

Module-2: Spectroscopic techniques and applications

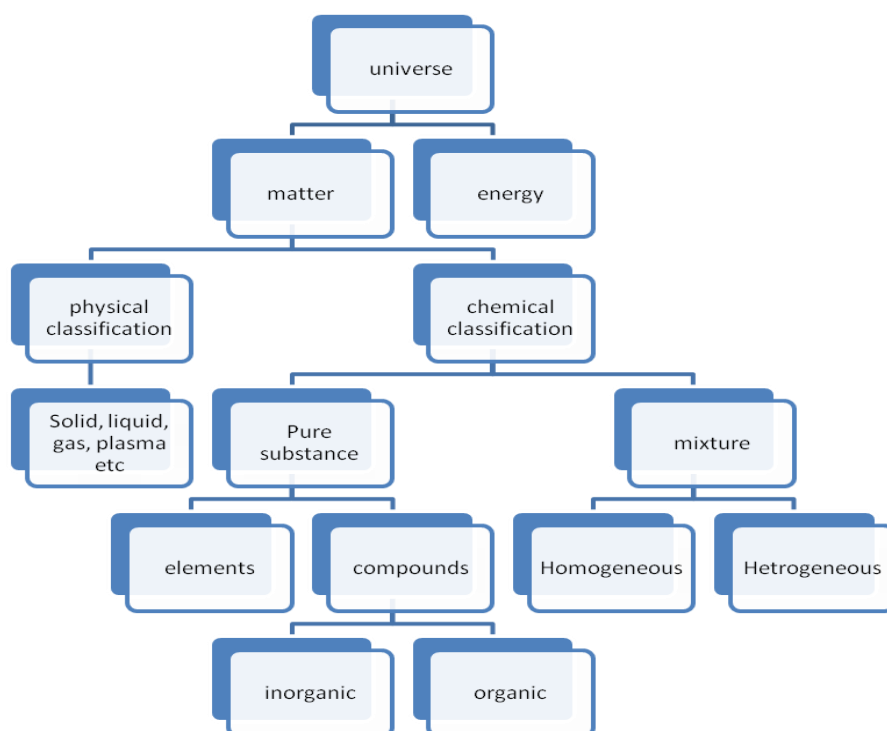
(Principles and Applications of Electronic spectroscopy and Nuclear magnetic resonance. Vibrational and rotational spectroscopy of diatomic molecules and its applications. Fluorescence and its applications in Medicine. Surface Characterisation Techniques (Scanning Electron Microscopy and Transmission Electron Microscopy))

Define Spectroscopy?

Spectroscopy is the branch of science which deals with the **interaction of electromagnetic radiation with matter**. The study of interaction provides a large scope to know what actually the atoms and molecules are.

Most of our present day knowledge about the structure of atoms and molecules has been derived on the basis of spectroscopic studies. Beside the elucidation of structure of atom and molecules, spectroscopy also serves a versatile tool in the chemical analysis of wide range of sample.

What is matter??



Spectrum??

The absorption of electromagnetic radiation by atoms and molecules induced a variety of transition in them. In atoms, the electrons get excited from lower electronic level to higher electronic levels. The excited electron in turn get de-excited is *quantised* and corresponds to a definite frequency or wavelength. A plot of intensities of radiation emitted or absorbed against the corresponding frequencies or wavelength is called a spectrum.

Principles of spectroscopy

“**Spectroscopy** is the analysis of the electromagnetic radiation scattered, absorbed or emitted by molecules. It deals with the transition that an electron in a molecule undergoes between energy levels upon absorption of suitable radiation”

A molecule in space can have many types of energies. It may possess rotational energy due to rotation of molecule about its centre of gravity, it may have Vibrational energy due to the periodic displacement of its atoms from their equilibrium positions, it will have electronic energy as the electrons associated with each atom are in motion.

As energy of an electron is quantized, similarly rotational, Vibrational and electronic energies of a molecule are also quantized, i.e., a particular molecule can exist in a variety of rotational, Vibrational etc, energy levels and can move from one level to another only by a sudden jump involving a finite amount of energy.

Consider two energy levels of atom/molecules. The one with lower energy is called **ground state energy level** (E_g) and the one with higher energy is called **excited state energy level** (E_e).

Absorption spectrum: results when an electron in an atom or molecule undergoes a transition from the lower energy levels to the higher one with the absorption of photon of energy $h\nu$, provided this energy is exactly equal to the energy difference ($\Delta E = E_e - E_g$) between two levels as shown in fig1.

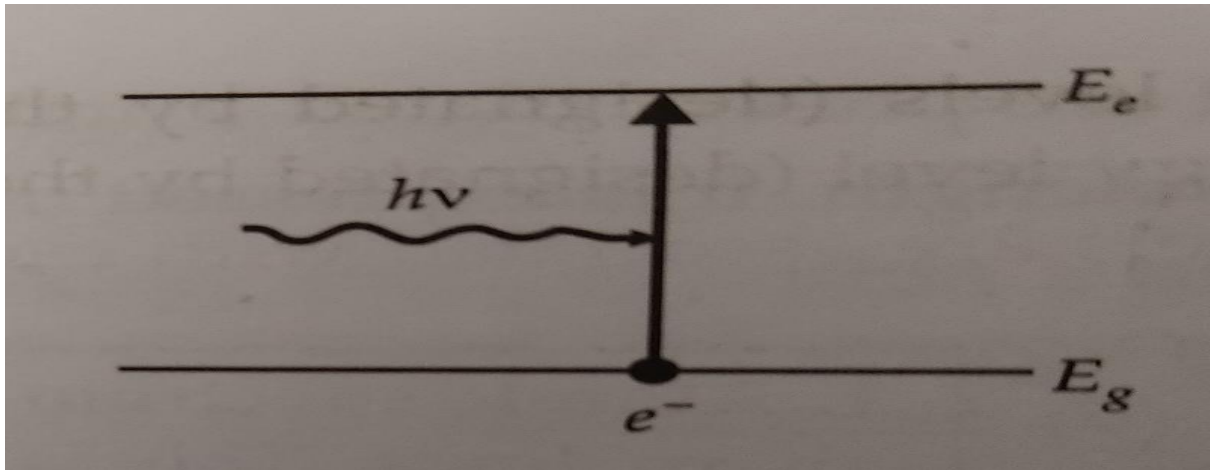


Fig1: Absorption spectrum

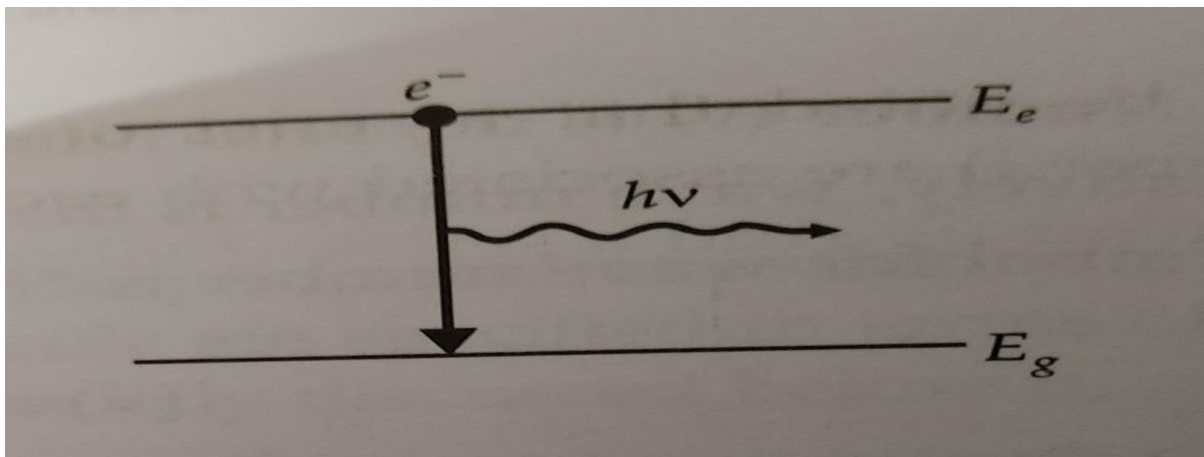


Fig2: Emission spectrum

Emission spectrum : results when an electron in an atom or molecule falls from the excited state to ground state with the emission of photon of energy ($h\nu$), as shown in fig2.

The frequency of absorbed or emitted radiation can be given by the formula,

$$\nu = \Delta E/h$$

Where h is plank's constant,

$$h = 6.63 \times 10^{-34} \text{ JS}$$

Question: Absorption (and not emission) spectroscopy is used to study the spectra of organic compounds. Explain why?

Solution: This is because emission of radiation from an organic compound requires very high temperature where organic compounds generally decompose.

The spectra can be broadly classified into two categories:

1. **Atomic spectra:** It arises from the transition of an electron between the atomic energy levels.
2. **Molecular spectra:** It arises from the transition of an electron between the Molecular energy levels.

In Atomic Spectroscopy, only electronic transitions are involved. That is why, electronic spectra can be used to obtain detailed information about electronic structure of atoms.

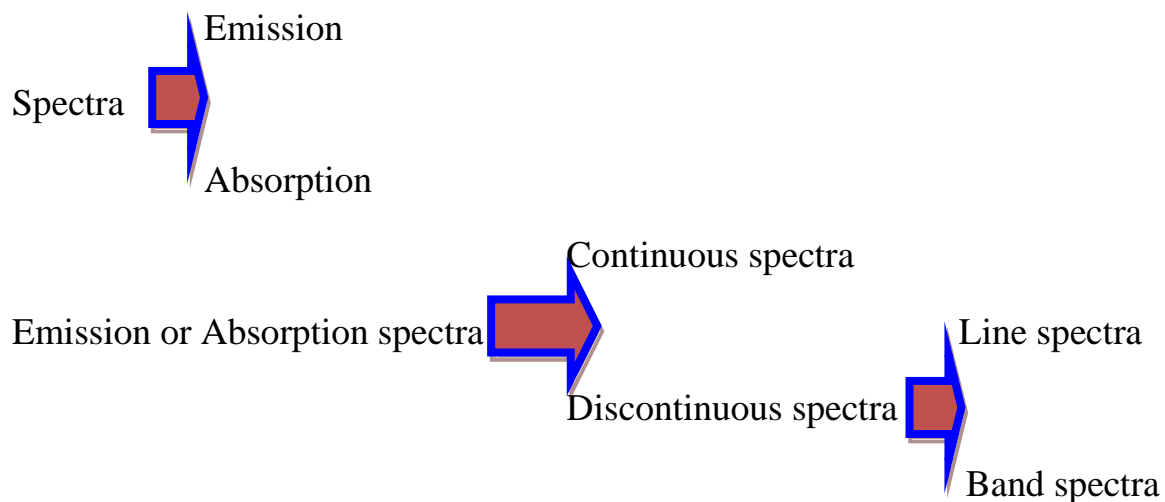
In contrast, the energy of a molecule can change not only as result of electronic transitions but also because it can make transition between its rotational and Vibrational states. Therefore the molecular spectra are more complicated and involve electronic, Vibrational and rotational transitions depending upon the nature of radiation absorbed.

Spectroscopy is the use of the absorption, emission or scattering of electromagnetic radiation by atoms or molecules to quantitatively study the atoms or molecule to study physical processes. The interaction of radiation with matter can cause redirection of the radiation and/or transition between the energy level of the atoms and molecules.

A transition from a lower level to a higher level with transfer of energy from the radiation field to the atom or molecules is called **absorption**.

A transition from a higher level to a lower level is called **emission** if transfer of energy from the radiation field.

Redirection of light due to its interaction with matter is called **scattering** and may or may not occur with transfer of energy, i.e. the scattered radiation has a slight different or the same wavelength.



Emission spectra:

When the radiation emitted by a luminous source of light is examined directly by a spectroscope then the spectra produced are known as emission spectra.

Example: - Visible spectra obtained from a filament lamp.

Absorption spectra:

When the light from a source is passed through an absorbing medium and the resulting radiation are examined by spectroscope, then the spectra obtained are known as absorption spectra. Dark lines or bands are obtained on bright background in these spectra, because certain radiations are obtained by absorbing medium under investigation.

Continuous spectra:

In the visible spectrum it is observed that colours diffuse into each other gradually. Thus the change in colour appears to be continuous and such spectra are called continuous emission spectra. These are obtained from incandescent solids, hot furnaces, hot iron, molten glass etc.

Line spectra:

Line spectra consist of line of definite wavelengths are given by gases and vapours of atoms. Hence, they are also called atomic spectra, line spectra of particular atoms.

Example: - Na- vapour

Electromagnetic spectrum and absorption of radiations:

The arrangement of all types of electromagnetic radiations in order of their increasing wavelengths or decreasing frequencies is called electromagnetic spectrum.

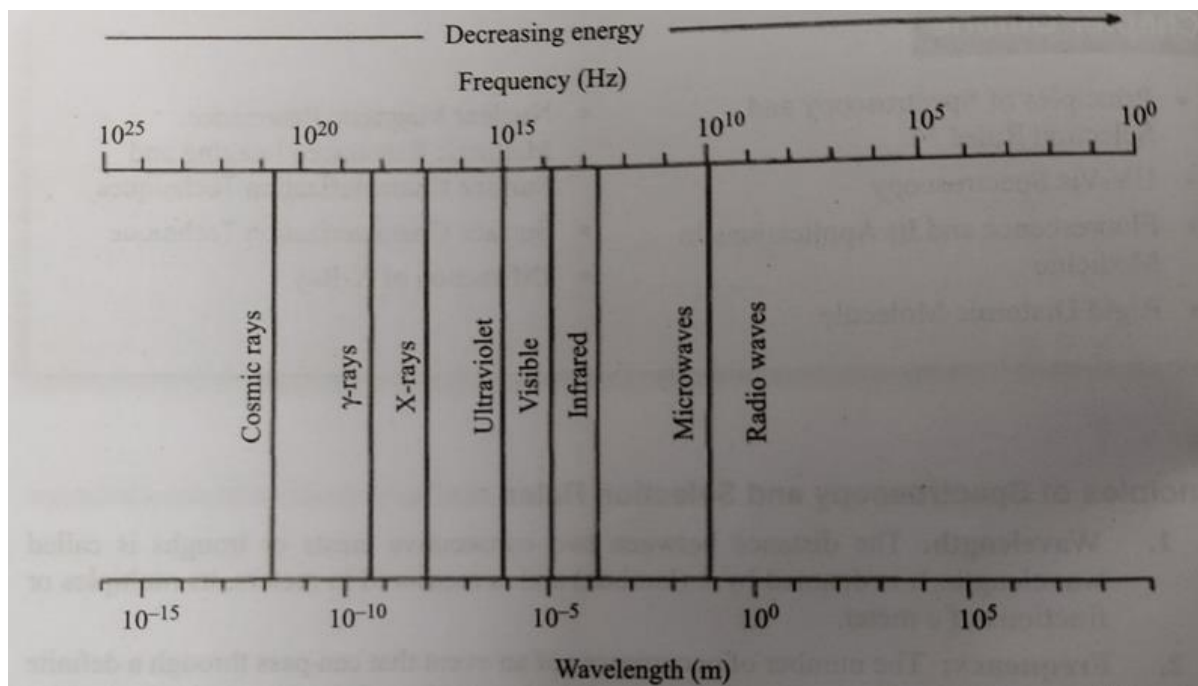


Fig1: Electromagnetic Spectrum

Infra red radiations have longer wavelengths and are thus less energetic cosmic rays carry high energy while radio waves are least energetic. Microwaves have larger wavelengths and are used in telephonic transmission. X- rays can pass through glass and muscle tissues. Radio waves can pass through air. Visible, UV, and IR radiations can be bent by reflection or by passing through a prism.

When light radiations are passed through an organic compound then electrons of the component atoms are excited. Due to the changes in the electronic, rotational and Vibrational energy levels, some of the wavelengths of light radiation are absorbed by the molecule. The wavelengths absorbed are measured with the help of a spectrometer.

If we plot the changes in absorption against wavelengths, we get certain absorption bands which are highly characteristics of a compound and the technique provides an excellent tool to ascertain the molecular structure of an unknown substance.

Principle:

“It involves the transition of electron(s) within a molecule or ion from a lower to a higher electronic energy level or vice versa by the absorption or emission of radiation falling in the UV-Visible rang of electromagnetic spectrum”

Electronic spectroscopy involves the promotion of electron (σ , π , n electrons) from the ground state to the higher energy state. It is very useful to measure the number of conjugated double bonds and also aromatic conjugation within the various molecules.

The region below 200 μ is called vacuum ultra violet region. Therefore ultra violet region below 200 μ is not much studied due to absorption by oxygen and nitrogen. Moreover, studies in these regions require Vacuum instruments.

Electronic Transitions

According to the molecular orbital theory, when a molecule is excited by the absorption of energy (UV or Visible light), its electron are promoted from a bonding to an antibonding orbitals. When a molecule is formed then valence electron taking part in bond formation exists in bonding orbital. Electrons forming sigma (σ) bond are called σ electrons and those which participate in π bond are called π electrons. Unshared electrons in a molecule which do not take part in any bond formation exist as non bonding orbitals. Such electrons are called n -electrons, non bonding electrons. There are higher energy levels termed as antibonding orbitals which are associated with σ and π bond as σ^* (*sigma star*) and π^* (*pi star*) orbitals. As n electrons do not take parts in bond formation thus is no antibonding orbital associated with it.

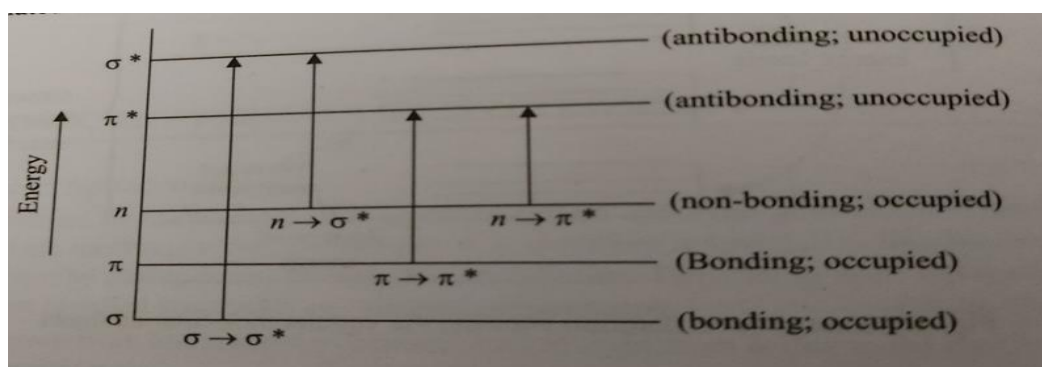


Fig: Relative energies required for various type of electronic transition.

Electronic transitions in molecules can be classified into:

(i) $\sigma\text{-}\sigma^*$ Transition: As σ electrons are held more firmly in the molecule, this transition takes place in UV or far UV region.

(ii) $\pi\text{-}\pi^*$ Transition: This transition takes place in the near UV and visible region.

(iii) $n\text{-}\pi^*$ transition: These transitions are generally of weak intensities and lie in the visible region.

Various transitions involved in ultraviolet spectroscopy:

A schematic arrangement showing the energy levels of all these orbitals is shown in above Fig.

(i) **Transition between bonding orbitals and antibonding orbitals e.g., $\sigma\rightarrow\sigma^*$ transition and $\pi\rightarrow\pi^*$ Transition.**

This excited state closely resembles the polar character.

(a) **$\sigma\rightarrow\sigma^*$ transition:** These are high energy transition as σ bond are generally Strong bonds. Saturated hydrocarbons like methane, propane etc shows such high energy transitions.

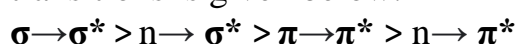
(b) **$\pi\rightarrow\pi^*$ Transition:** Molecules having unsaturation show this kind of Transition and absorb in ultraviolet or visible region for e.g. alkene, alkynes, carbonyl compounds etc

(ii) **Promotion of non-bonding electron** (i.e., unshared pair n) into or antibonding π , i.e. $n\rightarrow\sigma^*$ or $n\rightarrow\pi^*$. These involve transitions of non-bonding lone pair of hetero atom (e.g., oxygen, nitrogen) to an empty antibonding molecular orbital. The absorption bands for such transitions occur in longer wave length with low intensity. Compounds like saturated alkyl halides, alcohols, amines shows $n\rightarrow\sigma^*$ transition. Compounds like saturated aldehydes or ketones also show $n\rightarrow\pi^*$ transition.


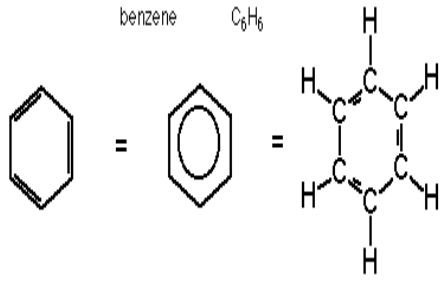
(iii) **Transition between bonding (occupied) σ and π orbitals to antibonding (unoccupied) σ^* and π^* orbitals** i.e., $\sigma\rightarrow\pi^*$ and $\pi\rightarrow\sigma^*$ can also take place.

According to the selection rules these radiations are forbidden and therefore have weak intensities.

All the above four types of electronic transition have been summarised in above figure. The relative energies required for these transitions have also been shown. The energy required is maximum for **$\sigma\rightarrow\sigma^*$ transition and minimum $n\rightarrow\pi^*$ transitions.** The energy requirements in the decreasing order of these transitions is given below:



Electronic transitions, λ_{\max} for some compounds:

Compound	Structure	Transitions	λ_{\max} (nm)
Water		$n-\sigma^*$	169
Methanol	$\text{CH}_3\text{-O-H}$	$n-\sigma^*$	188
Ethane	$\text{CH}_3\text{-CH}_3$	$\sigma-\sigma^*$	135
Acetylene	$\text{CH}\equiv\text{CH}$	$\pi-\pi^*$	173
Ethylene	$\text{CH}_2=\text{CH}_2$	$\pi-\pi^*$	194
Benzene	<div style="text-align: center;"> benzene C_6H_6  </div>	$\pi-\pi^*$	About 180
		$\pi-\pi^*$	About 200
		$\pi-\pi^*$	About 255

Selection Rule:

We know that atomic spectra as well as molecular spectra are obtained due to transitions taking place between energy levels. To make the spectral study simpler and easy interpretation of the data obtained from it, certain restrictions are imposed on quantum numbers during transitions, which are known as “Selection Rule”. If a spectral transition is as per the selection rule, it is called as allowed transition.

If these rules are not followed, the transition cannot take place and it is called a **forbidden** transition. Examples of forbidden transition are from s to s orbital and from p to p orbital. For these transition $\Delta\ell = 0$. In general allowed transitions are intense strong where as forbidden transition are weak.

The selection rules followed depend upon the type of transition (in the case of molecular spectra). The selection rules are generally expressed in terms of changes in quantum number for the allowed transitions. For examples:

- (i) In generally $\Delta\ell = \pm 1$, where ℓ is called azimuthal quantum number. Transition from s to p orbital and from p to d orbital are allowed transitions, because $\Delta\ell = \pm 1$.
- (ii) For the pure rotational transition, the selection rule is $\Delta J = \pm 1$ Where J represents rotational quantum number, ($\Delta J = +1$ corresponds to absorption and $\Delta J = -1$ corresponds to emission).
- (iii) For pure Vibrational transition, the selection rule $\Delta v = \pm 1$, where v represents the Vibrational quantum number.

Thus

(a) The transition which involves a change in the spin quantum number of an electron during the transition does not occur. Thus singlet-triplet transitions are forbidden.

(b) The transition between orbitals of different symmetry does not occur e.g. $n \rightarrow \pi^*$ transition is symmetry forbidden.

Chromophore and Auxochrome:

Chromophore may be defined as any isolated covalently bonded group that shows a characteristic absorption in the UV or the visible region, irrespective of the fact whether colour is produced or not.

There are two types of chromophores:

(i) Chromophore, which contain π - electrons and they undergo $\pi-\pi^*$ transitions. For examples, ethylene, acetylenes etc.

(ii) Chromophore, which contain both π electrons and n (non bonding) electrons and undergoes $\pi-\pi^*$ and $n-\pi^*$ transitions. For examples carbonyls and nitriles.

Auxochrome is basically colour enhancing group. It has the ability to extend the conjugation of the Chromophore by the sharing of the non-bonding electrons and thus shifts the absorption band towards the longer wavelength (the red end of the spectrum). Auxochrome by itself doesn't act as Chromophore.

Some common Auxochrome groups are $-\text{OH}$, $-\text{OR}$, $-\text{NH}_2$, $-\text{SH}$, $-\text{X}$ groups etc

Molecule	λ_{max} ($m\mu$)
$\text{CH}_2 = \text{CH}_2$	171
$\text{CH}_2 = \text{CH} - \text{Cl}$ Auxochrome	185

Factors Affecting λ_{\max} Intensity of Spectral line

The position and the intensity of absorption maximum is shifted for a particular Chromophore by changing the polarity of the solvent. In general, the absorption maximum for the non-polar compounds is the same in ethanol (polar) as well as in hexane (non-polar) solvent. The various effects are as follows:

1. Bathochromic Shift (Red Shift)
2. Hypsochromic Shift (Blue Shift)
3. Hyperchromic Effect
4. Hypochromic Effect

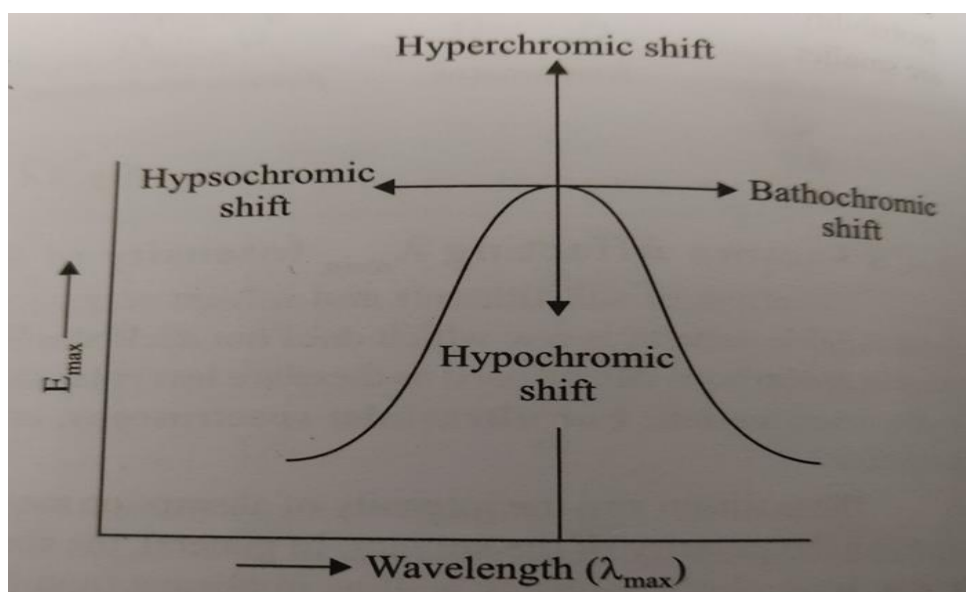


Fig: Absorption and Intensity Shift

1. Bathochromic Shift (Red Shift)

It is an effect by virtue of which the absorption maximum is shifted towards longer wavelength due to presence of an Auxochrome or by change of solvent.

If an electron donor group like OH, CH₃, NH₂ etc., is substituted in a compound, then the absorption band is shifted to longer wavelength. Such shifts of absorption bands towards longer wavelengths due to substitution or solvent effect are known as **bathochromic or red shifts**.

For Example the λ_{\max} for C₆H₆ and C₆H₅CH₃ are 254 nm and 261 nm. Thus, the substitution of hydrogen by CH₃ group in benzene causes bathochromic shifts in the absorption band. Conjugation of double bonds also cause bathochromic shifts.

2. Hypsochromic Shift (Blue Shift)

It is an effect by virtue of which the absorption maximum is shifted towards shorter wavelength. If an electron acceptor group like acetyl group is substituted in compound, then the absorption band shifted to shorter wavelength. Such shifts of absorption band towards shorter wavelength due to substitution or solvent effect is known as **Hypsochromic or blue shifts**. If CH_3 group is introduced in an unsaturated chain of an organic compound, then conjugation is destroyed and absorption band of the compound is shifted to shorter wavelengths. Hydrogen bonding also produces Hypochromic shifts.

For Example:



For example:

Compound	λ_{max} (nm)	Compound	λ_{max} (nm)	Compound	λ_{max} (nm)
$\begin{array}{c} \text{H}-\text{C}=\text{O} \\ \\ \text{N} \end{array}$	290	$\begin{array}{c} \text{H}-\text{C}=\text{O} \\ \\ \text{Cl} \end{array}$	235	$\begin{array}{c} \text{H}-\text{C}=\text{O} \\ \\ \text{NH}_2 \end{array}$	205

Even change in solvent can also bring the Hypsochromic shift. For example: $\text{CH}_3-\text{C}(=\text{O})-\text{CH}_3$ (acetone) absorbs at $\lambda_{\text{max}} = 279 \text{ nm}$ in hexane and at $\lambda_{\text{max}} = 264.5 \text{ nm}$ when dissolved in water.

3. Hyperchromic Effect:

It is an effect due to which the intensity of absorption maximum i.e., ϵ_{max} increases. If a substituent group causes increase in the intensity of band, then the effect is called *Hyperchromic effect*. The increase in the intensity of absorption band is due to the fact that the substituent group causes the increase in the molar extinction coefficient ϵ of the compound. For Example, Benzene (C_6H_6) shows

absorption at $\lambda_{\text{max}} = 254 \text{ nm}$ with $\epsilon_{\text{max}} -200$ whereas toluene ( ) shows absorption at $\lambda_{\text{max}} = 261 \text{ nm}$ with $\epsilon_{\text{max}} -300$, showing the Hyperchromic effect.

4. Hypochromic Effect:

If a substituent group causes the intensity of the band to decrease, then the effect is called Hypochromic effect. As an example ϵ_{max} for C_6H_6 and $\text{C}_6\text{H}_5\text{Cl}$ are 200 and 170 respectively. Thus, substitution of Cl causes Hypochromic effect.

Application of UV-spectroscopy:

There are extensive applications of Ultraviolet and visible spectroscopy in different field of chemical interest. However, a few important application are given below:--

(i) Identification of Organic Compounds:

It is also useful in establishing the identity of the new and unknown compounds. The spectrum of the new compound can be compared with the spectrum of known compound. For instance, it helps in deciding whether a particular compound is cis or trans geometry isomer. The resemblance of the two spectra establishes the nature of the new compound.

The absorption spectrum of unknown compound is compared with those of a number of known compounds.

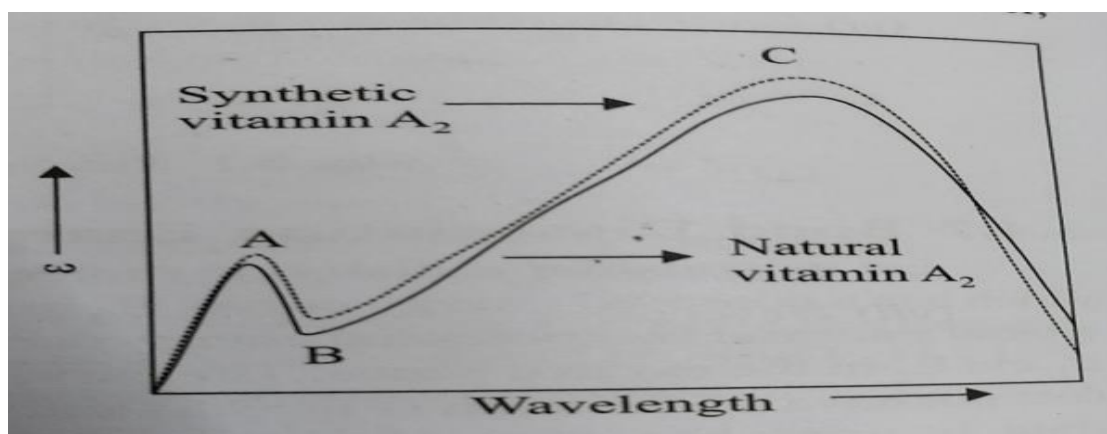


Fig: Absorption spectrum of Vitamin A₂ in Ultraviolet Region

The absorption spectrum of synthetic (unknown) and natural vitamin A₂ are shown in above figure. The similarity between two spectra proved helpful in the identification and assignment of structure of synthetic vitamin A₂.

(ii) Determination of dissociation energy:

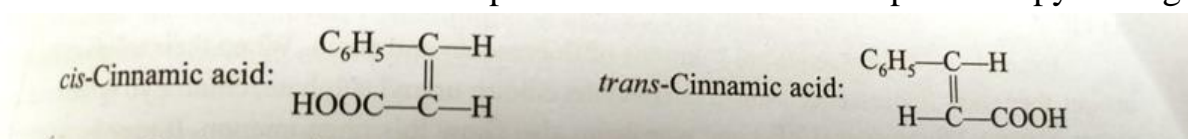
The dissociation energy for a molecule can be determined with great accuracy from this spectrum. The dissociation energy is calculated from the wavelength that separates the discontinuous part of the spectrum from the continuous part of the spectrum resulting from the dissociation of the molecule into two parts in varying amount of kinetics energy.

(iii) Presence of impurities:

It can be used to detect the presence of impurities in a substance. The spectrum of the compound is compared with the spectrum of pure compound. The impurities will certainly cause extra absorption bands, characteristic to their structure. Ultraviolet and visible Spectrophotometry is used for the control of purification of a compound.

(iv) Confirmation of cis and trans hydrogen:

Determination of Geometry isomer i.e., whether compound is cis or trans is also established with the help of UV-visible spectroscopy. e.g.,



In case of cis isomer, due to steric repulsion, coplanarity is lost and thus conjugation is limited. It absorbs at shorter λ i.e., 268nm as compared to trans which absorbs at 272nm.

(v) Determination of extent of conjugation:

It is a very useful technique to know the extent of multiple bonding in a molecule. It can also be used to distinguish between conjugate diene and conjugated triene, conjugated and non-conjugated molecules, α , β unsaturated ketones and β , γ unsaturated ketones (these differ in the position of carbon-carbon double bond). The value of λ_{max} and ϵ_{max} are more for extended conjugated molecules as compared to those for unconjugated alkenes. Bathochromic as well as Hyperchromic effect are observed when the spectrum of conjugated triene is compared to that of conjugated diene.

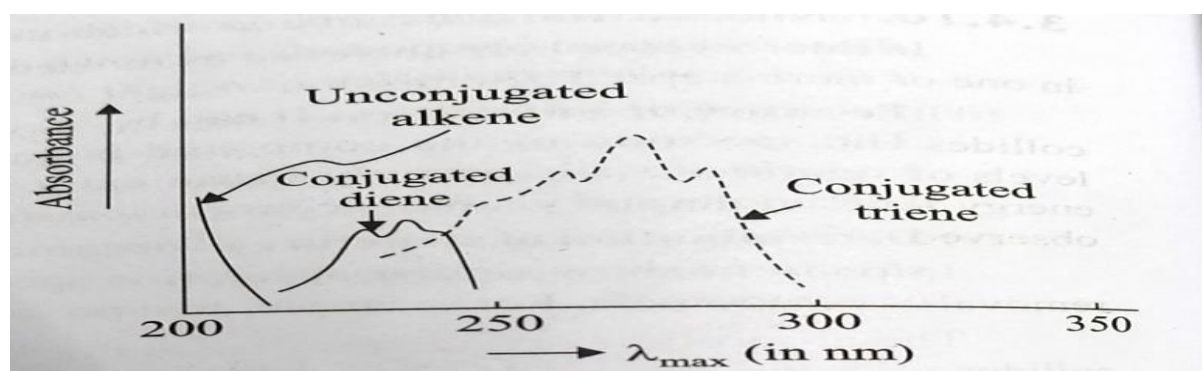


Fig: Absorbance in conjugated and unconjugated system

(vi) Study of Kinetics:

If the λ_{\max} values of reactants and products of a chemical reaction are quite different, then the change in the concentration of reactants or products can be followed spectrophotometrically and kinetics of the reaction can be studied. Spectrophotometric methods are particularly useful when the reaction are fast and the solutions are very dilute, e.g., photochemical transformation of ergosterol to vitamin D₂ is well suited for Spectrophotometric method.

(vii) Determination of structure of organic compounds.

(viii) Other physico-chemical studies:

Apart from these , ultraviolet and visible Spectrophotometry has been very useful in other physico-chemical studies, such as determination of dissociation constants of acids and bases, heat of formation of molecular addition compounds and complexes in solution, empirical formulae of complexes in solutions.

Question: Identify the molecular energy state which is excited by radiation in the UV and visible region.

Answer : Electronic energy state, these radiations cause an electron transition from the ground state to an excited state. More specifically, the electron goes from an occupied molecular orbital (bonding or non bonding) to an unoccupied antibonding MO's. The larger the energy gap between the ground and excited states, the shorter must be the wavelength and the higher must be the energy and frequency of the radiation.

Question: Are all the molecules of a substance that are exposed to a specific radiation excited?

Answer: No, absorption is a random event. Relatively few molecules may be excited. Intensity is measure of the concentration of excited molecules.

Question: What is the meaning of ϵ_{\max} ,the molar extinction co-efficient?

Answer: The molar extinction co-efficient is related to the molecule's probability of absorbing a photon at λ_{\max} or to the intensity of excited molecules.

Values for The molar extinction co-efficient $\geq 10^4$ are termed high intensity absorption, while values $<10^3$ are low intensity absorption.

Question: What happen to the excited molecules, when the radiation is removed?

Answer: The excited molecules give off energy and return to the ground state, or may undergo chemical change, e.g. cis-trans isomerism of an alkene. The return to the ground state is called deactivation.

Question: Explain how an electrochemically excited species returns to the ground state producing only heat.

Answer: It can lose all its excess energy through collisions with other molecules, the released energy appears as heat.

Question: Describe and compare the absorbance of visible light by a (a) White solid, (b) colourless liquid or gas, (c) black solid, (d) red gas, liquid or solid.

Answer: (a) and (b) A white substance absorbs no light in the visible region. All visible light is reflected if the substance is solid, the light is all transmitted through a colourless gas or liquid (pure or a solvent).

(c) A black solid absorbs all colours in the visible region, no light is reflected.

(d) A coloured substance absorbs the complement of the observed transmitted or reflected colour. Thus a red substance absorbs light in the green spectral region.

Vibrational and rotational spectroscopy of diatomic molecules and its applications.

Vibrational-Rotational spectroscopy is a branch of molecular spectroscopy concerned with infrared and Raman spectra of molecules in the gas phase. Transitions involving changes in both Vibrational and rotational states.

When such transitions emit or absorb photons (electromagnetic radiation), the frequency is proportional to the difference in energy levels and can be detected by certain kinds of spectroscopy. Since changes in rotational energy levels are typically much smaller than changes in Vibrational energy levels, changes in rotational state are said to give fine structure to the Vibrational spectrum. For a given Vibrational transition, the same theoretical treatment as for pure rotational spectroscopy gives the rotational quantum numbers, energy levels, and selection rules. In linear and spherical top molecules, rotational lines are found as simple progressions at both higher and lower frequencies relative to the pure vibration frequency. In symmetric top molecules the transitions are classified as parallel when the dipole moment change is parallel to the principal axis of rotation, and perpendicular when the change is perpendicular to that axis.

IR Spectroscopy or Vibrational (Infrared) Spectroscopy

Principal/Theory/Condition

- IR radiation is a part of the electromagnetic spectrum between the visible and microwave region.
- IR is absorbed by organic molecules and converted into energy of molecular vibration, either stretching or bending.
- Different type of bonds and different functional groups IR radiation at different wavelength.
- These vibrations produce a change in the bond length and consequently the dipole moment of the molecule may change. If the oscillating dipole is exposed to IR radiations, it may couple up with the electric field of radiation and absorption takes place which gives rise to Vibrational spectrum.
- *Homonuclear diatomic molecules* are unable to absorb radiation because they possess zero dipole moment and are thus *IR-inactive*.
- *Heteronuclear diatomic* or polyatomic molecule which possess permanent dipole moment or develop a dipole moment on account of vibrations, are able to absorb IR-radiation and *IR active*.

- CO₂ molecule has zero dipole moment but it develops a dipole moment due to asymmetric stretching or bending mode of vibration and become IR active.
- Stretching absorptions of a bond appear at higher wave number in the IR spectrum than the bending absorptions of the same bond.
- Infrared region divided into three sub-region (Near IR , Mid IR, Far IR)

Region	Wavelength (nm)	Wave number range (cm ⁻¹)
Near IR	0.78-2.5	12800-4000
Mid IR	2.5-50	4000-200
Far IR	50-1000	200-10

The most useful region lies between 4000-667 cm⁻¹.

- All three sub regions of IR part of the electromagnetic spectrum are associated with the change in the molecular vibration.
- In IR region, the region below 1500 cm⁻¹ is called finger print region, which is used to determine the identity of two compounds. Some molecules containing the same functional group show similar absorptions above 1500 cm⁻¹ but their spectra differ in finger print region.

Two conditions which are essential for a molecule to be IR- active

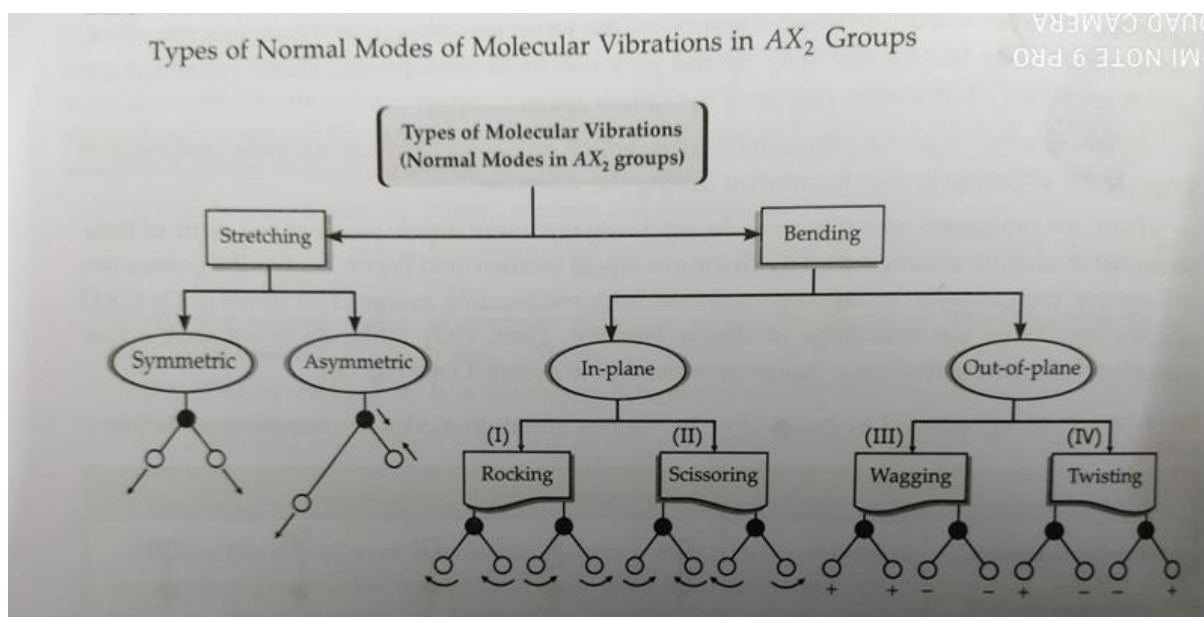
1. There should be change in dipole moment or there should be permanent dipole moment in the molecule.
2. Natural frequency of vibration of the molecule should be match with that of infrared radiation.

Mechanism of Interaction between the Vibrating Molecules with Infrared Radiation

During the vibration of a molecule, if there occurs a change in dipole moment, it will lead to generation of an oscillating electrical field. Now if the frequency of this oscillating electric field is equal to the frequency of fluctuating electric field of radiation (oscillating in a plane perpendicular to the direction of propagation of electromagnetic radiation) , there occurs the phenomenon of resonance and hence energy can be transferred from the radiation to the molecule or vice-versa. This will lead to generation of absorption IR-spectra or emission IR-spectra respectively.

Fundamental Mode of Vibration and Types

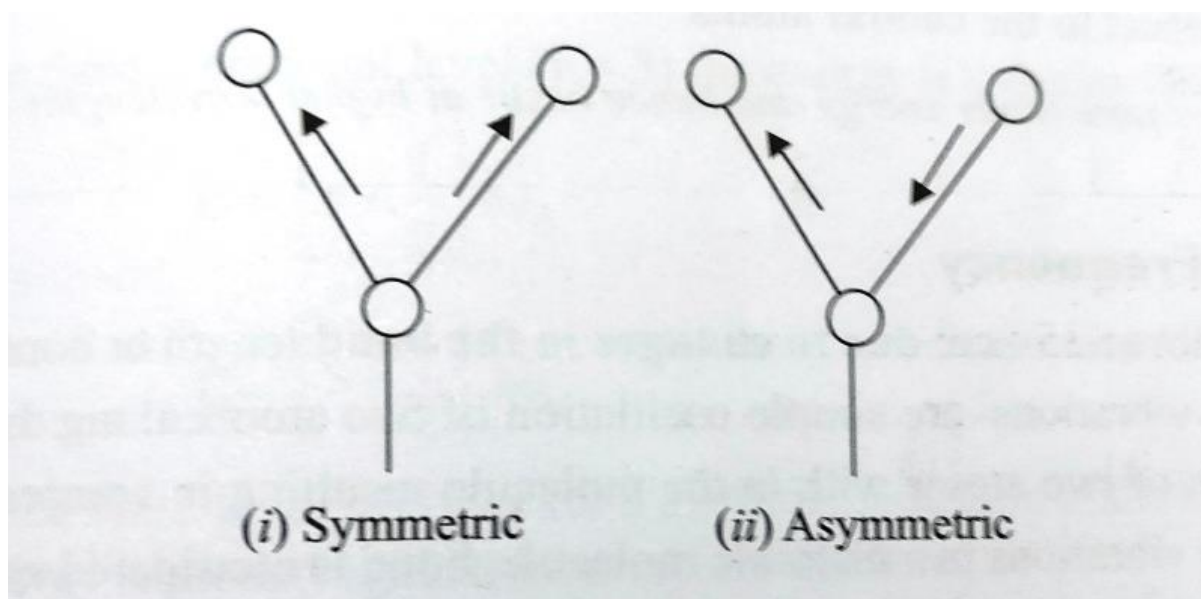
The various vibration modes are summarized below:



Stretching: In this type of vibrations, the distance between the two atoms increases or decreases but the atoms remain in the same bond axes.

Types of stretching vibration: There are two types of stretching vibrations

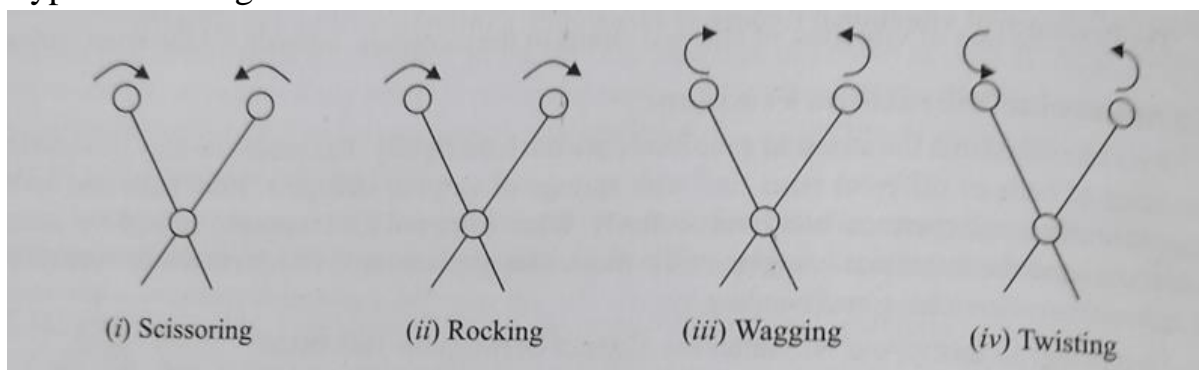
Symmetric stretching: - In this type, the movement of atoms with respect to a particular atom in a molecule is in the same direction.



Asymmetric stretching: - In this type, one atom approaches the central atom while the other departs from it.

Bending: In this type of vibrations, the position of the atoms changes with respect to original bond axis.

Type of bending vibration:



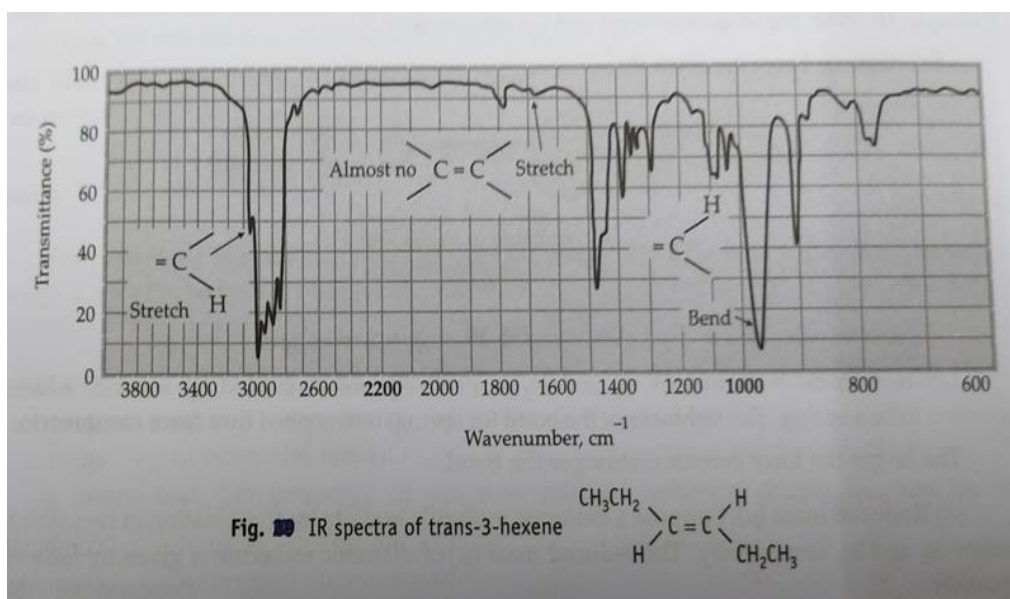
Scissoring: In this type, two atom approaches each other.

Rocking: In this type, the movement of the atoms takes place in the same direction.

Wagging: Two atoms move up and down the plane with respect to the central atom.

Twisting: In this type, one of the atoms moves up the plane and other move down the plane with respect to central atom.

Bending vibrations require lesser energy and hence occur at higher wavelengths or lower wave numbers than stretching vibrations.



IR absorption frequencies of some common structural units:

Stretching frequencies (cm^{-1})

Single bond		Double bonds	
Structural unit	Structural frequencies	Structural unit	Structural frequencies
-O-H(Alcohols)	3200-3600	$>\text{C}=\text{C}<$	1620-1680
-O-D	2100	$>\text{C}=\text{O}$	
-O-H (Carboxylic acids)	2500-3600	Aldehydes & ketones	1710-1750
$>\text{N}-\text{H}$	3350-3500	Carboxylic acid	1700-1725
		Acid anhydride	1800-1850
$\text{C}_{\text{sp}}-\text{H}$	3310-3320	Esters	1730-1750
$\text{C}_{\text{sp}}^2-\text{H}$	3000-3100	Amides	1680-1700
$\text{C}_{\text{sp}}^3-\text{H}$	2850-2950	Triple bonds	
$\text{C}_{\text{sp}}^3-\text{H}$	1200	$-\text{C}\equiv\text{C}-$	2100-2200
$\text{C}_{\text{sp}}^3-\text{H}$	1025-1200	$-\text{C}\equiv\text{N}-$	2240-2280

Bending frequencies (cm^{-1}) of hydrocarbon

Alkane	CH_3 1420-1470	$=\text{CH}_2$ 1430-1470	$\text{CH}(\text{CH}_3)_2$ Doublet of equal intensities at 1370, 1385 also 1170	$\text{CH}(\text{CH}_3)_3$ Doublet at 1370(strong) 1395 (moderate)
Alkenes out of plane	$\text{RCH}=\text{CH}_2$ 910-920 990-1000	$\text{R}_2\text{C}=\text{CH}_2$ 880-900		$\text{RCH}=\text{CHR}$ Cis 675-730 Trans 985-975
Aromatic C-H Out of plane	Mono substituted 690-710 730-770	Disubstituted		
		Ortho 735-770	Meta 690-710 750-810	Para 810-840

Sample preparation method in IR spectroscopy:

In IR spectroscopy sample preparation is one of the most important factors due to following reasons:

- (i) If concentration of sample is very high then the peak will become very broad and will go out of the axis.
- (ii) On the other hand, if the sample concentration is very low, the absorbance will be very low resulting in weak absorption bands.
- (iii) The sample must be very pure; else the peaks due to impurities will interfere.
- (iv) The sample should be free from moisture, if moisture is present; this will not only interfere with the spectra but also will spoil the cell (sample holder) which is made of alkali halides.

Various methods:

Neat method: - In this method a drop of the liquid is placed at one end of the NaCl plate, subsequently with the help of another plate this is spread uniformly and combination is directly placed in a sample holder.

Solvent method: In this method, about 3.5 mg of the sample is dissolved in a suitable solvent (say CHCl_3 (Chloroform), CCl_4 etc) the solution is slowly added in the cell and then stoppered.

For solids: There are two methods:

KBr pellet method: In this method about 2-4 mg of the sample is mixed with 40-50 mg of anhydrous KBr. The mixture is thoroughly ground using a mortar and pestle. The uniform mixture is then placed in a die, covered with the solid stainless steel cylinder and pressed using a hydraulic pressure. On pressing for 2-5 minute this forms a thin transparent disc, which is now directly placed in sample holder.

Nujol Mull Method: In this method about 2-5 mg of the compound is mixed with Nujol (high molecular wt. paraffin) using mortar and pestle a homogeneous suspension is formed, which can be directly used for recording spectra.

Fundamental Vibrational absorption bands in polyatomic molecule:

Polyatomic molecules may exhibit more than one fundamental Vibrational absorption bands.

The number of these fundamental bands is related to the degree of freedom in a molecule.

The total number of degree of freedom in a molecule will be equal to $3n$. Where 'n' is the number of atoms in a molecule.

$3n$ degree of freedom = translational + rotational + Vibrational

Linear molecule: -

A linear molecule has always 3 translational degree of freedom and only two degree of rotations, because the rotation of such a molecule about its axis of linearity does not bring about any change in the positions of the atoms while rotation about the two other axis change the position of the atoms. Thus for a linear molecules of 'n' atoms,

Total degree of freedom = $3n$

Translational degree of freedom = 3

Rotational degree of freedom = 2

Vibrational degree of freedom = $3n - 3 - 2$
= $3n - 5$

Total number of Vibrational degrees of freedom = $3n - 5$

Total number of stretching vibrations = $n - 1$

Total number of bending vibration = $2n - 4$

Degree of freedom which describe **rotation** = 2

Degree of freedom which describe **translational motion** = 3

For example: CO_2

Number of atoms (n) = 3

Total degree of freedom = $3n = 3 \times 3 = 9$

Translational = 3

Rotational = 2

Vibrational = $9 - 3 - 2 = 4$

For CO_2 , the theoretical number of fundamental band should be equal to 4.

Non-linear molecule:

In case of non-linear molecule, three translational and three rotational degree of freedom because rotation about all the three axes will result in a change in the position of the atoms.

Thus for non-linear molecule:

Total degree of freedom = $3n$

Translational = 3

Rotational = 3

Vibrational = $3n - 3 - 3$
= $3n - 6$

Total number of Vibrational degrees of freedom = $3n - 6$

Total number of stretching vibrations = $n - 1$

Total number of bending vibration = $2n - 5$

Degree of freedom which describe **rotation** = 3

Degree of freedom which describe **translational motion** = 3

For example:

C_6H_6 (benzene)

Number of atoms (n) = 12

Total degree of freedom = $3 \times 12 = 36$

Translational = 3

Rotational = 3

Vibrational = $36 - 3 - 3$
= 30

For benzene, theoretically there should be 30, fundamental bands in the IR spectra

.

It has been observed that theoretical numbers of fundamental vibrations are seldom obtained, it is because of the following reasons: -

(i) Fundamental vibrations that fall outside the region under investigation
i.e., 2.5 to 15 μ .

(ii) Fundamental vibrations those are too weak to be observed as bands.

(iii) Fundamental vibrations that are so close that they overlap
i.e., degenerate vibrations.

(iv) Certain Vibrational bands donot appear for want of the required change in
The dipole moment in a molecule

(v) They may appear some additional bands called combination bands,
difference bands and overtones.

Fundamental bands:

Transition from the ground state ($v=0$) to the first excited state ($v=1$) absorb light strongly and give rise to intensive bands called the fundamental bands. The energy difference can be represented as:

$$\begin{aligned}\Delta E_{\text{vib}} &= E_{\text{vib}}(v=1) - E_{\text{vib}}(v=0) \\ &= (1+1/2) h\nu - (0+1/2) h\nu \\ &= 3/2 h\nu - 1/2 h\nu \\ \Delta E_{\text{vib}} &= h\nu\end{aligned}$$

Overtone:

Transitions from the ground state ($v=0$) to second excited state ($v=2$) with the absorption of IR radiation give rise to weak bands, called overtones. The energy of first overtone is given by:

$$\begin{aligned}\Delta E_{\text{vib}} &= \Delta E_{\text{vib}(v=2)} - \Delta E_{\text{vib}(v=0)} \\ &= (2+1/2)h\nu - (0+1/2) h\nu \\ &= 2 h\nu\end{aligned}$$

Q. For methane molecule, calculate the number of Vibrational degree of freedom. How many of these would be bending modes ?

Solutions: Methane (CH_4) is a tetrahedral (non-linear) molecule containing 5 atoms.

Total number of Vibrational degrees of freedom $= 3n-6 = 3 \times 5 - 6 = \mathbf{9}$

Total number of bending vibration $= 2n-5 = 2 \times 5 - 5 = \mathbf{5}$

Applications of IR spectroscopy: -

1. Identification of functional groups and determination of structure:

Different functional group in a polyatomic molecule absorb at characteristic frequencies this phenomenon has been helpful in the determination of structure of organic compound. Structure determination by IR is possible because it gives information regarding molecular symmetry, band length, dipole moment etc.

The absorption frequencies of some typical functional group are given below: -

For example: Alcohol

O-H stretching: $3590 - 3650 \text{ cm}^{-1}$

O-H Bending: $1050 - 1150 \text{ cm}^{-1}$

2. Determination of purity:

IR is used for the detection of impurities by Q.C department of many chemical industries. In presence of impurities extra absorption bands will appear in the spectrum, reduces the sharpness of individual bands.

3. To distinguish between intra and inter molecular H- bonding

Generally, it is not possible to distinguish between intra and inter molecular H-bonding, but it can be possible by IR spectra of the compound at different concentrations. As the concentration is increased, the absorption band, due to intermolecular H- bonding increases, while those due to intramolecular H-bonding remain unchanged.

O-H stretching for H-bond at $3590-3650 \text{ cm}^{-1}$

4. Identification of an unknown compound

This is done by fingerprint region/technique which involves matching the IR spectrum of unknown compound with that a known compound.

5. Study of reaction kinetics

In a chemical reaction some bonds break producing new bonds. Accordingly certain bonds in the IR spectrum would disappear and new bands will appear in the course of time. Thus progress of organic reaction can be studied.

Comparison of UV-VIS and IR spectroscopy:

S. No.	UV-visible spectroscopy	IR spectroscopy
1.	The region of UV-Vis spectroscopy extends from 10-800 nm.	The region of IR spectroscopy extends from 800-20000 nm.
2.	As UV-Vis radiation possesses more energy, they are strong and so can cause electronic excitation.	IR radiations are weak and so cannot produce electronic excitation. They cause bending and stretching vibration of organic bonds.
3.	Unsaturated and especially conjugated groups only absorb in this region.	All organic bonds and groups absorb in this region.
4.	Uv- absorption spectrum exhibits broad absorption bands.	IR spectrum exhibits sharp peaks.
5.	This spectroscopy gives an idea about the presence of carbonyl group and a conjugated system.	This spectroscopy gives an idea about nature of the functional group in the molecule.
6.	The spectra are usually expressed in the terms of wavelength in nm units.	The spectra are usually expressed in terms of frequencies in wave number (ν) unit.

Important questions:

1. Describe in detail the principle and application of IR spectroscopy.
2. What changes are responsible for a molecule to absorb radiation in the IR region.
3. What type of molecules are IR active?
4. Explain why CO_2 molecule IR active although it has zero dipole moment?
5. Name the type of vibration exhibited by polyatomic molecules.
6. How many degree of freedom of motion are possessed by linear and non linear polyatomic molecules.
7. IR spectra is often characterised as molecular finger prints, comments on it.
8. What is the origin of Vibrational spectroscopy? Discuss the Vibrational spectra of diatomic molecules.
9. A molecule like HCl can undergo stretching vibration only, while a molecule like CO_2 can undergo stretching as well as bending vibration, explain.
10. Differentiate between IR and FT-IR.
11. Describe the IR analysis of (a) a liquid (b) a solution and (c) a solid.
12. Compare the relative wave numbers for stretching and bending vibrations.
13. Select the diatomic molecules that do not absorb in the IR from the following: HCl, N_2 , ClBr, O_2 , and H_2 and why?
14. Compare the relative stretching frequencies for C-C, C=C, and C \equiv C bonds.
15. How does conjugation affect the stretching frequencies of C=O in a carbonyl compound?

Introduction/Theory/Principal

Nuclear Magnetic Resonance (NMR) Spectroscopy is a commonly used technique in analytical chemistry for quantitative analysis of mixtures having identified compounds, to determine the content and pureness of a compound and derive its molecular libraries or conclude the basic structure directly. On deriving the basic structure, NMR can then be used to determine molecular conformation in solution as well as to analyze the physical properties at the molecular level such as phase changes, conformational exchange, diffusion and solubility. NMR encompasses (constitute) the interaction of oscillating magnetic field of electromagnetic radiation and the magnetic field of the hydrogen nucleus or other nuclei when placed in an external static magnetic field.

- NMR (^1H NMR and ^{13}C NMR) spectroscopy depends on the absorption of energy when the nucleus of an atom is excited from its lowest energy state to the next higher one. ^1H NMR spectrum tells us about the environments of various hydrogen in a molecule; ^{13}C NMR spectrum does the same for the carbon atom. Separately and together ^1H NMR and ^{13}C NMR take towards determining a substance's molecular structure.
- NMR performing experiments on the *nuclei* of the atoms not on the electrons. It has been found experimentally that any nucleus consisting of either an odd number of protons or an odd number of neutrons or both has the property of nuclear spin. e.g., ^1H , ^{13}C , ^{19}F nuclei possess spin.
- NMR differs from other spectroscopy because the different energy state (nuclear spin states) exists only in presence of magnetic field.

Principal of NMR:

Many nuclei have spin and all are electrically charged. In presence of an external magnetic field, energy transfer is possible between the base energy level and higher energy level at a wavelength that corresponds to radio frequencies. When the spin return to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in numerous ways and processed to yield an NMR spectrum for the concerned nucleus.

Q. What property of certain atomic nuclei is involved in NMR Spectroscopy?

Ans: The atomic numbers “spin” state. Nuclei of ^1H and ^{13}C can exist in either of two spin state $+1/2$ or $-1/2$ and are called NMR active nuclei.

Q. What property must a nucleus have to exhibit NMR? Which of the following atoms are not NMR active ^{12}C , ^{16}O , ^{14}N , ^2H (deuterium), ^{15}N , ^{19}F , ^{31}P , ^{13}C , ^{32}S ?

Ans: The nucleus must have an odd number of proton and/or neutron.

Inactive atoms have even number of protons and neutrons. These are ^{12}C (6p, 6n), ^{16}O (8p, 8n) and ^{32}S (16p, 16n)

Interpretation of the NMR Spectra:

The simplest types of NMR spectra are those which involve absorption of radiation by hydrogen nuclei only. This is because the hydrogen atom has the smallest spin quantum number i.e., $\frac{1}{2}$. Hence it would require only one absorption frequency.

The recorded NMR of a substance gives the following information:

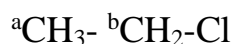
(i) Number of signals: *The number of signals in the spectrum indicates about the various types of protons present in substance e.g. if two peaks are seen in NMR spectrum of a compound, then these are two types of proton in a compound.*

(ii) Position of Peaks: *The position of peaks indicates about electronic environment of each kind of proton.* For example, if signal for a proton downfield (high δ -value) then the proton is attached to carbon which has electronegative atom.

(iii) Intensities of peaks: The intensities (relative height) of signal indicate number of protons of each kind. For example, if two signals are recorded on NMR spectrum and ratio of height of signal is 3:2, then there are 3 protons of one type and 2 protons of the other type.

(iv) Splitting of signals in the form of multiplets:

The splitting of signals indicates number of protons on the neighbouring carbon. If the protons have same chemical shift, then are called *chemical shift equivalents* and are designated by the same letter. In other words, protons with the same environment absorb at the same applied field strength and such a set of protons are equivalent protons. The equivalent protons can be judged by looking at the molecular structure of a molecule. For example, ethyl chloride has two types of protons labeled a and b.



Number of Signals (Equivalent and Non-equivalent Protons)

The number of signals in the NMR spectrum tells us how many different environments are there in a molecule. In other words, number of signals tells the number of different sets of equivalent protons in a molecule. Each signal corresponds to a set of equivalent protons.

“It may be noted that magnetically equivalent protons are chemically equivalent Protons”

(A) Example of compounds having only Equivalent Protons

(i) *Acetone molecule*: As all the six protons are in exactly similar environment. Thus, only **one signal** is observed when NMR spectrum of acetone is recorded.

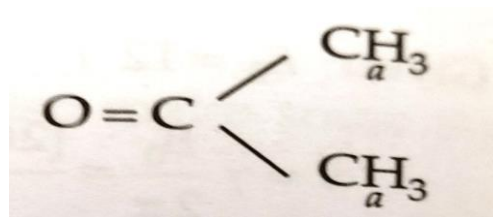


Fig: Acetone

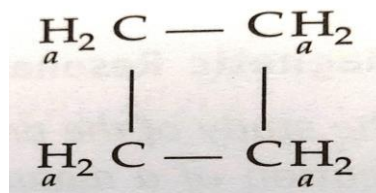
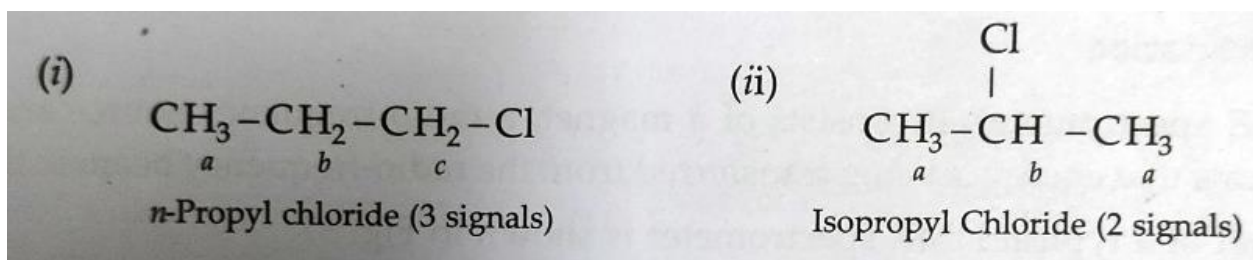


Fig: Cyclobutane

(ii) *Cyclobutane Molecule*: As can be seen from the stereo-chemical formula that all the 8 protons in Cyclobutane are positioned in structurally and chemically Equivalent areas, so they are equivalent protons. Thus, Cyclobutane also Shows only one signal in its NMR spectrum.

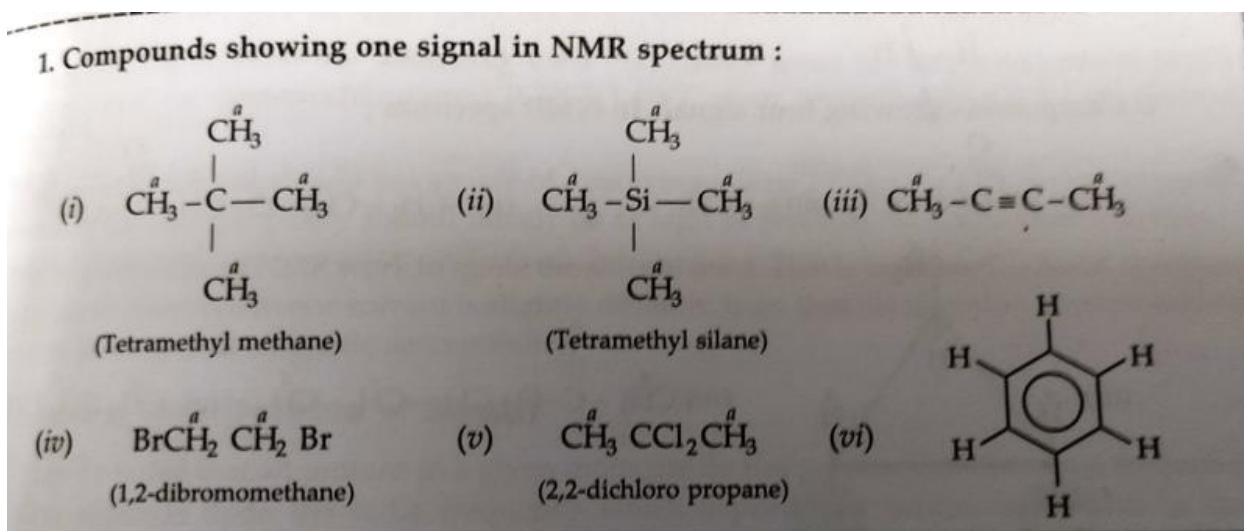
(B) Example of Compounds having both Equivalent as well as Non-equivalent Protons:

Consider the usefulness of NMR in distinguishing the n-propyl chloride from its isomer isopropyl chloride.

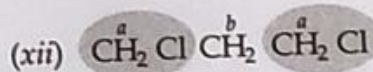
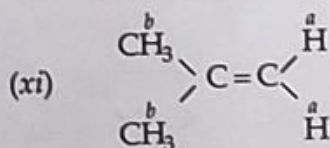
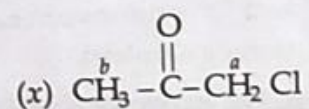
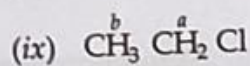
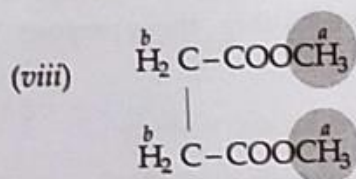
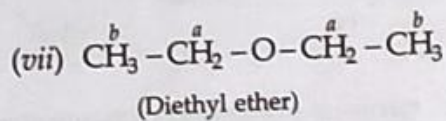
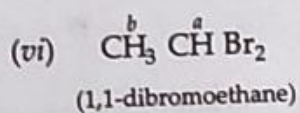
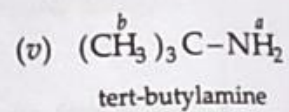
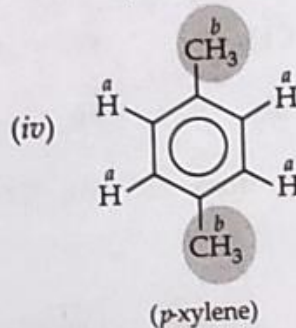
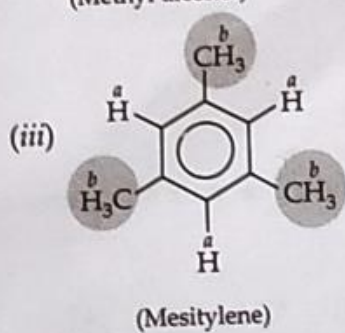
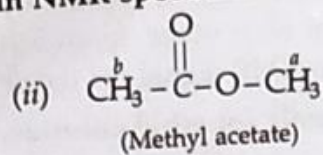
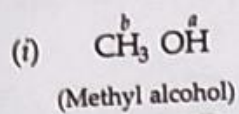


n-propyl chloride possesses three sets of non-equivalent protons and thus gives **three signals** in its NMR spectrum.

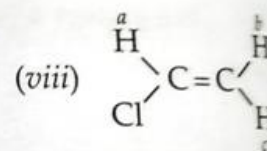
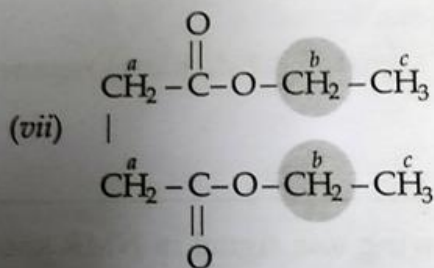
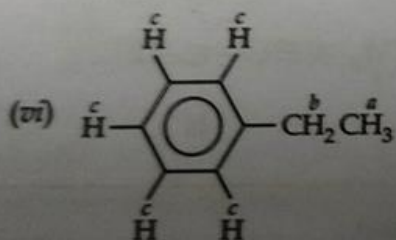
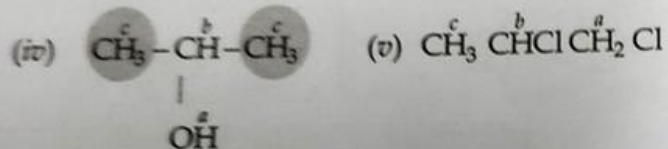
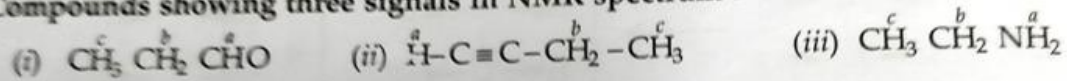
However, isopropyl chloride has two sets of equivalent protons and one non-equivalent proton. Thus, this compound gives **two NMR signals**.



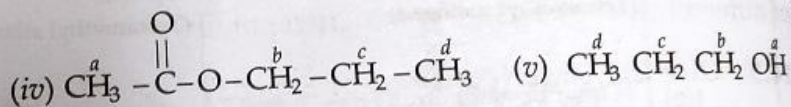
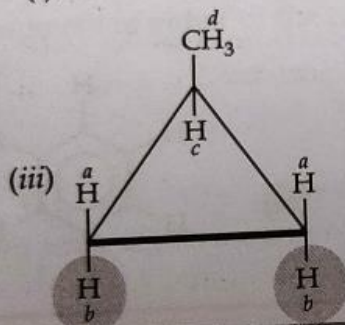
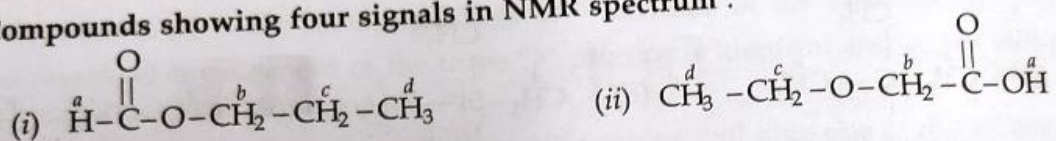
2. Compounds showing two signals in NMR spectrum :



3. Compounds showing three signals in NMR spectrum :



4. Compounds showing four signals in NMR spectrum :



Question: Predict the number of signals in the NMR spectrum of
 (i) $\text{CH}_3\text{-CH}_2\text{-OH}$ (ii) $\text{CH}_2\text{Cl-CHCl}_2$ (iii) $\text{CH}_3\text{-CH}_2\text{-CH}_3$

Answer:

S.No.	Molecule	Sets of non-equivalent protons	No. of signals in the NMR spectrum
(i)	$\text{CH}_3\text{-CH}_2\text{-OH}$ c b a	3	3
(ii)	$\text{CH}_2\text{Cl-CHCl}_2$ b a	2	2
(iii)	$\text{CH}_3\text{-CH}_2\text{-CH}_3$ b a b	2	2

Question: Predict the number of signals in the NMR spectrum of
 (i) $\text{CH}_3\text{-O-CH}_3$ (ii) $\text{CH}_3\text{COOCH}_3$ (iii) $\text{CH}_3\text{CH}_2\text{C}_6\text{H}_4\text{CH}_2\text{CH}_3$

Answer:

S.No.	Molecule	Sets of non-equivalent protons	No. of signals in the NMR spectrum
(i)	$\text{CH}_3\text{-O-CH}_3$ a a	1	1
(ii)	$\begin{array}{c} \text{O} \\ \\ \text{CH}_3\text{-C-O-CH}_3 \end{array}$ a b	2	2
(iii)	$\begin{array}{c} \text{c} \quad \text{c} \\ \text{H} \quad \text{H} \\ \quad \\ \text{CH}_3\text{-CH}_2\text{-} \text{C}_6\text{H}_4 \text{-CH}_2\text{-CH}_3 \\ \quad \\ \text{H} \quad \text{H} \\ \text{c} \quad \text{c} \end{array}$	3	3

Position of Signals (Chemical Shift)

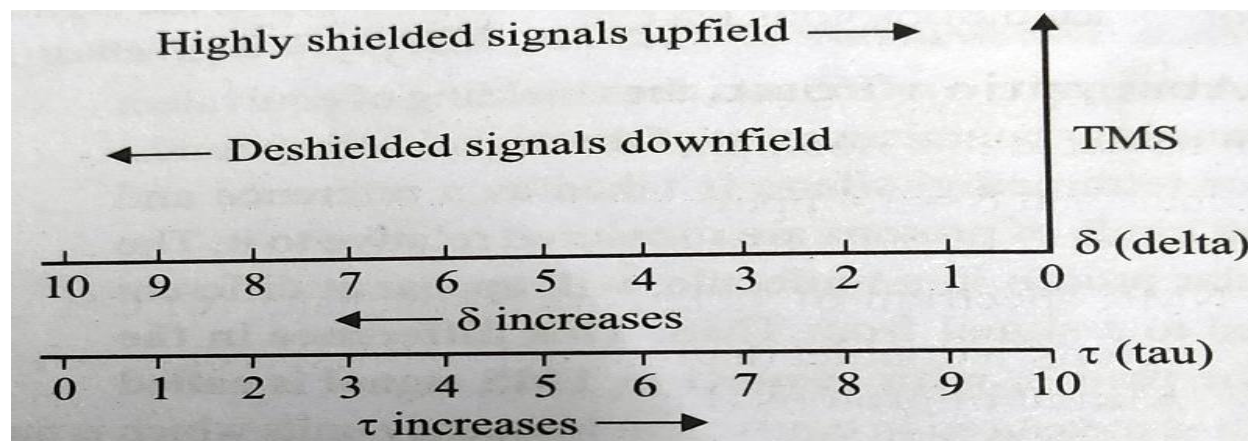
“The difference in the absorption of the proton with respect to TMS (Tetra methyl Silane) signal is called chemical shift (δ -value).

The chemical shift arises due to shielding and deshielding of the proton by the electrons, shielding shifts the absorptions upfield (low δ -value) and deshielding shifts the absorption position to the downfield (high δ -value).

The chemical shift denoted by δ
(δ is dimensionless and expressed in ppm)

$$\delta = \frac{\nu_{\text{sample}} - \nu_{\text{reference}}}{\text{operating frequency in megahertz (MHz)}}$$
$$= \frac{\Delta \nu}{\text{operating frequency in megahertz (MHz)}}$$

Where $\Delta \nu$ is the frequency shift. The value δ is expressed in parts per million (ppm). The most chemical shifts have δ values between 0 and 10. In the τ (tau) scale, signal for the standard reference, TMS, is taken as 10ppm. [$\tau = 10 - \delta$].



Tetra methyl silane (TMS) is used as the standard because the protons are very rich and the resulting absorption peak is upfield from most other protons.

Factors Influencing Chemical Shift:

1. Inductive effect
2. Vanderwaal's deshielding
3. Hydrogen bonding
4. Anisotropic effect

Question: Why is TMS taken as reference standard in NMR spectroscopy?**Answer:**

Characteristics of TMS that make it a molecule of choice to act as a reference:

- (a) TMS has 12 protons in magnetically equivalent positions so it gives intense sharp signal even at low concentration.
- (b) Due to the (+I) effect, silicon pushes electrons into the methyl groups of TMS. Due to the greater electron density, TMS protons come to resonance at low Frequency (defined as zero). In other words, precessional frequency (precessional frequency in NMR refers to the rate of precession of the magnetic moment of the proton around the external magnetic field.) of TMS proton is zero due to the powerful shielding effect.
- (c) TMS has low boiling point (26.5°C) so it can easily removed from recoverable sample of an organic compound.
- (d) It is chemically inert and soluble in organic solvent.

Splitting of Signals: (n+1) peak:

In an NMR spectrum each signal represents one kind or one set of proton in a molecule. Signal splitting is a phenomenon that arises from magnetic influences of hydrogen on atoms adjacent to those bearing the hydrogen atoms causing the signal to be considered.

It is found that in certain molecules, a single peak (singlet) is not observed, but instead a multiple (group of peaks) is observed.

Consider a simple molecule $^a\text{CH}_3\text{-}^b\text{CH}_2\text{-Br}$

This molecule has two kinds of proton in it and thus, two signals are expected in its NMR spectrum

- (a) a Kind of protons(CH_3) : These three equivalent proton see two($-\text{CH}_2$) vicinal (adjacent) non equivalent protons, there NMR signal split into $2+1 = 3$ peaks (triplet i.e , a group of three peak)

Signals and their Absorption position: (i) a three portion triplet at $\delta = 1.65\text{ppm}$

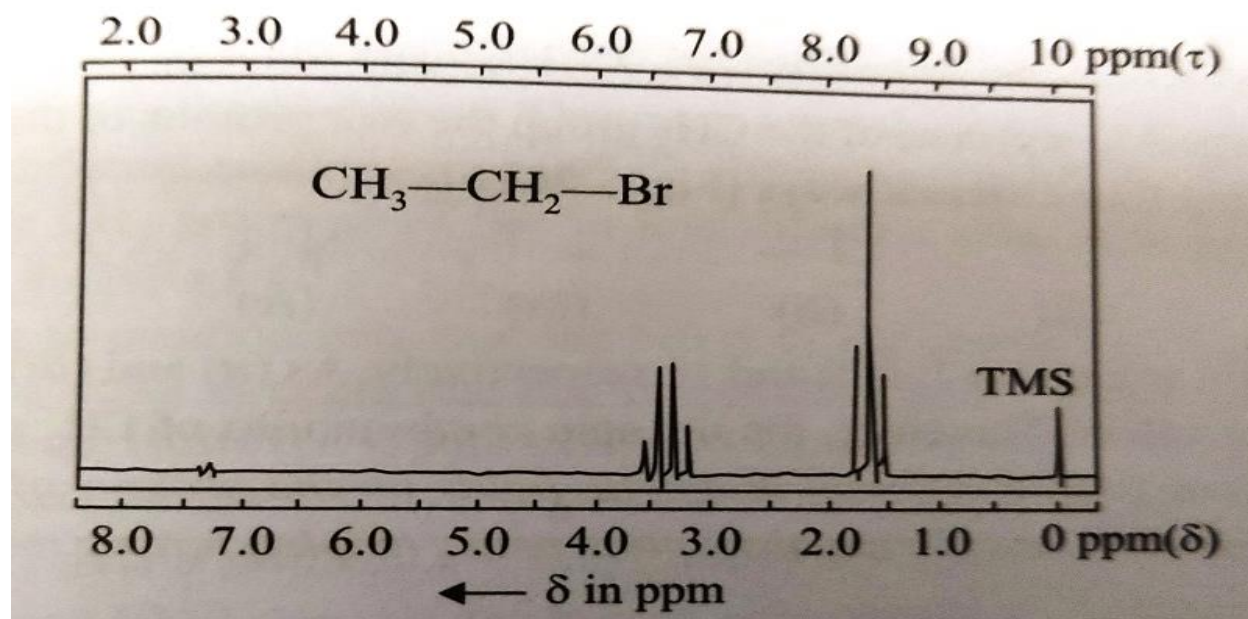
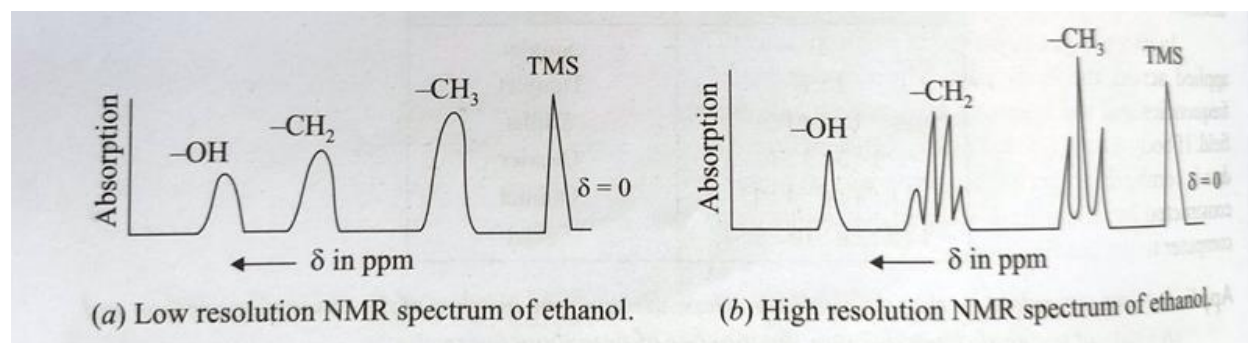


Fig: NMR Spectrum of ethyl bromide

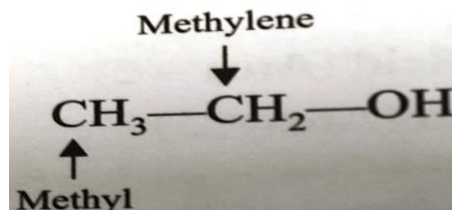
- (b) b Kind of protons(CH_2) : These three equivalent proton see two($-\text{CH}_3$) vicinal (adjacent) non equivalent protons, there NMR signal split into $3+1 = 4$ peaks (quartet i.e., a group of four peaks)

Signals and their Absorption position: (i) a two portion quartet at $\delta = 3.4\text{ppm}$

Spin-Spin Coupling:



Consider the structure of ethanol



The ¹H NMR spectrum of ethanol (above) shows the methyl peak has been split into three peaks (*a triplet*) and the methylene peak has been split into four peaks (*a quartet*). This occurs because there is a small interaction (*coupling*) between the two groups of protons. The spacing between the peaks of the methyl triplet are equal to the spacing between the peaks of the methylene quartet. This spacing is measured in Hertz and is called the *coupling constant* (*J*).

To see why the methyl peak is split into a triplet, let's look at the methylene protons. There are two of them, and each can have one of two possible orientations (aligned with or opposed against the applied field). This gives a total four possible state.

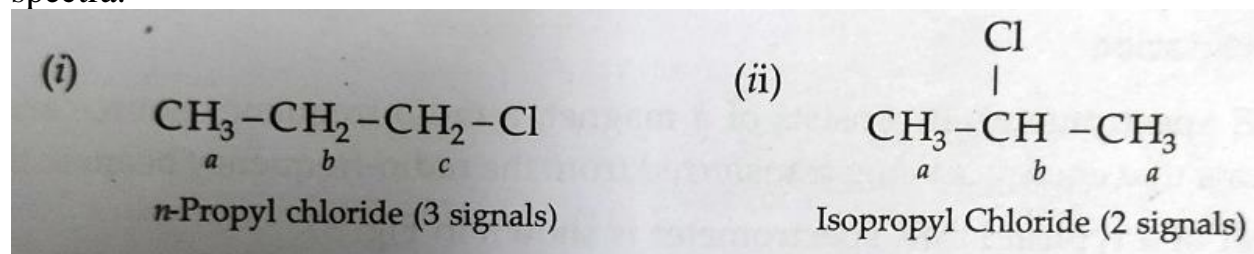
In the first possible combination, spins are paired and opposed to the field. This has the effect of reducing the field experienced by the methyl protons; thereafter a slight higher field is needed to bring them resonance, resulting in an up field shift. Neither combination of spins opposed to each other has an effect on the methyl peak. The spins paired in the direction of the field produce downfield shifts. Hence, the methyl peak is split into three, with the ratio of areas 1:2:1. Similarly, the effect of the methyl protons on the methylene protons is such that there are eight possible spin combinations for the three methyl protons.

Out of these groups, there are two groups of three magnetically equivalent combinations. The methylene peak is split into a quartet. The areas of the peak in the quarter have the ratio 1:3:3:1. In a *first –order* spectrum (where the chemical shift between interacting groups is much larger than their coupling constant), interpretation of splitting patterns is quite straight forward.

Application of NMR

1. Identification of structural Isomers:

The distinction between the following isomers can be easily made from their nmr spectra.



2. Detection of Hydrogen bonding:

Intermolecular H-bonding shifts the absorption for a concerned proton downfield. The extent of H-bonding varies with the solvent concentration of the solution and the temperature, Intramolecular H-bonding also shifts the absorption downfield. The two types of H-bonding can be distinguished as the intramolecular H-bonding is not concentration dependent.

3. Detection of Electronegativity:

It is known that the presence of an electronegativity atom or group in the neighborhood of the proton causes deshielding and signal shifted downfield. Greater the electronegativity of the adjacent atom, smaller is the τ (tau) value of absorption for the concerned proton. Fluorine causes more downward shift as compared to oxygen and oxygen in turn causes more downward shift as compared to nitrogen and so on.

4. Detection of some double bond character due to resonance

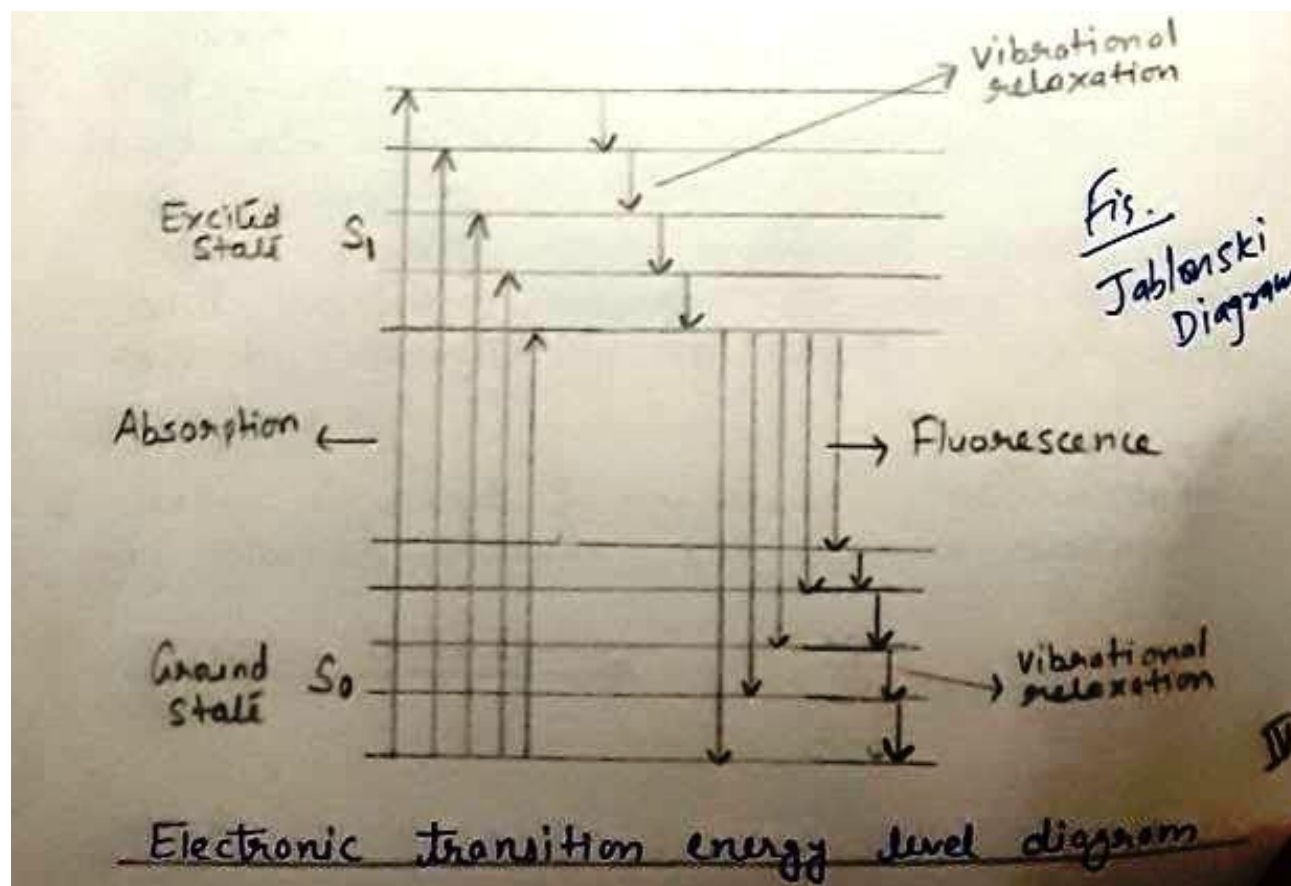
5. Importance in quantitative analysis

Fluorescence Spectroscopy

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Fluorescence is a spectrochemical method of analysis where the molecules are excited by irradiation at a certain wavelength and emit radiation of a different wavelength. The emission spectrum provides information for both qualitative and quantitative analysis.

When light of an appropriate wavelength is absorbed by a molecule, the electronic state of the molecule changes from the ground state to one of many Vibrational levels in one of the excited electronic states. The excited electronic state is usually the first excited singlet states S_1 . Once the molecule is in the excited state, relaxation can occur via several processes. Fluorescence is one of these processes and results in the emission of light.



If the molecule is in a high Vibrational state after electronic excitation, then excess Vibrational energy may be lost by intermolecular collisions.

The Vibrational energy is converted to kinetic energy and appears as heat in the sample. Such transfer between energy levels is referred to as radiation less.

When the excited molecule has reached a lower Vibrational state, it may then emit radiation and revert to the ground state, the radiation emitted are called the fluorescence spectrum, is normally of lower frequency than that of the initial absorption, but under certain conditions, it may be of higher frequency. The time between initial absorption and return to the ground state is very small, of the order of 10^{-8} s.

Applications of fluorescence in medicine:

There are many diagnostic techniques and methods available for diagnosis of medically important micro organisms like bacteria, virus, fungi and parasites. But almost all these techniques and methods have some limitations or inconvenience. Most of these techniques are laborious, time consuming negative results. It warrants the need of diagnostic technique which can overcome these limitations and problems. At present there is emerging trend to use fluorescence spectroscopy as a diagnostic as well as research tool in many field of medical sciences.

1. Diagnostic technique for microorganisms:

Fluorescence emission spectra derived from auto fluorescence property of many medically important bacteria make it possible to distinguish between various bacterial species and also enable to classify the bacteria into genus, species and groups.

Virus particles can monitored inside cells and various processes of viral infection can be detected by means of fluorescence spectroscopy.

2. Laser induced fluorescence spectroscopy of human tissues for cancer diagnosis.

3. DNA sequencing: -it is a process of determining the precise order of nucleotide within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases adenine (A), guanine (G), Cytosine(C) and Thymine (T)

Following the development of fluorescence based sequencing method with a DNA sequence, DNA sequencing has become easier and order of magnitude faster.

Difference between fluorescence and Raman Spectroscopy

S.N	Fluorescence Spectroscopy	Raman Spectroscopy
1	Radiation must be absorbed by the molecule and excited electronic state is formed	Energy is transferred from radiation to molecule but no excited electronic state is formed.
2	The exciting radiation is equivalent to the energy difference between electronic states.	The exciting radiation can be of any frequency except that which would induce an electronic transition.
3	In fluorescence spectroscopy, absorption occurs	In Raman spectroscopy Scattering occurs

Surface characteristics of a material means the properties associated with its surface. Typically measurement of surface area, surface roughness, pore size and reflectivity constitute surface characteristics. Information on surface characteristics is very important for the possible applications of surfaces as *semiconductors*, *heterogeneous catalysts* and also in *biological research*.

Surface area is an attribute that is used by catalyst manufacturers and users to monitor the activity and stability of a catalyst. There are different methods used to measure different surface properties. Most methods are based on isothermal adsorption of nitrogen.

These techniques could be classified into two categories:

1. Classical Methods
2. Instrumental Methods/
Microscopic methods of characterisation

Classical Methods may be divided into (A) Langmuir Model (B) BET equation (Brunauer, Emmett and Teller)

Microscopic methods of characterisation

Even microscopic methods make use of more or less the same principles but these methods are generated by bombarding the surface with *high-energy electron*, which are then multiplied several times so as to produce a magnified image of the surface on the fluorescent screen.

Few methods like STM (Scanning Tunneling Microscope) , AFM (Atomic Force Microscope) work based on *quantum mechanical principle*.

1. Scanning Electron Microscopy (SEM)
2. Transmission Electron Microscopy (TEM)

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy has great depth of focus. It transmits a beam which focuses at a specific point and scans each line of the surface of the sample in a rectangular raster pattern and provides a three-dimensional image of high resolution. These images provide information on:

- (A) Topography
- (B) Morphology
- (C) Composition

In this technique, electrons can penetrate deeply into a sample and give averaged chemical information with depth.

Instrumentation:

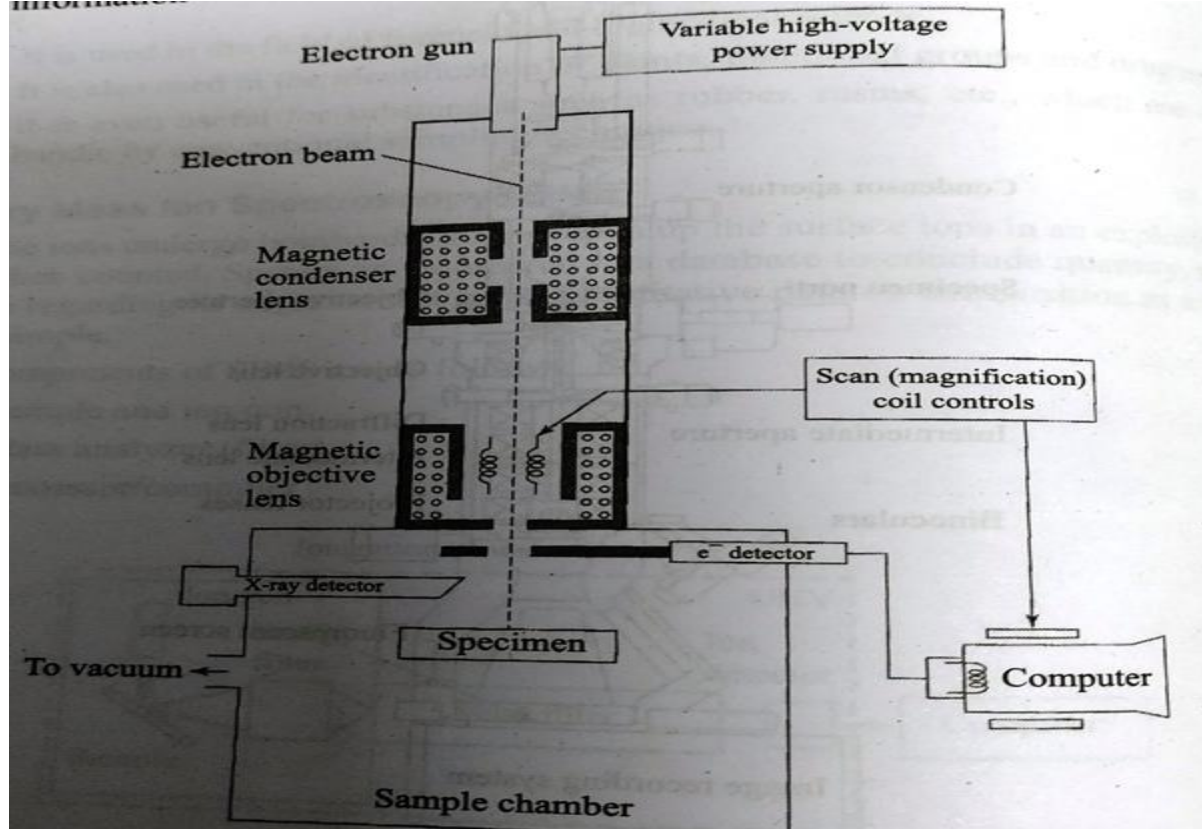


Fig: Schematic diagram of SEM

Application of SEM:

1. SEM is used to research, control the quality and analyze the failure in the field of materials science.
2. It is used in the field of criminal and forensic investigations to reveal the evidence and develop further forensic insight.
3. SEM is used in the field of biological science for insects, animal tissues, bacteria and viruses.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a microscopy technique. This technique transmits a broad static beam of electrons through a specimen which can be either an ultrathin section which is less than 100nm thick or a suspension on a grid, to form an image.

Instrumentation:

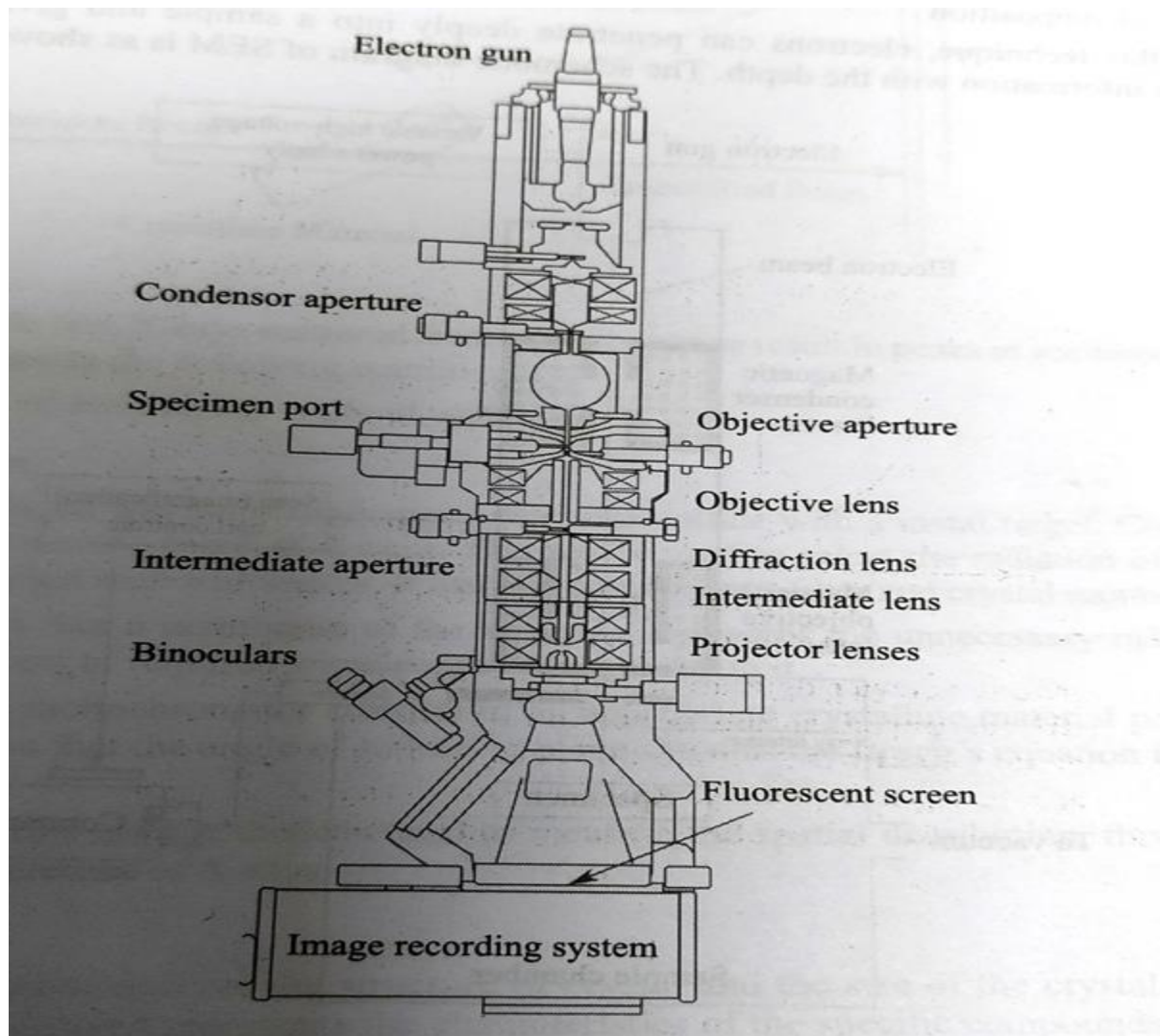


Fig: Schematic diagram of SEM

Application of TEM:

1. TEM is used to examine the configuration, properties and composition of a sample in detail.
2. It is also used to study general biological and medical specimens. It finds extensive use in the field of materials science, geology and environmental science.
3. It also produces characteristic X-rays from samples for microanalysis.

Spectroscopy Questions

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Q.1. Indicate the regions of the electromagnetic spectrum in which the following photons will lie.

- (a) $\lambda = 600\text{nm}$ (b) $\nu = 10^6\text{Hz}$ (c) $\lambda = 2000\text{cm}^{-1}$ (d) $\nu = 40\text{cm}^{-1}$ (e) $\lambda = 200\text{nm}$

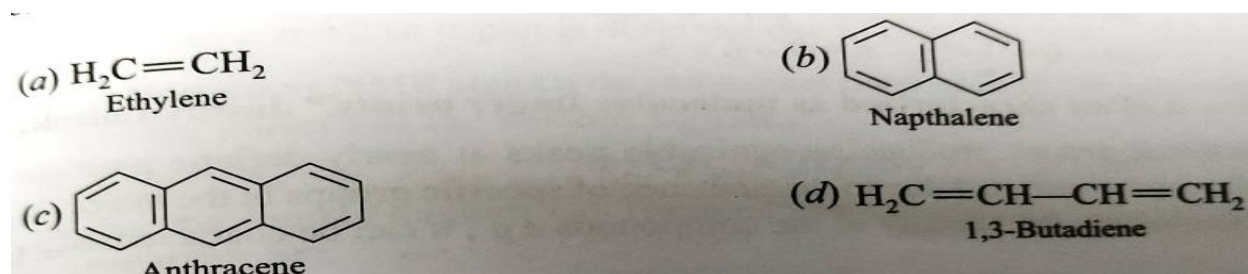
Solution: (a) Visible (b) Radio frequency (c) IR (d) Microwave (e) UV

Q.2. In which region of the spectrum the electronic spectra occur? What is its spectral range?

Answer: The electronic spectra occur in visible and ultra violet regions of the electromagnetic spectrum. The spectral range for visible region is 12500 to 25000 cm^{-1} and UV region is 25000 to 70000 cm^{-1}

Q.3. Arrange the following compounds in the increasing order of their UV absorption maxima. (a) Ethylene (b) Naphthalene (c) Anthracene (d) 1,3-Butadiene

Answer: The structure of the given compounds are:-



The absorption maxima (λ_{max}) are directly proportional to the extent of conjugation. Therefore, the increasing order of absorption maxima of the given compound would be:

(a) < (d) < (b) < (c)

Q.4. Most of absorption bands in the UV-Visible spectra are very broad. Give reason.

Answer:

UV-visible spectra involve transitions between electronic energy level which are associated with Vibrational and rotational energy levels. The electronic transition may occur from any of the several Vibrational and rotational states of one electronic level to any of the various corresponding states of higher level. Since a large number of transitions are possible, so a large number of absorption will take place corresponding to different wavelengths. Hence, spectra are very broad.

Q.5. what kind of molecules shows IR spectra?

Answer:

Molecules must possess permanent dipole moment e.g., CO, NO, CN etc . for a diatomic molecules if they do not have a permanent dipole moment then these molecules during vibration must show a change in the dipole moment. For example- O₂ , N₂ , H₂ are IR inactive because they are not satisfying either of the above conditions.

Q.6. Calculate the normal mode of vibration of the following molecules-

(i) CH₄ (ii) C₂H₂ (iii) H₂S (iv) HCl (v) C₆H₆ (vi) SO₂

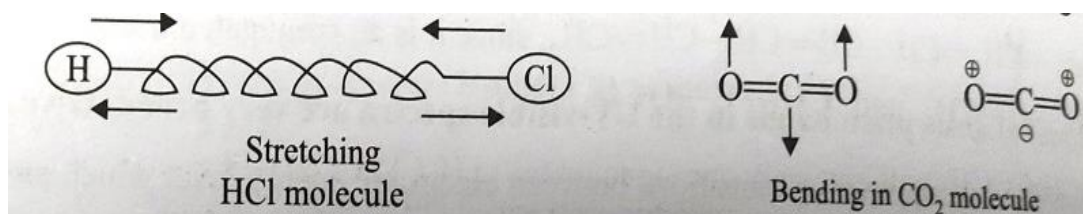
Answer:

S.No	Molecule	Vibrational degree of freedom
(i)	CH ₄	$3n-6=3 \times 5-6=9$,where n –number of atoms (non-linear molecule)
(ii)	C ₂ H ₂	$3n-5=3 \times 4-5=7$ (linear molecule)
(iii)	H ₂ S	$3n-6=3 \times 3-6=3$ (non-linear molecule)
(iv)	HCl	$3n-5=3 \times 2-5=1$ (linear molecule)
(v)	C ₆ H ₆	$3n-6=3 \times 12-6=30$ (non-linear molecule)
(vi)	SO ₂	$3n-6=3 \times 3-6=3$ (non-linear molecule)

Q.7. Molecule like HCl can undergo stretching vibration only; while CO₂ molecule can Undergo Stretching as well as bending vibration. Why?

Answer:

HCl molecule may regarded as spring, whose atom can undergo back and forth vibrations (i.e., stretching). On the other hand, CO₂ molecule can undergo symmetric as well as asymmetric stretching with respect to C-atom, also it undergo in-plane and out of plane bending.



Q.8. How many NMR signals do you expect from each of the following compounds, indicate also the splitting pattern of the various signals.

(a) CH₃OCH₃ (b) CH₃OCH₂CH₃ (c) CH₃CH₂OH

Answer:

(a) one signal (Singlet) (b) Three signals (singlet, quartet, triplet) and
(c) Three signals (singlet, quartet, triplet)

- Q.9. What is molecular spectroscopy? How does it differ from atomic spectroscopy?
- Q.10. Define the following terms (short notes): (i) Bathochromic shift
(ii) Hypochromic shift (iii) Chromophore (iv) Hyperchromic effect.
- Q.11. what do you understand by nuclear spin? When will a nuclei show nuclear Magnetic resonance.
- Q.12. Describe in detail the principle and application of IR spectroscopy.
- Q.13. what changes are responsible for a molecule to absorb radiation in the IR region.
- Q.14. What types of molecules are IR active? Explain why CO_2 molecule IR active although it has zero dipole moment?
- Q.15. Name the type of vibration exhibited by polyatomic molecules. How many degree of freedom of motion are possessed by linear and non linear polyatomic molecules.
- Q.16. IR spectra is often characterised as molecular finger prints, comments on it.
- Q.17. What is the origin of Vibrational spectroscopy? Discuss the Vibrational spectra of diatomic molecules.
- Q.17. A molecule like HCl can undergo stretching vibration only, while a molecule like CO_2 can undergo stretching as well as bending vibration, explain.
- Q.18. Compare the relative wave numbers for stretching and bending vibrations. Select the diatomic molecules that donot absorb in the IR from the following: HCl, N_2 , O_2 , and H_2 and why?
- Q.19. write principle and application of NMR spectroscopy. What information obtained from nmr absorption peaks?

- Q.20. What do you understand by position of signals in nmr spectrum ? How many signals are expected in each of the following compounds :- propane, isobutane , ethanol.
- Q.21. what is meant by term chemical shift. Write with examples the shielding and the deshielding effects involved in nmr spectroscopy. Describe with examples the various factors which affect the magnitude of the chemical shift.
- Q.22. what do you understand by the term splitting of signals? Explain with Examples.
- Q.23. Why TMS used as standard reference.
- Q.24. Write notes on Scanning Electron Microscopy (SEM).
- Q.25. Write notes on Transmission Electron Microscopy (TEM).