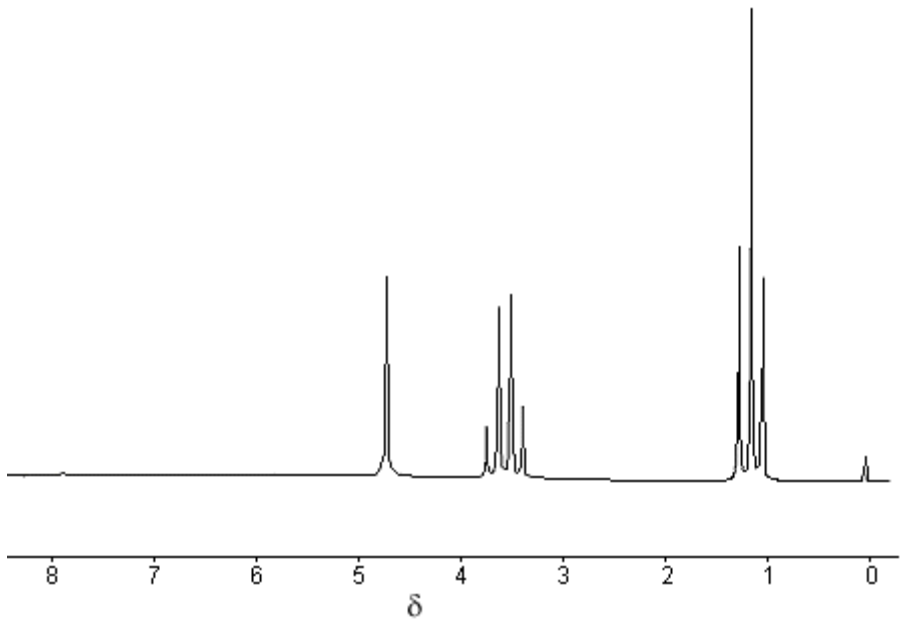
9.5 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear magnetic resonance (NMR) allows us to detect atomic nuclei and say what sort of environment they are in, within their molecule. Thus, the hydrogen of, say, propanol's hydroxyl group is different from the hydrogens of its carbon skeleton. NMR (actually ^{1}H, or proton, NMR) can easily distinguish between these two sorts of hydrogens. Moreover, it can also distinguish between all the other different sorts of hydrogen atoms present. Likewise, carbon (or rather ^{13}C) NMR can easily distinguish between the three different carbon atoms.

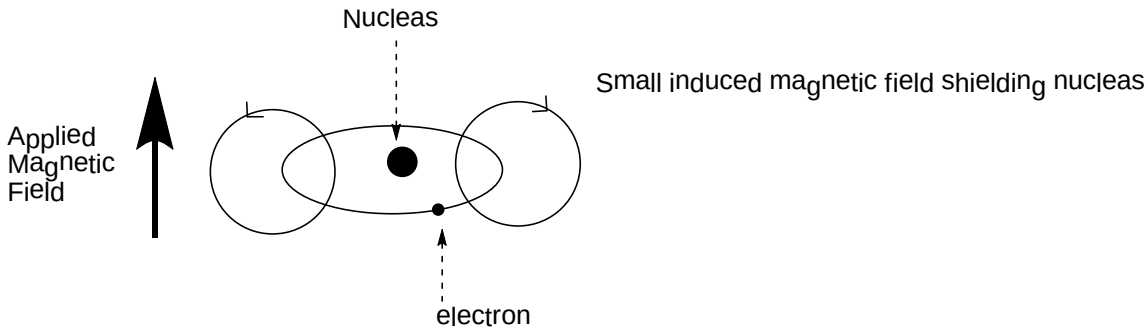
- Some atomic nuclei act like tiny compass needles and have different energy levels when placed in a magnetic field. Nuclear energy levels are quantized, just like the energy levels of an electron, and there are only certain specific energy levels it can adopt. Some nuclei (including 'normal' carbon-12) do not interact with a magnetic field at all and cannot be observed in an NMR machine. ^{1}H and ^{13}C do interact and have just two different energy levels. When a magnetic field is applied to these nuclei, they can either align themselves with it, which would be the lowest energy state, or they can align themselves against the field, which is higher in energy. nucleus in a magnetic field, the difference in energy between the nuclear spin aligned with and against the applied field depends on how strong the magnetic field is, and also on the properties of the nucleus itself. The stronger the magnetic field we put our nucleus in, the greater the energy difference between the two alignments. However, the energy difference between the nuclear spin being aligned with the magnetic field and against it is really very small—so small that a very, very strong magnetic field is required to see any difference at all.
- Nuclei that interact with magnetic fields are said to possess nuclear spin. The exact number of different energy levels a nucleus can adopt is determined by this nuclear spin, I, of the particular isotope. The nuclear spin I can have various values such as 0, 1/2, 1, 3/2 and the number of energy levels is given by 2I+ 1. Some examples are: ^{1}H, I= 1/2; ^{2}H (= D), I= 1; ^{11}B, I= 5/2; ^{12}C, I= 0.

- The amount of energy needed to flip the nucleus can be provided by electromagnetic radiation of radiowave frequency. Radio waves flip the nucleus from the lower energy state to the higher state. The nucleus now wants to return to the lower energy state and, when it does so, the energy comes out again and this (a tiny pulse of radiofrequency electromagnetic radiation) is what is detected. The operation of a NMR machine is summarized below.
- The sample of the unknown compound is dissolved in a suitable solvent and put in a very strong magnetic field. Any atomic nuclei with a nuclear spin now have different energy levels, the exact number of different energy levels depending on the value of the nuclear spin.
- The sample is irradiated with a short pulse of radiofrequency energy. This disturbs the equilibrium balance between the two energy levels: some nuclei absorb the energy and are promoted to a higher energy level
- The energy given out when the nuclei fall back down to the lower energy level is detected using a sophisticated radio receiver.
- After lots of computational analysis involving complex mathematical operations, the results are displayed in the form of intensity (i.e. number of absorptions) against frequency. This is known as one dimensional NMR spectrum.

The proton NMR spectrum of ethanol is given below.

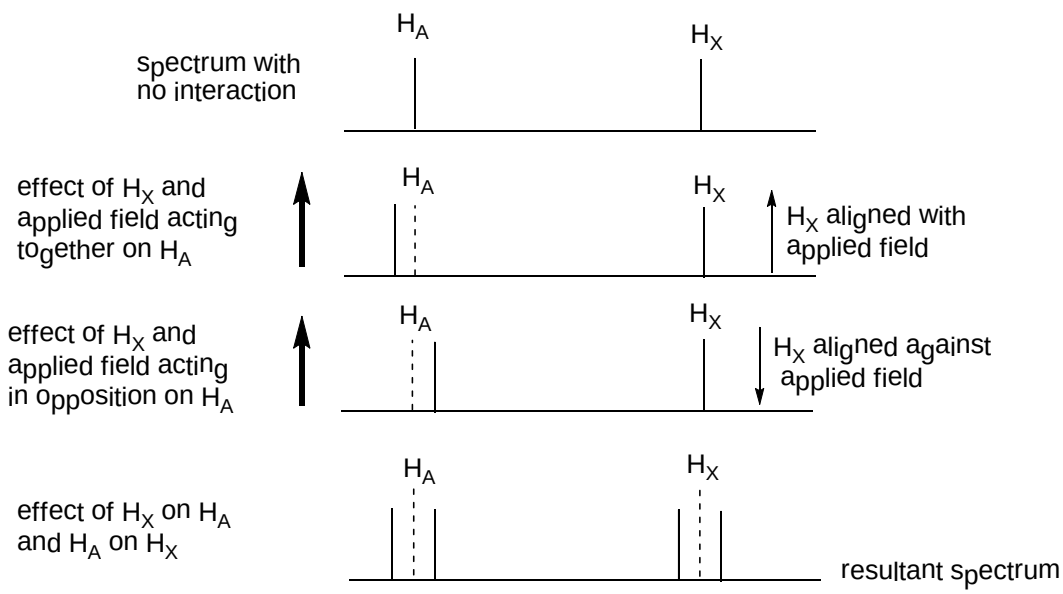


- In the spectrum, each line represents a different kind of carbon atom: each one absorbs energy (or resonates—hence the term nuclear magnetic resonance) at a different frequency. The reason that each carbon does not feel the same magnetic field is because of the electronic cloud around them. Each nucleus is surrounded by electrons, and in a magnetic field these will set up a tiny electric current. This current will set up its own magnetic field (rather like the magnetic field set up by the electrons of an electric current moving through a coil of wire or solenoid), which will oppose the magnetic field that is applied. The electrons are said to shield the nucleus from the external magnetic field. If the electron distribution varies from ^{13}C atom to ^{13}C atom, so does the local magnetic field, and so does the resonating frequency of the ^{13}C nuclei. A change in electron density at a carbon atom also alters the chemistry of that carbon atom. NMR tells us about the chemistry of a molecule as well as about its structure.



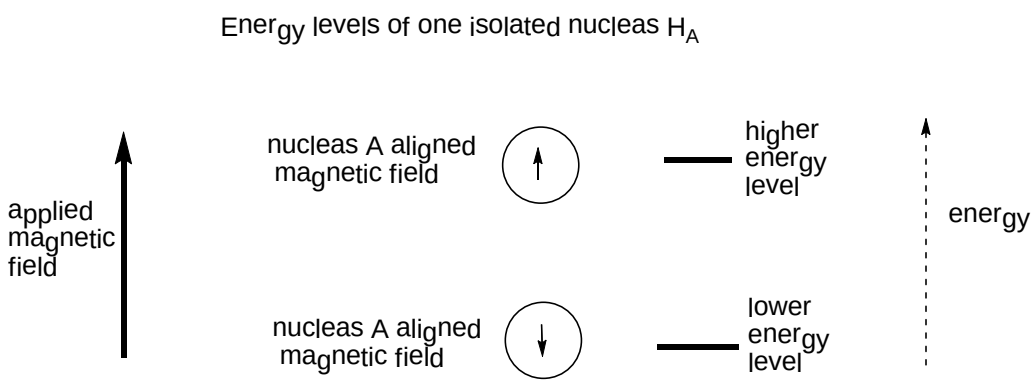
- In the spectrum above, the peak centred on 1ppm is for the CH_3 protons, the next are for CH_2 protons and the peak at around 5 ppm is for the OH proton. There are two things to be noted, the first is the so called "ppm" scale and second one is the splitting of the peaks in the proton signals. The splitting of the signal is due to coupling of the nuclear spins of one atom with another. An explanation can be provided for this phenomenon. If a molecule has two types of proton H_A and H_X having no interaction between them, then they can be aligned either along the external magnetic field or against it and only two lines are obtained. This can be seen 4,6-diaminopyrimidine-where there are only two single lines (called singlets). However, if there is an interaction between the two types of protons, then a different scenario arises. Now each proton, say, H_A, is near enough to

experience the small magnetic field of the other proton H_X as well as the field of the magnet itself. The diagram shows the result.

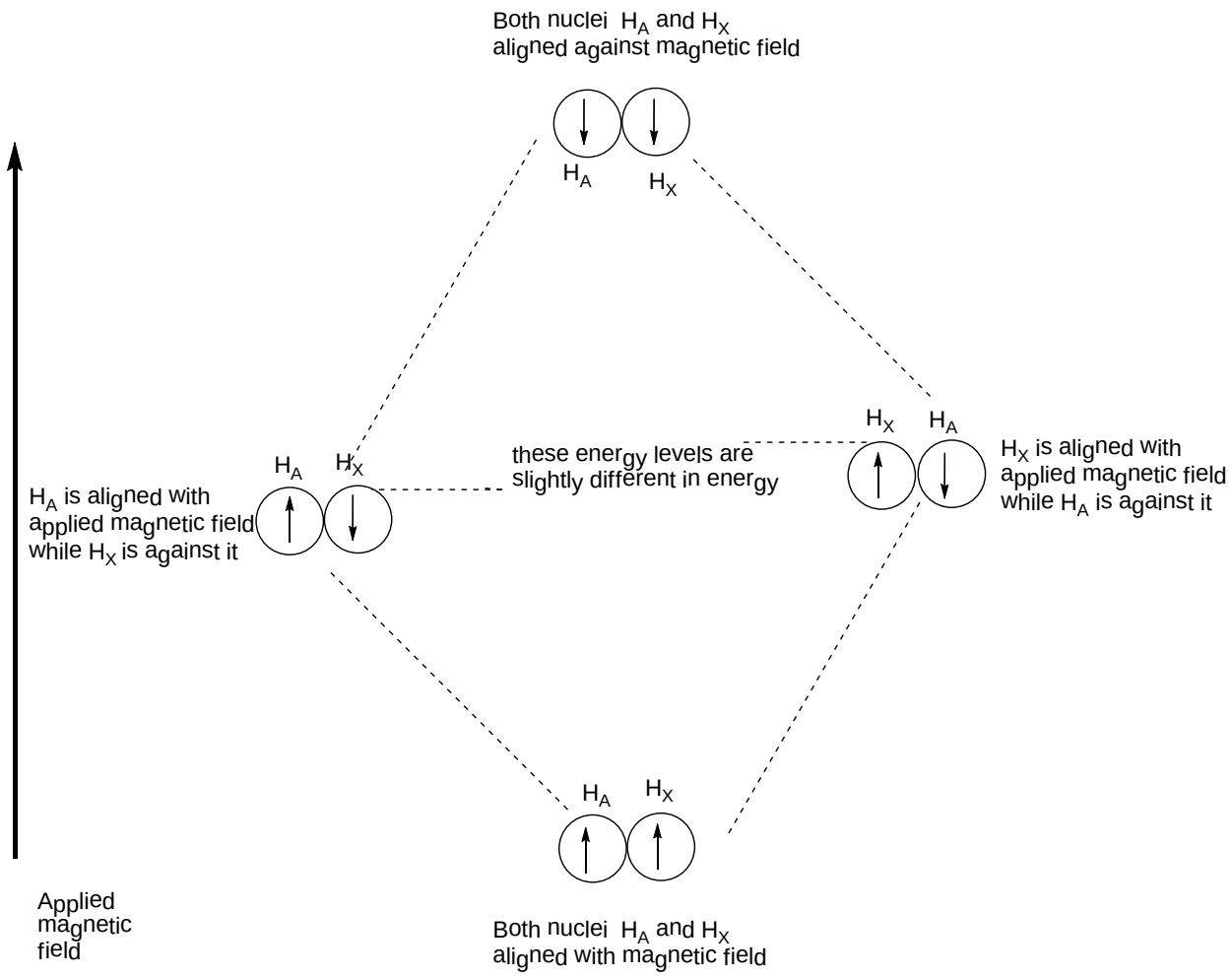


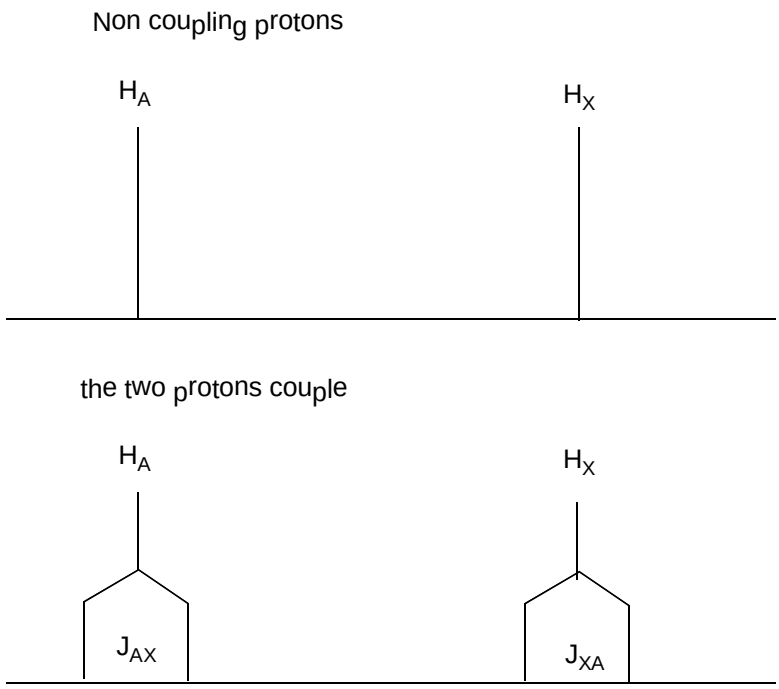
- If each proton interacted only with the applied field two singlets only would be obtained. But proton H_A actually experiences two slightly different fields: the applied field plus the field of H_X or the applied field minus the field of H_X. H_X acts either to increase or to decrease the field experienced by H_A. The position of a resonance depends on the field experienced by the proton so these two situations give rise to two slightly different peak-a doublet as it is called. And whatever happens to H_A happens to H_X as well, so the spectrum has two doublets, one for each proton. Each couples with the other. The field of a proton is a very small indeed in comparison with the field of the magnet and the separation between the lines of a doublet is very small.
- The second explanation takes into account the energy levels of the nucleus. Electronic energy levels on neighbouring atoms interact with each other and splitting to produce new molecular energy levels, some higher in energy and some lower in energy than the original atomic energy levels. When hydrogen nuclei are near each other in a molecule, the nuclear energy levels also interact and split and produce new energy levels. If a single hydrogen nucleus interacts with a magnetic field there are two energy levels as the nucleus can be aligned with or against the

applied magnetic field, there is one energy jump possible, and there is a resonance at one frequency.

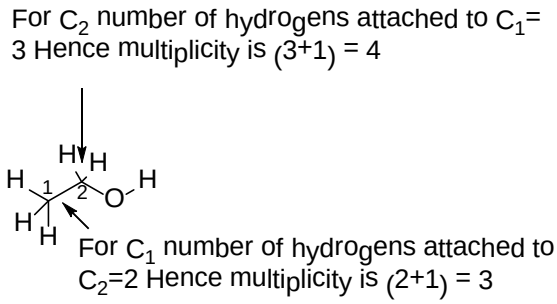


- This representation shows H_A protons independent of the influence of H_X. Each had two energy levels, each gave a singlet, and there were two lines in the spectrum. But, if each proton has hydrogen nucleus nearby and then there are now four energy levels. Each nucleus H_A and H_X can be aligned with or against the applied field. There is one most stable energy level where they are both aligned with the field and one least stable level where they are both aligned against. In between there are two different energy levels in which one nucleus is aligned with the field and one against. Exciting H_A from alignment with to alignment against the applied field can be done in two slightly different ways, shown as A_1 and A_2 on the diagram given below. The result is two resonances very close together in the spectrum. If there are two slightly different energy jumps to excite H_A, there must also be two slightly different energy jumps to excite H_X . The difference between $A_1$and A_2 is exactly the same as the difference between $X_1$and X_2. Each proton now gives two lines (a doublet) in the NMR spectrum and the splitting of the two doublets is exactly the same. This situation is described as coupling. We say ‘A and X are coupled’ or ‘X is coupled to A’(and vice versa, of course). Now look back at the spectrum of cytosine at the beginning of this section. Each is split by the same amount and the separation of the lines is the coupling constant and is denoted by J. Coupling constants J are measured in Hz because the same number regardless of the frequency of the spectrometer.


Both nuclei H_A and H_X aligned against magnetic field
H_A
H_X
H_A is aligned with applied magnetic field while H_X is against it
H_A
H_X
these energy levels are slightly different in energy
H_X
H_A
H_X is aligned with applied magnetic field while H_A is against it
H_A
H_X
Applied magnetic field
Both nuclei H_A and H_X aligned with magnetic field



- The true spectrum has a pair of doublets each split by an identical amount. It is to be noted that no line appears at the true chemical shift, but it is easy to measure the chemical shift by taking the midpoint of the doublet. The multiplicity (splitting of peaks) can be determined by counting the protons on the carbons attached immediately to carbon to which the referred proton is attached and using the formula n+1. Thus for the CH_3 group in ethanol, there is a CH_2 group attached to it hence the formula gives (2+1) or 3 as multiplicity.



- As for the ppm scale, the exact frequency at which the nucleus resonates depends on the external applied magnetic field. This means that, if the sample is run on a machine with a different magnetic field, it will resonate at a different frequency. It would be very difficult if as it couldn't be said exactly where the signal was, so

instead how far it is from some reference sample, as a fraction of the operating frequency of the machine is mentioned. All protons resonate at approximately the same frequency in a given magnetic field and that the exact frequency depends on what sort of chemical environment it is in, which in turn depends on its electrons. This approximate frequency is the operating frequency of the machine and simply depends on the strength of the magnet—the stronger the magnet, the larger the operating frequency. The precise value of the operating frequency is simply the frequency at which a standard reference sample resonates. In everyday use, rather than actually referring to the strength of the magnet in tesla, chemists usually just refer to its operating frequency. A 9.4 T NMR machine is referred to as a 400 MHz spectrometer since that is the frequency in this strength field at which the protons in the reference sample resonate; other nuclei, for example ^{13}C, would resonate at a different frequency, but the strength is arbitrarily quoted in terms of the proton operating frequency. The compound we use as a reference sample is usually tetramethylsilane, TMS. The four carbon atoms attached to silicon are all equivalent and, because silicon is more electropositive than carbon, are fairly electron-rich (or shielded), which means they resonate at a frequency a little less than that of most organic compounds. The chemical shift, δ, in parts per million (ppm) of a given nucleus in our sample is defined in terms of the resonance frequency as:

$$\delta = \frac{\text{frequency (Hz)- frequency TMS (Hz)}}{\text{frequency TMS (MHz)}}$$

- No matter what the operating frequency (i.e. strength of the magnet) of the NMR machine, the signals in a given sample (e.g. ethanol) will always occur at the same chemical shifts. In ethanol the carbon attached to the OH resonates at 3.56 ppm whilst the carbon of the methyl group resonates at 0.98 ppm. By definition TMS itself resonates at 0 ppm. The proton nuclei in most organic compounds resonate at greater chemical shifts, normally between 0 and 10 ppm. Similarly, the carbon nuclei in most organic compounds resonate at greater chemical shifts, normally between 0 and 200 ppm.
- In the ^{1}H NMR spectrum, it is also possible to obtain the number of protons that a particular signal corresponds to. Thus the signal at 0.98 corresponds to 3 protons for ethanol. This is because the area under each peak is directly proportional to the number of protons. This peak area is called integration value of the peak. Thus ^{1}H NMR spectra can be quantitative in nature but the same cannot be said for ^{13}C spectrum. For reasons beyond the scope of this text, they cannot be integrated to give the number of carbons.
- Similarly, the protons attached to particular type of carbon usually resonate in the same region. Thus, it is very useful to know the different regions on a ^{1}H spectrum.

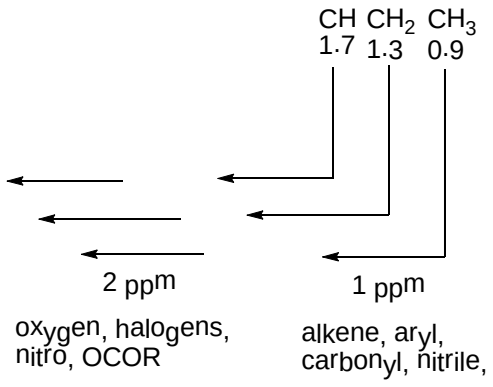
protons on unsaturated carbons next to oxygen: aldehydes	protons on unsaturated carbons: benzene, aromatic hydrocarbons	protons on unsaturated carbons: alkenes	oxygen saturated CH_3 CH_2 CH next to oxygen	CH_2 CH_3 saturated CH not next to oxygen	
10.0	8.5	6.5	4.5	3.0	0.0

A similar region wise classification may be made for ^{13}C NMR spectrum.

Unsaturated Carbon next to O (C=O)	Unsaturated Carbon atoms (C=C and aromatic carbons)	Saturated atoms next to oxygen	Saturated carbon atoms (CH_3, CH_2 etc)

200 150 100 50 0.0

- If adding electronegative atoms are added to a carbon atom, electron density is progressively removed from it and the carbon nucleus and the hydrogen atoms attached to it are progressively deshielded. In general an oversimplified chart can be constructed regarding the values of chemical shifts in ^{1}H NMR. However, it should be kept in mind that such a chart is purely a simplication of the situation and therefore exceptions are very common. These chemical shifts are additive. This means that if in a molecule there are two factors that should lead to an increase in the value of chemical shift of the proton concerned, then the additive effect due to these is to be considered.
- Methyl groups are to be started at 0.9, methylenes (CH_2) at 1.3, and methines (CH) at 1.7 p.p.m. Any functional group is worth a one ppm downfield shift except oxygen and halogen for which the value goes two ppm downfield.



In order to determine the structure of an organic compound, it is advised to proceed in the following manner.

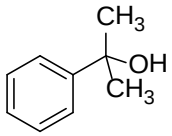
1. The history of the compound has to be determined. This includes the source from which the compound is obtained, or in case of a synthetic compound the series of steps which were carried out to obtain the compound.
2. The UV-Visible spectrum may be recorded and λ_{max} and its corresponding ε_{max} is to be determined. It may give information about the degree of conjugation in the molecule and well as information about certain functional groups like carbonyl group.
3. The IR spectrum also provides information about functional groups such as carbonyl group (aldehydes, ketones, carboxylic acid and its derivatives) and amine functionality.
4. NMR spectrum provides the most exhaustive source of information regarding the structure of the molecule

As an example, if a $C_9H_{12}O$ compound has strong infrared absorption at 3300 to 3400 cm^{-1}. The ^{13}C NMR spectrum of this compound has six discrete signals. It's ^{1}H NMR spectrum has three sets of lines: singlets at δ1.1 (6H), 1.9 (1H) and 7.3 (5H) ppm.

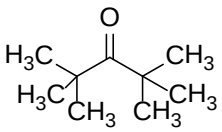
In this case the molecular formula has been deduced by mass spectroscopy. A calculation to determine the degree of unsaturation is usually helpful. It is defined as follows.

$$\text{Degree of Unsaturation} = (\text{Number of carbon atoms}+1) - \frac{(\text{Number of monovalent atoms})-(\text{number of trivelent atoms})}{2}$$

In this case the value comes out to be 4. This is indicative of a benzene ring or 4 double bonds or two triple bonds or a triple bond and a double bond. A strong absorption at 3300-3400 cm^{-1} indicates a O-H group. In the ^{1}H spectrum, the five 5 peaks at 7.3 ppm are indicative of the presence of monosubstituted benzene ring. Similarly, a singlet at 1.1ppm is indicative of two methyl groups which are not connected to each other. The peak at 1.9 could be due to the O-H proton. Hence the structure that could be deduced is- 2-phenylpropan-2-ol.



Now in the ^{13}C NMR spectrum, this compound should show 6 signals which is same as observed. Hence the deduction is correct.

Similarly, if a compound $C_9H_{18}O$ has a strong infrared absorption at 1710 cm^{-1}. Its ^{1}H NMR spectrum has a single sharp peak (a singlet) at δ 1.2 ppm while ^{13}C NMR spectrum shows three lines at δ210, 45 and 25 ppm. The logic for the elucidation of structure may be as follows:

Degree of unsaturation is 1. This surely means there is only one double bond in the molecule. A strong IR absorption at 1710 cm^{-1} indicates a carbonyl group. A peak in ^{1}H spectrum at 1.2 ppm indicates the presence of a methyl group, however there must be multiple methyl groups with a symmetrical environment to satisfy the molecular formula. A further clue may be obtained from the ^{13}C spectrum that there are two peaks in the aliphatic region (45 and 25 ppm) but only one peak in ^{1}H spectrum. This means the other carbon (at 45 ppm) is a tertiary carbon. This overall scenario leads to the assumption of the presence of two t-butyl groups. Hence the compound is 2,2,4,4-tetramethylpentan-3-one.