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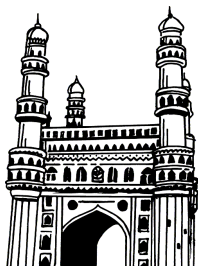

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UNIT - I

(Molecular Spectroscopy)

S5-E-A-I: Introduction to electromagnetic radiation, interaction of electromagnetic radiations with molecules, various types of molecular spectra.

Rotational Spectroscopy (Microwave spectroscopy)

Rotational axis, moment of inertia, classification of molecules (based on moment of inertia), rotational energies, selection rules (No derivation), determination of bond length of rigid diatomic molecules eg. HCl.

Infra Red Spectroscopy

Energy levels of simple harmonic oscillator, molecular vibration spectrum, selection rules. Determination of force constant (Problems). Qualitative relation of force constant to bond energies. Anharmonic motion of real molecules and energy levels. Modes of vibrations in polyatomic molecules. Characteristic absorption bands of various functional groups. Finger print nature of infrared spectrum.

Electronic Spectroscopy

Bonding and antibonding molecular orbitals,electronic energy levels of molecules (σ, π, n), types of electronic transitions: σ-σ^*, n-σ^*, n-π^*, π-π^* with suitable examples. Selection rules, Terminology of chromophore, auxochrome, bathochromic and hypsochromic shifts.

Absorption of characteristics of chromophoses: diene, enone and aromatic chromophores. Representation of UV-visible spectra. General features of absorption – spectroscopy, transmittance, absorbance, and molar absorptivity. Beer Lambert's law and its limitations.

S5-G-2: MOLECULAR SPECTROSCOPY

Q1. Explain the interaction of electromagnetic radiation with molecules.

Ans : **(Imp.)**

Electro Magnetic Radiation

Electromagnetic radiation is a form of energy which is transmitted through space at a constant velocity of 3×10^8 ms^{-1} electromagnetic radiations have dual characterization i.e., both particle and wave nature.

Electromagnetic radiations travel in the form of waves. These wave motion consists of oscillating electric and magnetic field directed perpendicular to each other and perpendicular.

Electromagnetic Spectrum

The region due to arrangement of different EMR in either increasing or decreasing order of wavelength is called electromagnetic spectrum (OR).

For spectroscopic purposes EMS electromagnetic spectrum is considered to be consisting of the region of radiant energy ranging from wavelength of 10 meters to 1×10^2 centimeters electromagnetic spectrum consists following regions.

1. γ-rays region
2. x-ray region
3. Visible and UV region
4. Infrared region
5. Microwave region
6. Radio frequency region.

Table Different regions of the electromagnetic spectrum

S.No.	Type of Radiation	Wave length(λ)	Wave number($\bar{\nu}$)	Type of molecular spectrum expected
1.	Radio frequency	> 100 mm	$< 3 \times 10^9$ Hz	NMR
2.	Microwave	1 – 100 mm	10×0.1 cm^{-1}	Rotational
3.	Far IR	50 mμ to 1 mm	200 – 10 cm^{-1}	Vibrational and rotational
4.	IR	2.5 – 50 mμ	4000 – 667 cm	Vibrational (fundamental)
5.	Near IR	780 mm to 2.5 mμ	$(13 - 4)\ 10^3$ cm^{-1}	Vibrational (overtone)
6.	Visible	380 – 780 nm	$(2.6 - 13) \times 10^4$ cm^{-1}	Electronic
7.	Near UV	200 – 380 nm	$(5 - 2.6) \times 10^4$ cm^{-1}	Electronic
8.	Vacuum	10 – 200 nm	$(10^6$ to $5) \times 10^6$ cm^{-1}	Electronic
9.	X-rays	10 pm to 10 nm	$10^9 - 10^6$ cm^{-1}	Electronic (core orbitals)
10.	γ-rays	10^{-10} cm	10^{10} cm^{-1}	Mossbauer

Q2. Write types of molecular spectra.

Ans :

Types of Molecular Spectroscopy

Depending upon the region of electromagnetic spectrum utilized by the molecule that leading to change in internal energy or causing transition b/w different spin orientation of nucleus in magnetic field, molecular spectroscopy is classified into following types.

(OR)

Molecular spectroscopy is classified into various types depending on the type of radiation absorbs (or) utilized by the molecules.

1. Infrared Spectroscopy

Absorption of infrared radiation leads to vibrational transitions of the molecules. The energy range involved is 4000 to 667cm^{-1}.

2. Pure Rotational (Microwave) Spectroscopy

Absorption of radiation in microwave region is responsible for rotational transitions energy involved is in the range from 100 to 100^{-1}.

3. This branch of spectroscopy deals with the transitions b/w electronic energy levels of a molecule. This transitions are brought in a organic molecule due to the absorption of ultraviolet or visible radiation.

Energy level involved is

13000 to 26000 cm^{-1} - visible

26000 to 50000 cm^{-1} - near UV

50,000 to 106 cm^{-1} - vacuum UV

4. Nuclear Magnetic Resonance Spectroscopy ^{13}CNMR/PMR Spectroscopy

This branch of spectroscopy is concerned with the study of interaction of energy with spin active nuclei which is magnetically active.

If the nuclei involved is proton, then it is termed as PMR.

It the nuclei involved is ^{13}C then it is termed as ^{13}CNMR spectroscopy.

This spectroscopy measures the energy necessary to bring about transitions b/w energy levels by subjecting the nuclei to powerful magnetic field and simultaneously irradiating it with radio frequency.

5. Mass Spectrometry

This technique is based on the principle that molecules in the gaseous phase are converted into parent and daughter positive ions by bombarding with electron beams of 70ev.

Types of Molecular Spectroscopy

1. IR Spectroscopy

An instrumental method for detecting functional groups. Give information regarding functional groups present in the molecule like aldehyde ketone, carboxylic acid etc.

2. UV Spectroscopy

Organic chemists use UV-vis spectroscopy mainly for detecting the presence and elucidating the nature of the conjugated multiple bond are aromatic rings.

Eg: Benzene $CH_2 = CH_2$, $CH_2 = CH - CH = CH_2$

3. Proton Magnetic Resonance Spectroscopy (CPMR)

PMR spectroscopy involves nuclear magnetic resonances which depend on the magnetic property of nuclei. It gives information regarding number and type of magnetic nuclei (protons).

4. Electron Spin Resonance Spectroscopy (ESR)

It is given only by free radicals. It gives information regarding presence of free radicals.

5. Mass Spectroscopy

Mass spectrometry is not an absorption spectra because the molecule is not absorbing any energy from the EMR.

Mass spectroscopy gives information regarding mol. wt. mol. formula etc.

IR and Raman Spectra

Introduction

An instrumental method for detecting functional groups.

Infrared spectroscopy deals with the recording of absorption of radiations in the infrared region of the electromagnetic spectrum. The position of a given infrared absorption is expressed in microns (m) or more commonly in terms wave number (cm^{-1}). Since it is directly proportional to energy. The ordinary infrared region 2.5 – 15 (4000 – 66 cm^{-1}) is of practical use to organic chemists. The absorption of infrared radiation by a molecules occurs due to vibrational and rotational energy. When it is subjected to infrared irradiation. Thus, IR spectra are often called vibrational rotational spectra.

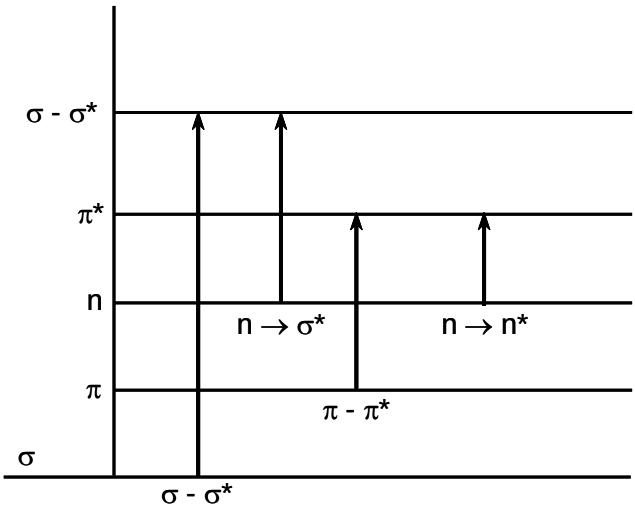
Q3. Explain the types of electronic transitions.

Ans : **(Imp.)**

According to molecular orbital theory when a molecule is excited by the absorption of energy (UV and visible light) its electrons are promoted from a bonding to an antibonding orbital. There are two types of electronic transitions.

(a) The transitions between bonding and antibonding orbitals.

(b) Transitions between non-bonding atomic orbitals and antibonding orbitals.



(a) Transitions b/w bonding and antibonding orbitals.

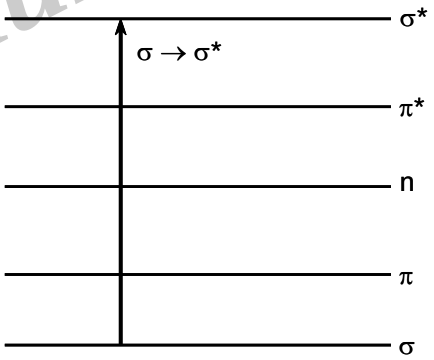
(i) $\sigma - \sigma^*$ (bonding o to antibonding σ^*)

(ii) $\pi \rightarrow \pi^*$ (bonding *n* to antibonding π)

(i) $\sigma - \sigma^*$ Transition (120 - 400 nm)

Excitation between bonding sigma and antibonding sigma orbitals ($\sigma - \sigma^*$) requires large energies corresponding to the UV region. Thus these transitions in saturated hydrocarbons containing only σ bonds remain transparent in the near UV or visible region.

Example: Methane, Propane, cyclohexane

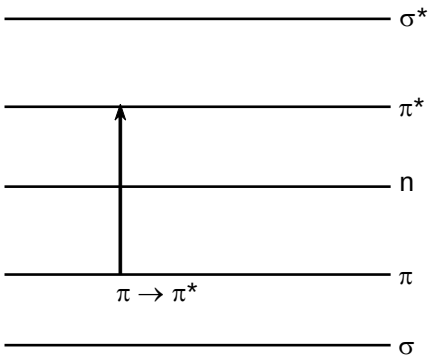


(ii) $\pi - \pi$ Transition

Transitions between bonding pi and antibonding pi are called $\pi - \pi^*$ transitions.

The transitions occur in compounds which contain double or triple bonds, aromatic rings and carbonyl group.

Ethylene absorbs at around 180 nm ig far or region where as butadiene absorbs at 217cm thus conjugated π electrons systems are readily excited $\left(\in x\ 1/\lambda\right)$.



(b) Transition between non bonding atomic orbitals to antibonding orbital.

(i) n – π* (non-bonding atomic orbital to antibonding n)

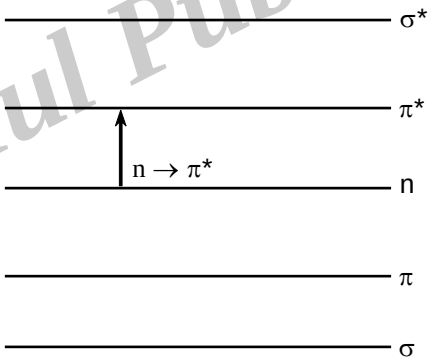
(ii) n – σ* (non bonding atomic orbital to antibonding σ)

(i) n - π* transition

Transition between non bonding atomic orbitals holding unshred pair of electrons and antibonding pi orbitals is known as n – π* transition.

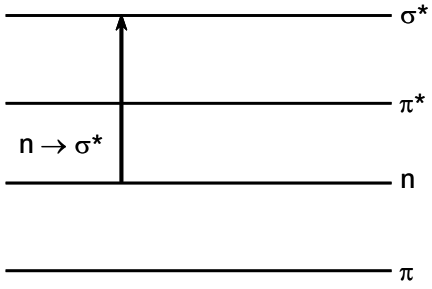
Non-bonding electrons are held loosely than s electrons and hence undergo transition at longer wavelength (because e = hv e = $\frac{hc}{\lambda}$ i.e., $\in \times \frac{1}{\lambda}$).

Example: Compounds containing double bonds involving hetro atom bearing unshared electrons. Aldehydes, ketones etc.



(iii) n – σ* Transition

Excitation of an electron in an unshared pair (non-bonding electrons) to an antibonding π* orbital is called n – σ* transition.

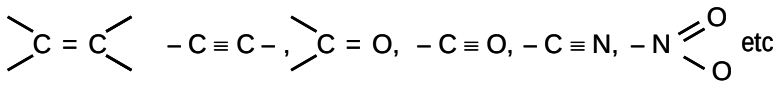


Q4. Define chromophores.

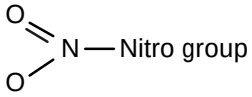
Ans : **(Imp.)**

It is defined as an unsaturated group covalently bonded unsaturated group that is responsible for absorption in the UV visible region.

Chromophore is responsible for imparting colour to the compound.

Example:  etc

Nitrocompounds are generally yellow in colour nitrogroup is chromophore which gives yellow colour to the compound.



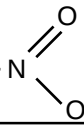
Chromophores which contains π electrons undergo $\pi - \pi^*$ transition.

Example: $H_2C = CH_2$; $HC \equiv CH$

Chromophore which contain both n and π electrons undergo two types of transitions.

$\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$

Example: Carboxyls  $C = O$, nitriles $C \equiv N$

Nitrocompounds ()

Q5. What is Auxochrome.

Ans: **(Imp.)**

Auxochrome is defined as the group which does not itself act as a chromophore but its presence brings shift of the absorption bond towards red end of the spectrum.

(or)

A convalently bonded saturated group which when attached to a chromophore changes both the wavelength and intensity of the absorption, maximum is known as auxochrome.

Example: –OH, –OR, –NH, –NHR, –SH etc.

Q6. Explain bathochromic and hypsochromic shifts.

Ans: **(Imp.)**

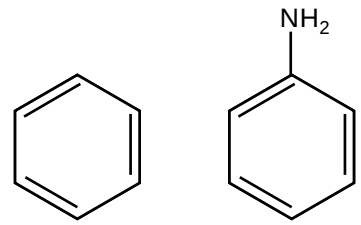
Absorption and Intensity Shifts

(i) Bathochromic Shift (or) Red Effect

Shift of an absorption maximum to a longer wave length due to the presence of an auxochrome (or) solvent effect is called as bathochromic shift (or) Red shift.

Eg: Ethylene

Benzene absorbs at 255 nm whereas aniline has absorption maximum at 280 nm (λ_{max}) Increase in λ_{max} in aniline is due to the presence of auxochrome group-NH_2.



λ_{max} 255 nm λ_{max} 280 nm

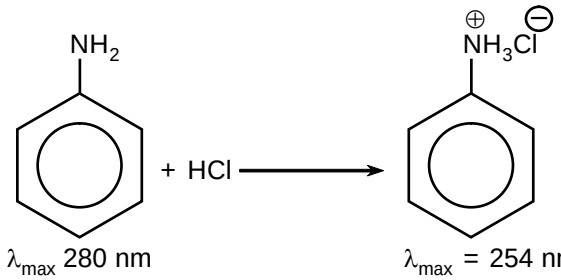
(ii) Hypsochromic Shift (OR) Blue Effect

The shift of an absorption maximum towards shorter wavelength is called hypsochromic or blue shift.

This is caused by the removal of conjugation or change in the solvent polarity.

Example: Aniline has λ_{max} at 280 nm whereas anilinium ion (acidic solution of aniline). Shown absorption maximum at 254 nm.

This is because of protonated aniline do not have lone pair of electrons for conjugation. Hence hypsochromic shift is due to the removal of n-π conjugation of pair of electrons of the nitrogen atom with π bonded electron system of the benzene ring on protenation.

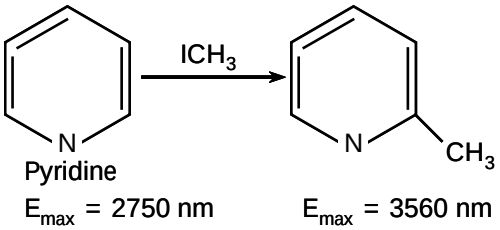


λ_{max} 280 nm λ_{max} = 254 nm

(iii) Hyperchromic Shift

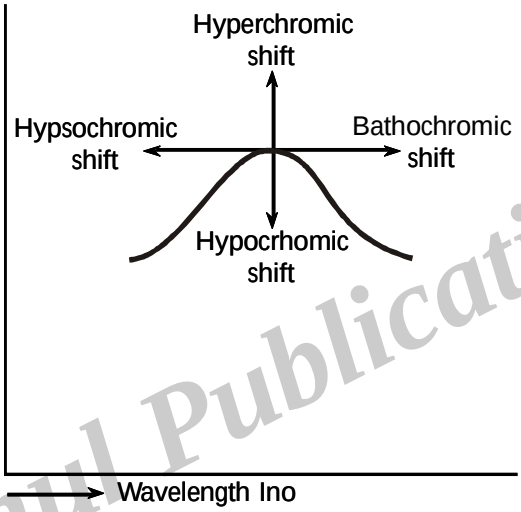
An effect which leads to an increase in absorption intensity E_{max} is called hyperchromic shift. Introduction of an auxochromes usually causes hyperchromic shift.

Example



(iv) Hyperchromic Shift

An effect which leads to a decrease in a absorption intensity E_{max} is called Hypochromic effect. This is caused by the introduction of a group which distorts the chromophore.

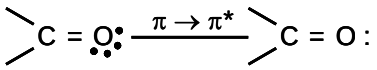


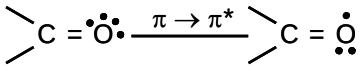
Q7. Describe the characteristics of chromophores, diene, enones.

Ans:

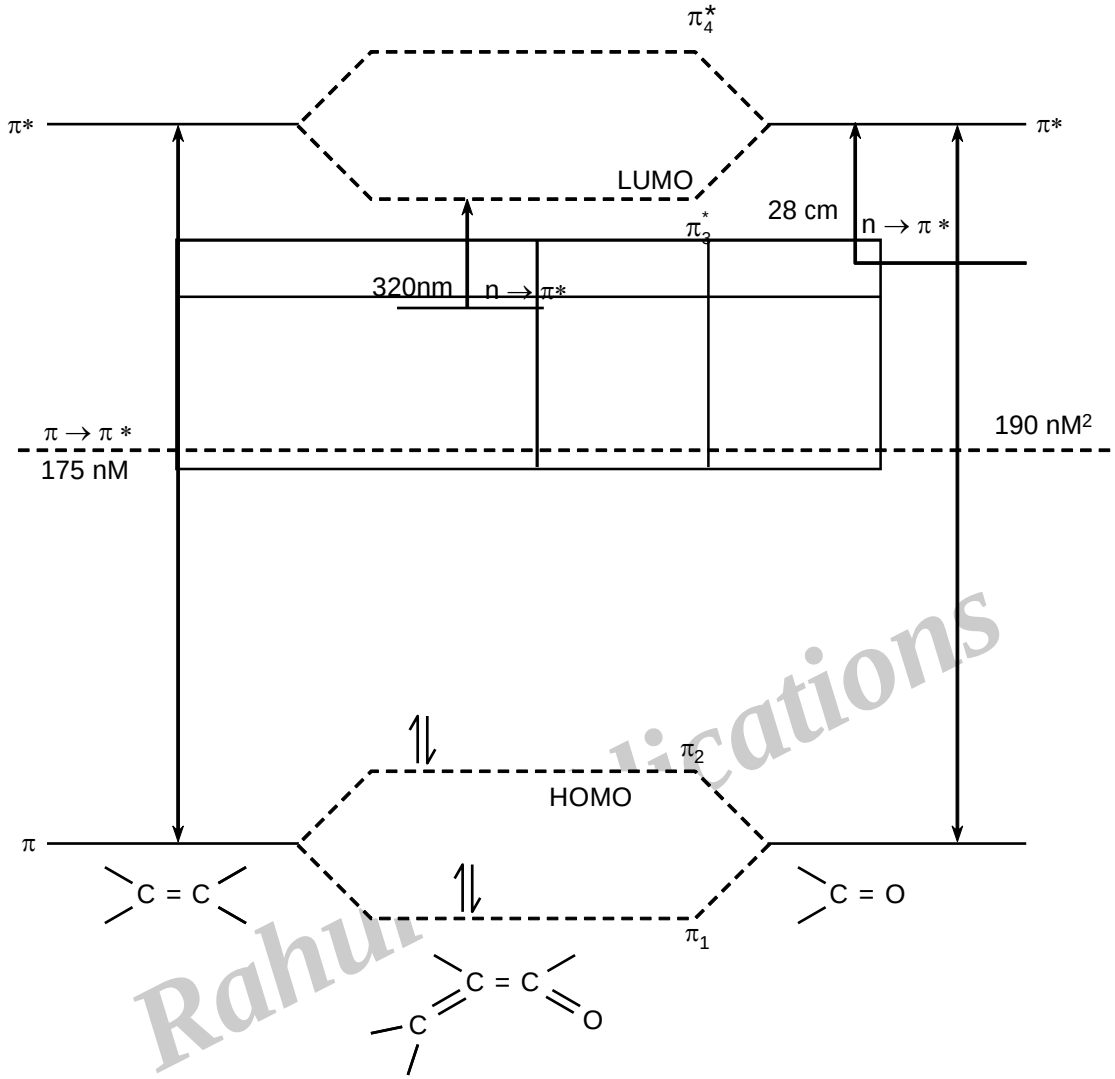
Ultra-Violet Absorption in Enone

For a carbonyl group 2 types of transitions takes place.

(i) 

(ii) 

$\pi \rightarrow \pi^*$ transition involves, the promotion of one p electron to an antibonding orbital ($\pi \rightarrow \pi^*$). Second transition i.e., $n \rightarrow \pi^*$ involves the promotion of one of the non-bonding electron to π_3^* orbital. In enone where ethylene and carbonyl groups are conjugated both $\pi \rightarrow \pi^*$ and n – π^* transitions show bathochromic shift.



Q8. Describe the UV-visible spectra of conjugated dienes.

Ans:

Conjugated Dienes

The wavelength of absorption is shifted to higher values (Bathochromic shift), if two or more chromophoric groups are present in conjugation in a molecule. For example, ethylene (one double bond) absorbs at 170 μm ($\pi \rightarrow \pi^*$ transition) while butadiene (two double bonds in conjugation) absorbs at 217 mμ. The bathochromic shift is more pronounced if the double bonds are in conjugation as compared to the isolated double bonds in which there is a little interaction between them. The absorption maximum is usually shifted 15 - 45 mμ towards higher wavelength in conjugated system (compared to unconjugated) as the electron density is spread over at least four atomic centres. The extinction coefficient also increases. In conjugated dienes, $\pi \rightarrow \pi^*$ transition results in the formation of a band, called K-band.

Table : $\pi \rightarrow \pi^*$ transition (K-band)

Compound	λ_{max}(mμ)	ε_{max}
Butadiene 1, 3	217	21,000
2, 3 dimethyl butadiene	226	21,400
1,3,5 Hexatriene	254	21,4000

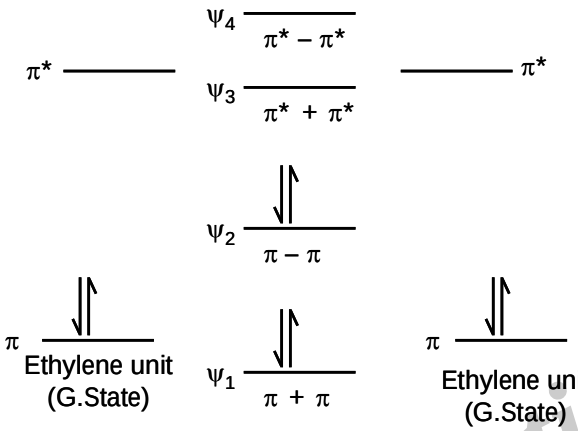


Fig.: Electronic Excitations in Conjugated Dienes

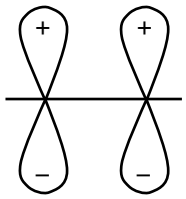
When ethylene molecule gets excited, it gives $CH_2 - CH_2$ diradical. The electron cloud is spread on two carbon atoms and the absorption maximum occurs at 170 mμ.

Consider the absorption maximum of butadiene 1, 3($CH_2 = CH - CH = CH_2$). It consists of two ethylene units. The various excitations are shown in figure.

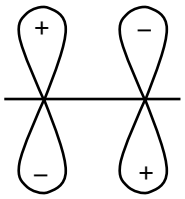
The two π bonding orbitals, one from each ethylene unit interact or mix up to give rise to two new bonding orbitals.

(i) $\pi + \pi = \pi_1$ or ψ_1 - having smaller energy.

(ii) $\pi - \pi = \pi_2$ or ψ_2 - having higher energy.



π-bonding orbitals



π^* Antibonding orbitals

The energy of ψ_1 is less than any one of the two combining atomic orbitals.

Also two π^* orbitals (antibonding) are formed from two ethylene units which are

(i) $\pi^* + \pi^* = \pi_1^*$ or ψ_3 - having smaller energy.

(ii) $\pi^* - \pi^* = \pi_2^*$ or ψ_2 - having higher energy.

The energies of ψ_3 and ψ_4 are compared with any one of the two (π^*) antibonding orbitals.

Thus, ψ_1 can be represented as shown in the figure.

In this case, all the four singly filled atomic orbitals have the same spin of electrons.

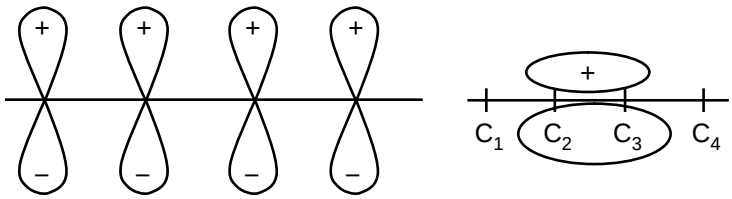


Fig.: Low Energy Atomic Orbital (ψ_1)

Thus mixing is complete and there is no nodal plane*.

$\pi - \pi = \pi_2 = \psi_2$ can be represented as shown in figure.

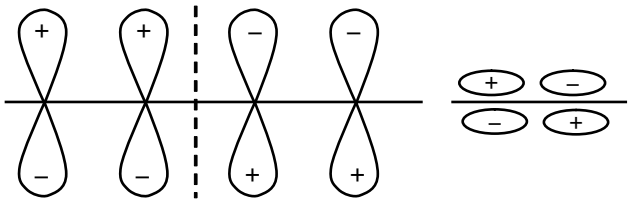


Fig.: High Energy Atomic Orbital (ψ_1)

In this case, we seen one nodal plane*.

Clearly there are double bonds between C_1, C_2 and C_3, C_4 and there is a single bond between C_2 and C_3.

$\pi^* + \pi^* = \pi_3^* = \psi_3$ can be represented as shown in figure.

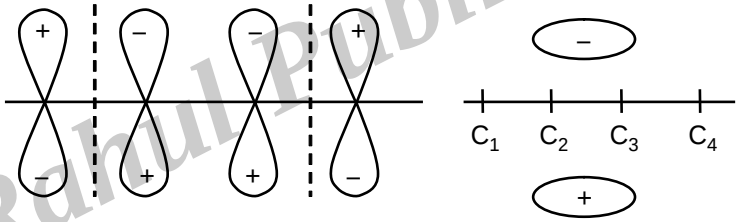


Fig.: Low Energy Atomic Orbital (ψ_3)

In this case, there are two nodal planes and one double bond between C2 and C3

$\pi^* - \pi^* = \pi_4^* = \psi_4$ can be represented as shown in figure.

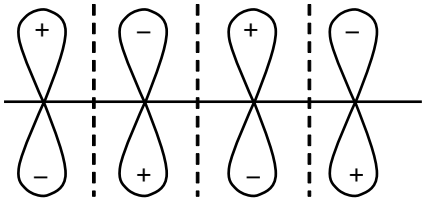


Fig.: High Energy Atomic Orbital (ψ_3)

This structure corresponds to high energy state since it involves three nodal planes.

Thus, in butadiene, four orbitals are involved. On absorption of energy, electron jumps from π_2 to π_3^*. Since the energy difference between π_2 to π_3^* is less, absorption occurs at higher wave length. This type of transition is called $\pi \rightarrow \pi^*$ transition. The net result is that when two double bonds are in conjugation, the energy level of higher occupied molecular orbital (HOMO) is raised and that of the lowest unoccupied molecular (antibonding) orbital (LUMO) is lowered.

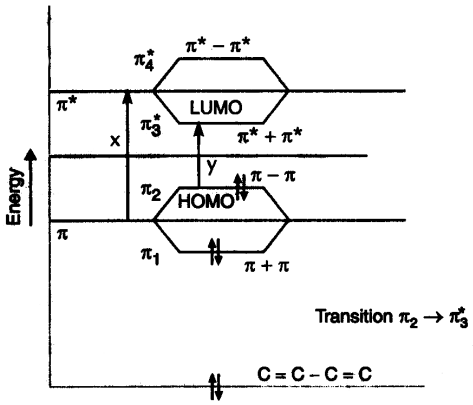


Fig.: Electronic Transition in Conjugated Diene

This absorption corresponds to the transition y (low energy or higher wavelength). Similarly, when dissimilar chromophores. In conjugation, absorption occurs at longer wavelength as compared to the isolated chromophores. In general longer the conjugated system, smaller will be the energy needed to cause $\pi \rightarrow \pi^*$ transition and therefore, absorption occurs at still longer wavelength. In a long conjugated system like carotene, absorption occurs in the visible region (higher wavelength region).

The values of absorption maximum (λ_{max}) as well as extinction coefficient for conjugated and conjugated alkenes can be compared from their spectra shown in Figure.

The values of λ_{max} and ε_{max} are more for conjugated diene as compared to those for an unconjugated alkene. A bathochromic as well as hyperchromic effect are observed when the spectrum of conjugated triene is compared to that of conjugated diene.

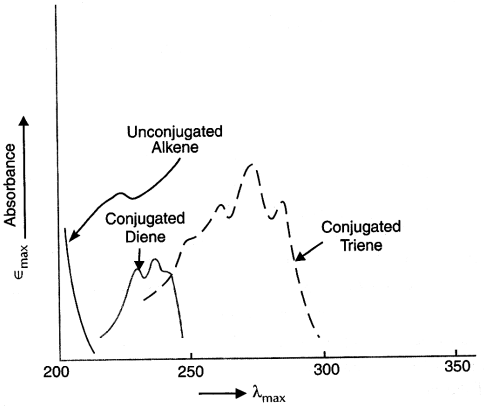


Fig.: Absorption in Conjugated and un-conjugated Systems

It is important to note that greater the number of conjugated double bonds, greater is the bathochromic shift. With continuous increase in conjugation, the absorption may even shift to the visible region. As the conjugation increases, the energy gap between HOMO and LUMO decreases, See Figure. 2.18). In case of (3-carotene which contain eleven double bonds, the absorption bands appear at (i) λ_{max} 478 nm (ε_{max} 139000) and (ii) λ_{max} 452 nm (ε_{max} 122000).

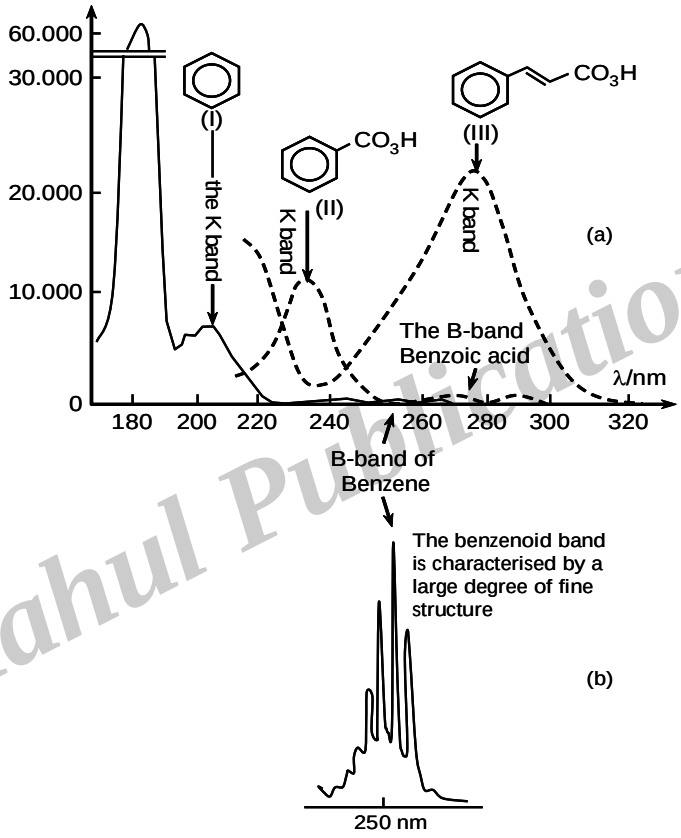
Q9. Describe the UVspectrum in benzene and its derivatives.

Ans :

Benzene (I) shows three absorption bands (solid line, figure) at 184 nm (ε_{max} 60,000); 203.5 nm (ε_{max} 7400, often called K-band) and at 254 nm (ε_{max} 204, often called B-band). The intense band near 180 nm is a result of an allowed transition, while the weaker K and B bands near 200 and 260 nm respectively result from forbiden transitions in the highly symmetrical benzene molecule.

The B-band of benzene and many of its homologues show a great deal of fine structure in the vapour phase or in non-polar solvents. The fine structure originates from sub-levels of vibrational absorption upon which the electronic absorption is superimposed. When benzene is substitute by simple alkyl groups the absorpiton are shifted slightly to longer wavelength.

This small bathochromic shift is ascribed to hyperconjugation between the alkyl group and π system of the ring. The second alkyl group is most effective in producing a red shift if it is in the para position. The para isomer absorbs at the longest wavelength where as the ortho isomer generally absorbs at the shortest wavelength. This effect is due to steric interaction between the ortho subsitutents, which effectively.



reduce hyperconjugation. When however, non-bonding pair substituents ($-OH_1$ – NH_2, – OR, etc.) are present on the benzene ring the absorptions are shifted subtantially to longer wavelengths and the fine structure of the B-band is either diminished eliminated, because of n - π conjugation.

A reference figure shows how the wavelength and intensity of the absorptions peaks increase with an increase in the extent of the chromophore. On adding more and more conjugation to the benzene ring, the K-band (at 203.5 nm is benzene) effectively 'moves' to longer wavelength, faster than the B-band. Thus benzoic acid shows the K-band at 230 nm the B-band being still clearly visible at 273 nm, however, with the longer chromophore of cinnamic acid the K-band shifts to 273 nm and the B-band is completely enveloped.

ROTATIONAL SPECTROSCOPY

Q10. Discuss rotation spectra and selection rules of diatomic linear molecules.

Ans : **(Imp.)**

Selection Rules

For a rigid diatomic rotating molecule, the transition may take place only between those rotational energy levels for which the change in rotational quantum number is unity i.e.,

$$\Delta J = \pm 1$$

Thus according to this selection rule, transitions from rotational levels $J = 0 \rightarrow J = 1$, $J = 1 \rightarrow J = 2$, $J = 2 \rightarrow J = 3$, etc. are possible, whereas transitions of the type $J = 0 \rightarrow J = 2$ or $J = 2 \rightarrow J = 4$, etc. are not possible. The transitions $\Delta J = +1$ and $\Delta J = -1$ correspond respectively to absorption and emission of radiation.

Q11. Determine the rotational energy of rigid diatomic molecules.

Ans : **(Imp.)**

Rotational Spectra of rigid Diatomic Linear Molecules

A rigid diatomic molecule means the distance between the atoms (bond length) does not change during rotation. No vibrational movement is taking place during rotation. Let us consider a diatomic molecule A–B in which the atoms A and B having masses m_1 and m_2 are joined together by a rigid bond of length $r_0 = r_1 + r_2$.

The molecule A – B rotates about a point C (the centre of gravity). At point C, the following condition is satisfied:

$$m_1 r_1 = m_2 r_2$$

From Schrodinger wave equation, the allowed rotational energies, Ej for such a rigid diatomic molecule is given as

$$E_J = \frac{h^2}{8\pi^2 I} J(J+1)$$

where 'J' is the rotational quantum number and can have values 0, 1, 2, 3,.. .etc., 'h' is Planck's constant and I is the moment of inertia of the diatomic molecule AB and is given by the expression

$$I = \left(\frac{m_1 m_2}{m_1 + m_2}\right) r_0^2 = \mu r_0^2$$

where μ is the reduced mass of the system, i.e.,

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

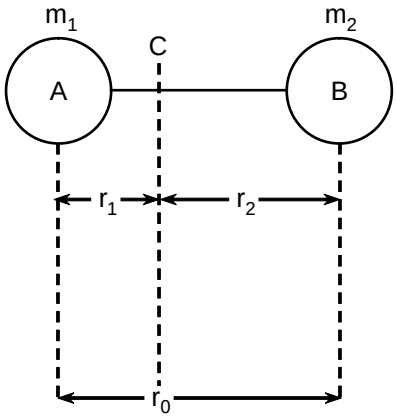


Fig.: A rigid diatomic molecule A – B having atomic masses m, and m_2 joined together by a rigid bond of length $r_0 = r_1 + r_2$ and rotates about a point C

Equation gives the allowed energies of various rotational levels in joules.

Q12. Determine the bond length and moment of Inertia from rotational spectra.

Ans : **(Imp.)**

Determination of Bond Length and Moment of Inertia from Rotational Spectra

In rotational spectra, we have,

$$\bar{\nu}_{j \to J'} = 2BJ' = 2B(J + 1)\ (cm^{-1})$$

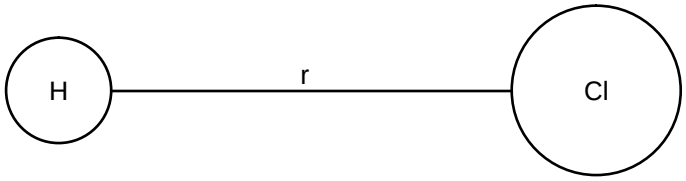
Once the value of B is determined, it is possible to determine the value of moment of inertia from the following relationship:

$$B = \frac{h}{8\pi^2 Ic}$$

which in turn gives the value of bond length, since $I = mr^2$, where

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

Let us calculate the bond length in HCl molecule.



The reduced mass of HCl is

$$m = \frac{m_H m_{Cl}}{m_H + m_{Cl}}$$

Therefore the moment of intertia is given by

$$I = \mu r^2$$

The rotational energy change during the transition $J \rightarrow J + 1$ is given by

$$\bar{\nu}_{j=0 \text{ to } J=(J+1)} = 2B(J + 1) = 2B \text{ cm}^{-1} \text{ (when } J = 0)$$

This equation shows that the moment of inertia (included in B) can be evaluated from the spectrum. The pure rotational spectrum of HCl has been measure by Hansler and Oetjen and the value of B was found to be 10.4 cm^{-1}. The value of I can be calculated as :

$$I = \frac{h}{8\pi^2 Bc} = \frac{6.62 \times 10^{-27}}{8(3.14)^2 \times 10.4 \times 3 \times 10^{10}}$$

$$I = 2.69 \times 10^{-40} \text{ g/cm2}$$

and since the reduced mass of HCl is

$$\mu = \frac{35.5 \times 1}{35.5 + 1} \times \frac{1}{6.023 \times 10^{23}} = 1.6148 \times 10^{-24} \text{ g/molecule}$$

Therefore, the bond length is calculated as

$$r^2 = \frac{2.69 \times 10^{-40}}{1.6148 \times 10^{-24}} = 1.6658 \times 10\text{–}16 \text{ cm2}$$

or $r = 1.29 \times 10^{-8}$ cm $= 1.29 \times 10^{-10}$ m $= 0.129 \times 10^{-9}$ m

or $r = 0.13$ nm

INFRARED SPECTROSCOPY

Q13. Explain the energy levels of simple harmonic oscillator.

Ans :

Harmonic Oscillations in Diatomic Molecules

The vibrational energies in a molecule are quantized. That is, only certain energies for the system are allowed, and only photons with certain energies will excite molecular vibrations. The symmetry of the molecule will also determine whether a photon can be absorbed. The allowed vibrational energies for a system can be calculated with the help of Schrodinger's equation, and for simple harmonic oscillators, the vibrational energy is given by

$$E_{vib} = \frac{h}{2\pi}\sqrt{\frac{f}{\mu}} (\nu + 1/2)$$

or $E_{vib} = (\nu + 1/2)\, h\nu$ (joules)

where 'ν' is the vibrational quantum number and can have values $\nu = 0, 1, 2, 3, 4, \ldots$ etc. In case of harmonic oscillations in a diatomic molecule the distance between the atoms will change as a result of vibration, therefore the vibrational frequency) can be replaced by equilibrium frequency Eqn. can be written as

$$E_{vib} = (\nu + 1/2)\, h\nu_e \text{ (joules)}$$

This vibrational energy in terms of wave numbers can be expressed as

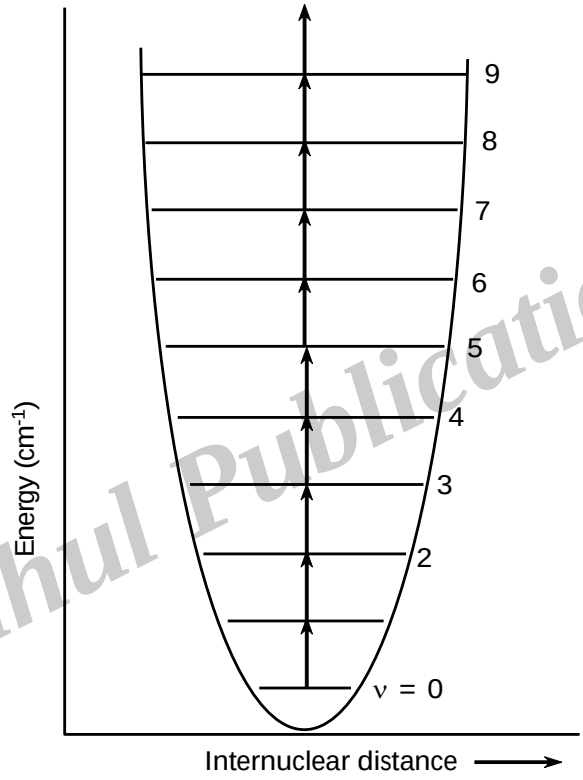
$$\bar{\nu} = \frac{E_{vib}}{hc} - = (v + 1/2)\ \bar{\nu}_e\ (cm^{-1})$$

The various allowed energy levels as described by equation is shown in figure.

It is seen that the vibrational energy levels of a harmonic oscillator are equally spaced and the energy levels are calculated by using Eqn. The simple harmonic oscillator provides a good fit to energies for the lowest energy levels, but fails at higher energies. In Eqn. if we put $v = 0$, the equation reduces to

$$E = 1/2\ h\nu_e$$

or $$\bar{\nu} = 1/2\ \bar{\nu}_e\ cm^{-1}$$



The quantity $1/2\ h\nu_e$ or $1/2\ \bar{\nu}_e$ is known as zero point energy of harmonic oscillator. This corresponds to the energy of molecules in the ground vibrational state.

Q14. Write the selection rules of IR spectroscopy.

Ans : **(Imp.)**

Selection Rules

The simple selection rule for vibrational transition in a simple harmonic osicllator in terms of quantum number is

$$\Delta\nu = \pm 1$$

Thus, if any transition is taking place from any virbational level ν to $\nu + 1$, then we have

$$\Delta E = E_{v+1} - E_v$$
$$= [(v + 1) + 1/2)]h\nu_e - [(v + 1/2)]h\nu_e$$
$$= h\nu_e \text{ (joules)}$$

or $\Delta E / hc = \bar{\nu} = 1/2\,\bar{\nu}_e$ (cm^{-1})

Therefore, only one spectral line will be obtained in the virbational spectrum of the diatomic molecule performing simple harmonic oscillations.

Q15. Describe the Anharmonic oscillations in Diatomic Molecules.

Ans : **(Imp.)**

Anharmonic Oscillations in Diatomic Molecules

In reality the movement of molecules is not perfectly harmonic. The bonds can be considered as perfectly elastic for small compressions and stretching. But for long distortions they do not obey Hooke's law. It was then suggested by P.M. Morse that equation as mentioned earlier for harmonic oscillators, must be modified for diatomic molecules making anharmonic oscillations. The allowed vibrational energy levels in this case is given by

$$E_{vib} = (v + 1/2)\, h\nu - (v + 1/2)^2\, h\nu > x_e + (v + 1/2)^3\, h\nu > y_e + \text{higher terms}$$

On replacing the frequency term v in the above Eqn. by equilibrium oscillation frequency $x>_e$, we have

$$E_{vib} = (v + 1/2)\, h\nu_e - (v + 1/2)^2\, h\nu_e\, x_e + (v + 1/2)^3\, h\nu_e\, y_e + \text{higher terms}$$

where x_e and y_e are the first and second anharmonicity constants respectively. These correction terms provide much better match of the calculated energies to the energies that are observed experimentally. Due to these anharmonicity constants, the spacing between two energy levels decreases with increasing value of v. The value of y_e and higher terms in equation are very small and can be neglected, therefore, equation can be written as

$$E_{vib} = (v + 1/2)\, h\nu_e - (v + 1/2)^2\, h\nu_e\, x_e \text{ (joules)}$$

and in terms of wave number, equation becomes

$$\bar{\nu} = (v + 1/2)\ \bar{\nu}_e\{1 - x_e(v + 1/2)\}\ (cm^{-1})$$

At v = 0, the energy of the ground vibrational level in terms of wave number from equation becomes.

$$\bar{\nu} = 1/2\bar{\nu}_e\{1 - 1/2x_e\}\ (cm^{-1})$$

This is known as zero point energy of an anharmonic oscillator which differs from that of the harmonic one by a factor $\{1 - 1/2\ x_e\}$.

On putting different values of v = 0, 1, 2, ... in Eqn. The energy levels of anharmonic oscillator may be obtained. For the transition from a level v to another level v + 1, according to the selection rule $\Delta v = \pm 1$, one can write the energy difference in terms of wave number ($\bar{\nu}$) as

$$\bar{\upsilon} = \bar{\upsilon}_e\{1 - 2x_e(v + 1)\} \quad (cm^{-1})$$

This suggests that the vibrational frequency in this case decreases with the increasing value of v. The vibrational energy levels in diatomic molecules making anharmonic oscillations are shown in Figure.

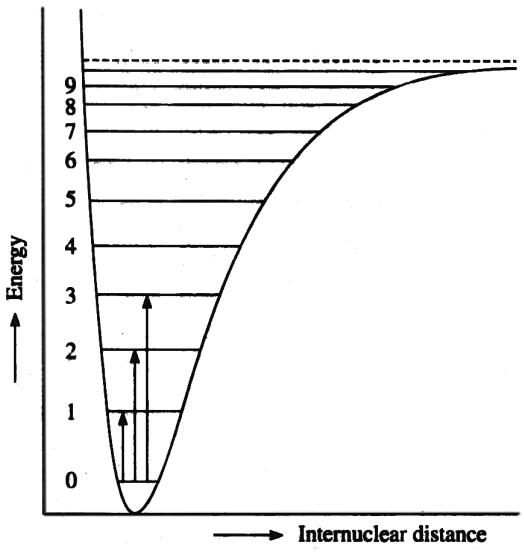


Figure: Potential energy curve for a diatomic molecule making anharmonic oscillations

When the transition occurs from v = 0 to v = 1, Equation becomes

$$\bar{\upsilon} = \bar{\upsilon}_e \{1 - 2x_e\} \quad (cm^{-1})$$

Q16. Discuss different types of vibrations in polyatomic molecules.

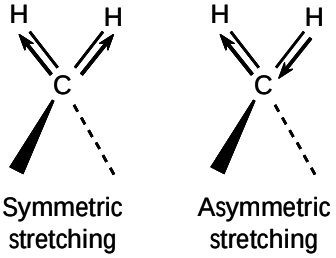
Ans :

Molecular Virbations in Polyatomic Molecules

A molecule may be regarded as a system of balls (atoms) and springs (bonds). When it absorbs infrared radiation, it is set into vibrations resulting in excitation of bond deformations-stretching and bending. A complex molecule has a large number of vibrational modes which involve the whole molecule. To a good approximation, however, some of these molecular vibrations are associated with the vibrations of individual bonds or groups (localized vibrations) while others must be considered as vibrations of the whole molecule. The localized vibrations are stretching, bending, rocking, twisting or wagging. For example, the various stretching and bending vibrations of the methylene group are discussed below:

1. Stretching Vibrations

In these vibrations, the atoms move along the bond axis and because of this movement, the bond length increases or decreases at regular intervals which give rise to a change in dipole moment The stretching vibrations are of two types: (i) Symmetric stretching and (ii) Anti symmetric stretching. In symmetric stretching both the H-atoms either simultaneously elongates or contracts towards the C-atom, whereas in anti symmetric stretching one H-atom elongates while the other one contracts towards the C-atom.

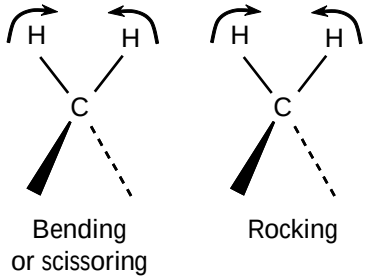


(Stretching vibrations of CH_2)

2. Bending Vibrations

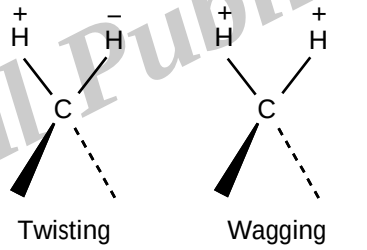
In bending vibrations there is a change in bond angle between the bonds connected to a common atom which give rise to a change in dipole moment. Also the movement of an atom, or a group of atoms in the molecule may lead to bending vibrations. The bending vibrations may occur either (i) in-plane, or (ii) out-of-plane.

(i) In-plane Bending Vibrations: These are of two types: (a) Scissoring - when the bond angle in a triatomic molecule increases and decreases simultaneously i.e., the bonds move in or out like a scissor, the vibration is known as scissoring and (b) Rocking – When two atoms in a triatomic molecule oscillate in the same direction, they are said to rock. The bond angle remains constant in this case.



(in-plane bending vibrations of CH_2)

(ii) Out-of-plane Bending Vibrations: It is also of two types: (i) Twisting - When one atom moves up (denoted by + sign) while the other moves down (denoted by - sign) of the molecular plane, the vibration is called twisting and (ii) Wagging - when both the atoms either move up (+) or down (–) simultaneously out-of-plane, the vibration is known as wagging.



(out-of-plane bending vibrations of CH_2)

All these vibrations may give rise to different absorption bands in IR spectra.

In general stretching frequencies are higher than corresponding bending frequencies because it« easier to bend a bond than to stretch or compress it.

Q17. Write characteristic absorption bands of varveys variety groups.

Ans :

Table: Characteristic IR Absorption Frequencies of Organic Functional Groups

Functional group	Types of vibration	Characteristic absorptions (cm^{-1})	Intensity
Alcohol			
0—H	(stretch, H-bonded)	3200-3600	strong, broad
O—H	(stretch, free)	3500-3700	strong, sharp
C—O	(stretch)	1050-1150	strong

Alkane			
C—H	stretch	2850-3000	strong
—C—H	bending	1350-1480	variable
Alkene			
=C—H	stretch	3010-3100	medium
=C—H	bending	675-1000	strong
C=C	stretch	1620-1680	variable
Alkyl halide			
C—F	stretch	1000-1400	strong
C—Cl	stretch	600-800	strong
C—Br	stretch	500-600	strong
C—I	stretch	500	strong
Alkyne			
C—H —C=C—	stretch stretch	3300 2100-2260	strong, sharp variable, not present in symmetrical alkynes
Amine			
N—H	stretch	3300-3500	medium (primary amines have two bands; secondary have one band, often very weak)
C—N	stretch	1080-1360	Medium-weak
N—H	bending	1600	medium
C—H	stretch	3000-3100	medium
C=C	stretch	1400-1600	Medium-weak, multiple bands
Analysis of C—H out-of-plane bending can often distinguish substitution patterns			
Carbonyl	**Detailed information on carbonyl IR**		
C=C	stretch (conjugation moves absorptions to lower wave numbers)	1670-1820	strong
Ether			
C—O	stretch	1000-1300 (1070-1150)	strong
Nitrile			
CN	stretch	2210-2260	medium
Nitro			
N—O	stretch	1515-1560 & 1345-1385	strong, two bands

Acid			
C=0	stretch	1700-1725	strong
O—H	stretch	2500-3300	strong, very broad
C—O	stretch	1210-1320	strong
Aldehyde			
C=Q	stretch	1740-1720	strong
=C—H	stretch	2820-2850 & 2720-2750	medium, two peaks
Amide			
C=0	stretch	1640-1690	strong
N—H	stretch	3100-3500	unsubstituted have two bands
N—H	bending	1550 -1640	
Anhydride			
C=0	stretch	1800—1830 & 1740-1775	two bands
Ester			
C=0	stretch	1735-1750	strong
C—O	stretch	1000-1300	two bands or more
Ketone			
acyclic	stretch	1705-1725	strong
cyclic	stretch	3-membered -1850	
		4-membered - 1780	
cyclic	stretch	5-membered - 1745	strong
		6- membered - 1715	
		7- membered - 1705	
unsaturated	stretch	1665-1685	strong
aryl ketone	stretch	1680-1700	strong

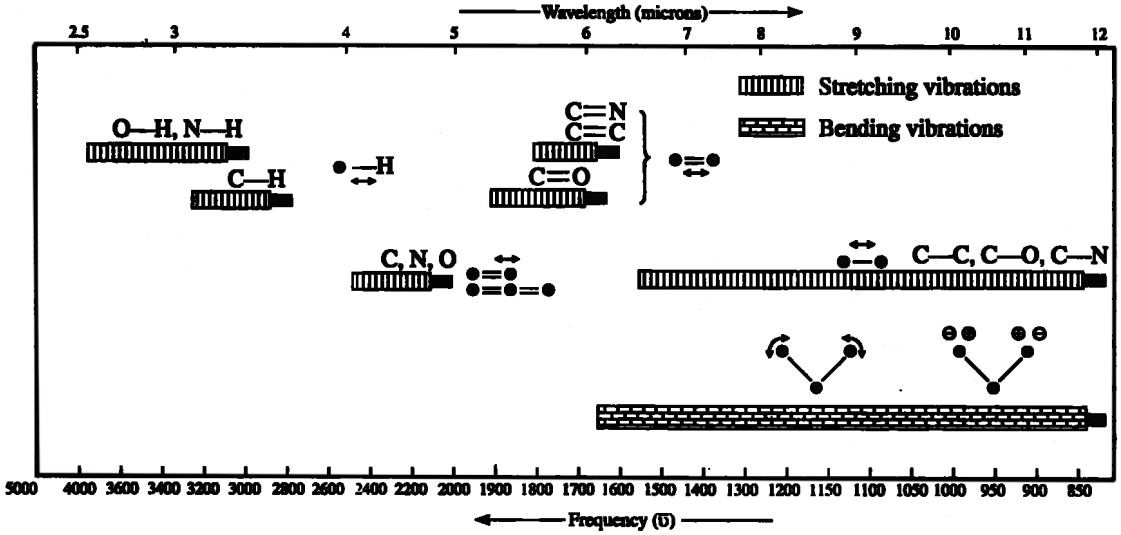
Q18. Give the fingerprint region of Infrared spectrum.

Ans :

Group Frequencies and Finger Print Region

The general regions of the infrared spectrum in which various kinds of vibrational bands are observed are outlined in figure. Note that the shaded sections above the dashed line refer to stretching vibrations, and below the line encompass bending vibrations. The complexity of infrared spectra in the 1450 to 600 cm^{-1} region makes it difficult to assign all the absorption bands, and because of the unique patterns found

there, it is often called the fingerprint region. Absorption bands in the 4000 to 1450 cnr.[1] region are usually due to stretching vibrations of diatomic units, and this is sometimes called the group frequency region. Detailed information about the infrared absorptions observed for various bonded atoms and groups is usually presented. Since most organic compounds have C—H bonds, a useful rule is that absorption in the 2850 to 3000 cm^{-1} is due to sp^3 C—H stretching; whereas, absorption above 3000 cm^{-1} is from sp^2 C—-H stretching or sp C—H stretching if it is near 3300 cm^{-1}.



ELECTRONIC SPECTROSCOPY

Q19. How the force constant quantitatively related to bond strength.

Ans :

The value of the stretching vibrational frequency of a bond can be calculated fairly accurately by the application of Hooke's law which may be represented as:

$$\frac{v}{c} = \bar{v} = \frac{1}{2\pi c}\left[\frac{k}{\frac{m_1 m_2}{m_1 + m_2}}\right]^{1/2}$$

$$= \frac{2}{2\pi c}\sqrt{\frac{k}{\mu}}$$

where μ is the reduce mass.

m1 and m2 are the masses of the atoms concerned in grams in a particular bond.

k = Force constant of the bond and relates to the strength of the bond. For a single bond, it is approximately 5×10^5 gm sec^{-2}. It becomes double and tripe for the double and tripe bonds respectively.

c = Velocity of the radiation = 2.998×10^{10} cm sec^{-1}

Thus, the value of vibrational frequency or wave number depends upon:

(i) Bond strength, and

(ii) Reduced mass.

If the bond strength increase or the reduced mass decrease, the value of the virbational frequency increases.

C = C stretching is expected to absorb at higher frequency than C – C stretching. It is due to the higher bond strength (value of k) of the double bond compared to the single bond.

Q20. Classification of Molecules based on moment of inertia.

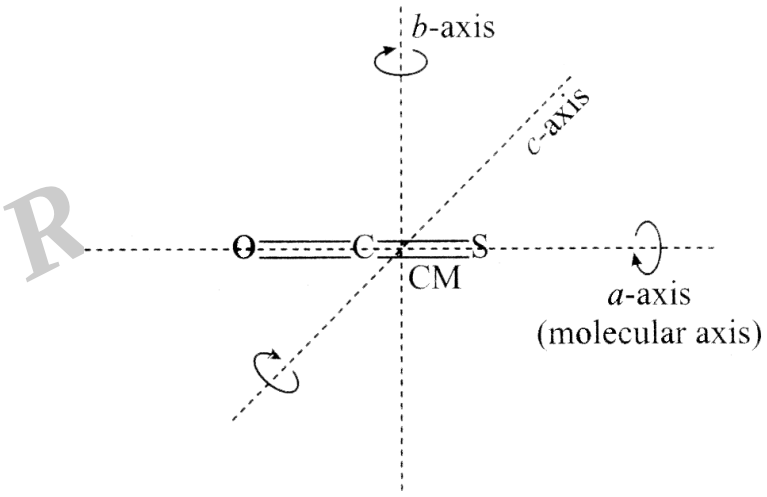
Ans : **(Imp.)**

A rotating molecule in space has three moments of inertia in 3-directions/axes, represented as I_a, I_b and Ic. Based on the relationship bettween these moment of inertia, molecules are classified into the following 4-groups. (1) Linear molecules (2) Spherical-top molecules (3) symmetrical-top molecules. (4) Asymmetrical-top molecules.

1. Linear Molecules

Molecules such as HCl, HBr, CO, OCS etc are Linear-molecules, in which all the atoms lie in the same line.

Ex: O ═ C ═ S molecules.



a-Axis : is the molecular axis passing through all the z-atoms and centre of mass (CM).

b-axis : is the passing only through CM and is perpendicular ($\perp$) to a-axis.

c-axis : is the axis which is ($\perp$) to both a and b-axes and passing through CM.

When the molecule is rotated about its molecular axis (a-axis), the equilibrium positions of atoms do not change. Hence, the moment of inertia about this axis (I_a) is zero.

$$\boxed{I_a = 0}$$

Rotation about b-axis and c-axis is all the same, *i.e.*, end-over-end rotation in which the equillibrium positions of all the atoms are changing for which the moment of inertia in these directions (I_b and I_c) is not equal to zero and are identical.

i.e., $\boxed{I_b = I_c \neq 0}$

$\therefore$ A linear molecule is defined as

$$\boxed{I_a = 0; I_b = I_c \neq 0}$$

2. Spherical-top Molecules

The molecules, in which all the moments of inertia are identical are called symmetrical-top molecules.

i.e., $\boxed{I_a = I_b = I_c}$

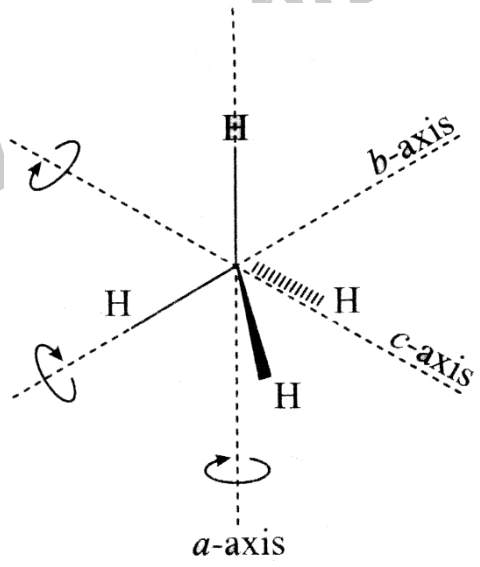
Example: CH_4, CCl_4, SF_6 etc.

When CH_4-molecule is rotated about a-axis, the equillibrium positions of 3-H-atoms are changing, i.e., the moment of inertia about a-axis is not equal to zero.

i.e., $\boxed{I_a \neq 0}$

Similarly, when CH_4-molecule is rotated about 6-axis and c-axis, the equillibrium positions of 3-H-atoms are changing and hence moment of inertia along 6-axis and c-axis is also not zero.

i.e., $\boxed{I_b \neq 0, I_c \neq 0}$



Hence, for all 3-rotations 3-H-atoms are undergoing displacement and all the 3-moments of inertia are the same.

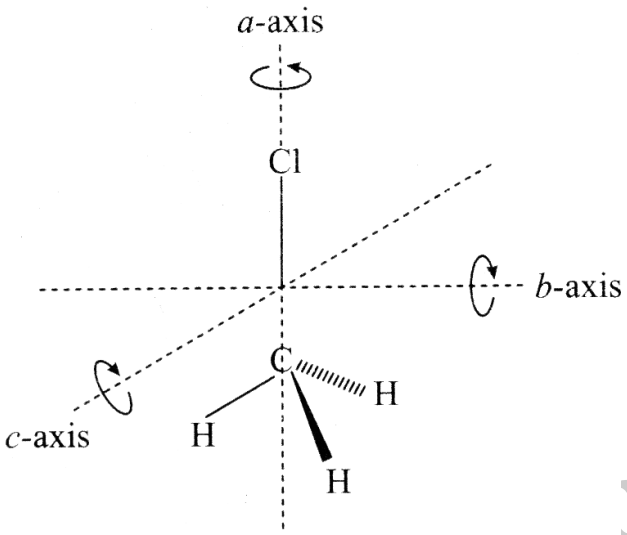
i.e., $\boxed{I_a = I_b = I_c}$

The dipole moments of such compounds is equal to zero and hence they will not give microwave spectra (microwave inactive).

3. Symmetrical-top molecules

The molecules with 3-non-zero moments of inertia, and two of them are same are called symmetrical-top molecules.

Example: CH_3Cl, $CHCl_3$, $CH_3 - CN$ etc.



a-axis : is the molecular axis passing through maximum no. of atoms and CM. when the molecule is rotated about a-axis, the equilibrium positions of 3 - H' are changing and the rotation is similar to spinning-top. The moment of inertia about this axis is **not zero.**

i.e., $\boxed{I_a \neq 0}$

Rotation about b-axis and c-axis are same, end-to-end rotation with the displacement of all 3-atoms.

i.e., $\boxed{I_b = I_c}$

$\therefore$ For Symmetrical-top molecule

$$\boxed{I_a \neq 0, I_b = I_c}$$

Based on the relationship between I_a, I_b and I_c, Symmetric-top-molecular are. further divided into two subgroups.

(a) Prolate symmetric-top molecules.

(b) Oblate symmetric-top molecules.

(a) Prolate symmetric-top molecules

The molecules with 3-non-zero moments of inertia, where I_a will be smaller than I_b & I_c.

Example : CH_3C1,$CHC1_3$ etc.

(b) Oblate symmetric-top molecules : The molecules with 3-non-zero moments of inertia, where I_c will be much greater than I_a & I_b.

i.e., $\boxed{I_a = I_b < I_c}$

Example : BF_3, $BC1_3$,C_6H_6 etc.

In case of $BC1_3$, $\boxed{2I_a = 2I_b = I_c}$

(4) Asymmetrical-top molecules

The molecules having 3-non-identical moments of inertia are called Asymmetrical-top molecules.

i.e, $\boxed{I_a \neq I_b \neq I_c}$

Example : H_2O, $CH_3 - OH$, $CH_2 = CH — Cl$ etc.

Hence, based on moments of inertia and their relationship, molecules are divided into four-groups, where.

(1) Linear molecules $I_a = 0$, $I_b = I_C$.

(2) Spherical-top molecules $I_a = I_b = I_C$.

(3) Symmetrical-top molecules $I_a \neq 0$, $1_b = I_C$.

(4) Asymmetrical-top molecules $I_a \neq I_b \neq I_c$.

Q21. Determination of Bond length of rigid diatomic molecules HCl.

Ans : **(Imp.)**

On absorbing microwave radiation, HCl-molecule undergo rotational motion involving transitions from lower energy level to higher level. From the transition, it gives equally spaced spectral lines with a gap of '2B' where the first line appears. The moment of inertia and the bond length is calculated from these spectral lines.

For example, in the calculation of the bond length of HCl. The first line is observed at 20.7 cm^{-1} in in its microwave spectra.

$\Rightarrow$ $2B = 20.7\ cm^{-1} \Rightarrow \boxed{B = 10.35\,cm^{-1}}$

But $B = \frac{h}{8\pi^2 IC} \Rightarrow \boxed{I = \frac{h}{8\pi^2 BC}}$

$\Rightarrow$ $\frac{6.625 \times 10^{-27}}{8 \times (3.14)^2 \times 10.35 \times 3 \times 10^{10}} = 2.705 \times 10^{-40}$ gm. cm^2

Reduced mass of HCl. $U_{HCl} = \frac{m_1 m_2}{m_1 + m_2} \times \frac{1}{N} = \frac{1 \times 3.5.5}{1 + 35.5} \times \frac{1}{6.023 \times 10^{23}}$

$\Rightarrow$ $U_{HCl} = 1.614 \times 10^{-24}$ gm/molecule.

$$1 = ur^2 \Rightarrow r^2 = \frac{1}{u} \boxed{\Rightarrow r = \sqrt{\frac{I}{u}}}$$

$$\Rightarrow \quad r = \sqrt{\frac{2.705 \times 10^{-40}}{1.614 \times 10^{-24}}} = \sqrt{1.71 \times 10^{16}}$$

$$= 1.31 \times 10^{-8} \text{ cm} = 1.31 \text{ A}^0$$

$\therefore$ The bond length of HCl = 1.31 A^0.

Q22. Define Terms Employed In Absorption Spectroscopy.

Ans : **(Imp.)**

Transmittance (T)

Transmittance (T) is the ratio of the intensity of the light transmitted by the sample P to the intensity of the light incident on the sample P_0

$$T = P/P_{0,} \qquad \%T = \frac{P}{P_0} \times 100$$

The transmitted light is measured as % transmittance, which can have any value from 0-100 (100% transmittance means no absorption by the compound). From the value of the % transmittance, we can determine the amount of incident light energy absorbed. The amount of light energy absorbed by the sample solution actually depends on the concentration of the compound in the sample and this forms the basis for a quantitative analysis.

Absorbance (A)

The absorbance (A) of a solution is defined as the logarithm to the base 10 of the reciprocal of transmittance T, i.e., $A = \log_{10}\left(\frac{1}{T}\right)$

$$A = -\log T$$

as $T = \frac{P}{P_0}$

$$A = -\log\frac{P}{P_0} = \log\frac{P_0}{P}$$

Absorptivity or extinction coefficient (a)

The absorptivity a of a solution is defined as the ratio of the absorbance, A, to the product of concentration c, and path-length b

$$a = A/bc \quad \text{or} \quad A = abc,$$

where a is a proportionality constant called absorptivity, since, generally 6 is 1 cm and c is g/1, the absorptivity a has units of lg^{-1} cm^{-}.

Molar absorptivity or molar extinction coefficient ∈

When the concentration, c, in the equation A - abc is expressed in moles per litre and the path-length b, is in centimeters, the proportionality constant is called the 'molar absorptivity' or 'molar extinction coefficient' and given the symbol e.

Thus,

$$A = \varepsilon bc \quad or \quad \varepsilon = \frac{A}{bc}$$

where b is in cm and c is in mol/1, and e has the units 1 mol^{-1} cm^{-1}.

Q23. Define Beer-Lambert's Law.

Ans : **(Imp.)**

The intensity of the light transmitted by a solution is dependent both on the concentration and the path-length of the solution. The absorption of light by a solution is generally expressed as the combined Beer-Lambert's law, which is stated as follows. The absorbance A of a solution is linearly related to the concentration c, of the absorbing species, and the path-length 6, of the radiation in the absorbing medium. That is,

$$A = \log \frac{P_0}{P} = abc \quad or \quad A = abc.$$

For a 1 M solution of a compound (c = l) and for a path-length of 1 cm (6 = 1), the molar absorptivity e is equal to the absorbance, A; or in other words, the absorbance, A, of a solution of 1 M concentration and 1 cm path-length is equal to its molar extinction coefficient ∈.

Since b is generally 1 cm and e is a constant characteristic of the compound analyzed, the Beer-Lambert's Law simply states that the absorbance of any solution is related to its concentration and is expressed graphically as in fig.

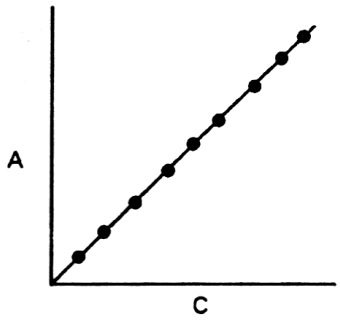


Fig.: Beer-Lamberf's law: A plot of concentration vs absorbances (calibration graph)

Beer-Lambert's law is extensively used in the quantitative analysis of organic compounds, as well as inorganic salts coloured as well as colourless.

Beer-Lamberts calibration graph for quantitative analysis by the Uv-visible method

The use of Beer-Lambert's law for quantitative analysis requires the preparation of a calibration graph, using the following steps.

(i) Prepare a stock solution of the pure analyte.

(ii) Prepare different concentration solutions from the stock solution by dilution, using standard flasks.

(iii) Measure the absorbance, A, of all these solutions using a colourimeter for coloured solutions or a spectrophotometer for coloured as well as colourless solutions, at a chosen wavelength, i.e., A_{max} of the analyte.

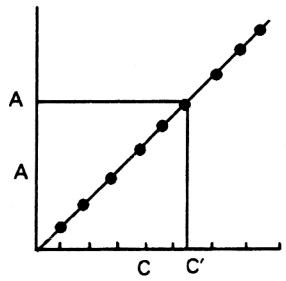


Fig. Determination of the concentration of an unknown solution by the use of Beer-Lambert's calibration graph

(iv) A plot of absorbances vs concentrations a straight line passing through its origin is obtained. This is known as Beer-Lambert's law calibration graph.

(v) The analyte of unknown concentration is suitably diluted and its absorbance, A, is measured. From the calibration graph, the concentration of the analyte is obtained from fig. A is the absorbance of the unknown solution and C is its concentration from the graph.

Deviations and Limitations of Beer-lamberts Law

Beer-Lambert's law is valid when:

(i) The plot of absorbance vs concentration in the calibration graph is a straight line passing through the origin,

(ii) The light of a single wavelength is used, and

(iii) The solution contains only one species capable of absorbing that particular wavelength.

Deviation from Beer-Lambert's law

There are certain cases where there are deviations from Beer-Lambert's law. In the case of such deviations, the plot of absorbance vs concentration is not a straight line fig.

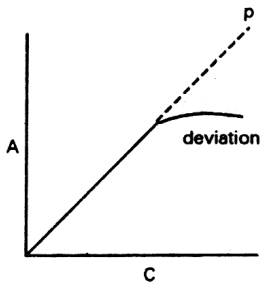


Fig.: Deviations from Beer-Lambert's law

The deviations from Beer-Lambert's law may be due to chemical or instrumental reasons.

Chemical deviations

Chemical deviations occur as a result of chemical changes associated with concentration changes. Beer-Lambert's law is not valid in case the absorbing material gets coagulated which causes the scattering of radiation, and is also not applicable in case of suspensions. Chemical deviations from Beer-Lambert's law are encountered when the absorbing species participates in a concentration dependent equilibrium,

such as dissociation or association reactions. For example, consider the spectrophotometric determination of unbuffered potassium dichromate solution, in which the following equilibrium exists

$$2\,CrO_4^{-2} + 2H^+ \longrightarrow Cr_2O_7^{-2} + H_2O$$

Dilution favours the formation of CrO_4^{-2} at the expense of $Cr_2O_7^{-2}$. The absorbance of any Cr(VI) solution is dependent upon the ratio of concentrations between the dimeric and monomeric forms. The above equilibrium ratio changes markedly with dilution, and the result may be a non-linear plot of absorbance vs total chromium concentration. Either high dilution or high acidity force the reaction virtually to one side, resulting in a linear plot.

Instrumental deviations

Instrumental deviations occur as a consequence of the manner in which absorbance measurements are made,

(i) Beer-Lambert's law applies only to absorbance measurements with monochromatic radiations (such as lasers, which are not practical for routine analytical measurements). In practice, a polychromatic continuous source is employed in conjunction with a grating or a filter that isolates a more or less symmetric band of wavelengths,

(ii) The radiation employed for absorbance measurements is usually contaminated with small amounts of stray radiation due to instrumental imperfection. "Stray radiation is the result of scattering and reflection off the surfaces of gratings, lenses, filters and windows. Stray radiation differs greatly from principal radiation, and may not have passed through the analyte or solvent.

Short Question and Answers

1. Write types of molecular spectra.

Ans :

Types of Molecular Spectroscopy

Depending upon the region of electromagnetic spectrum utilized by the molecule that leading to change in internal energy or causing transition b/w different spin orientation of nucleus in magnetic field, molecular spectroscopy is classified into following types.

(OR)

Molecular spectroscopy is classified into various types depending on the type of radiation absorbs (or) utilized by the molecules.

1. Infrared Spectroscopy

Absorption of infrared radiation leads to vibrational transitions of the molecules. The energy range involved is 4000 to 667cm^{-1}.

2. Pure Rotational (Microwave) Spectroscopy

Absorption of radiation in microwave region is responsible for rotational transitions energy involved is in the range from 100 to 100^{-1}.

3. This branch of spectroscopy deals with the transitions b/w electronic energy levels of a molecule. This transitions are brought in a organic molecule due to the absorption of ultraviolet or visible radiation.

Energy level involved is

13000 to 26000 cm^{-1} - visible

26000 to 50000 cm^{-1} - near UV

50,000 to 106 cm^{-1} - vacuum UV

4. Nuclear Magnetic Resonance Spectroscopy ^{13}CNMR/PMR Spectroscopy

This branch of spectroscopy is concerned with the study of interaction of energy with spin active nuclei which is magnetically active.

If the nuclei involved is proton, then it is termed as PMR.

It the nuclei involved is ^{13}C then it is termed as ^{13}CNMR spectroscopy.

This spectroscopy measures the energy necessary to bring about transitions b/w energy levels by subjecting the nuclei to powerful magnetic field and simultaneously irradiating it with radio frequency.

5. Mass Spectrometry

This technique is based on the principle that molecules in the gaseous phase are converted into parent and daughter positive ions by bombarding with electron beams of 70ev.

Types of Molecular Spectroscopy

1. IR Spectroscopy

An instrumental method for detecting functional groups. Give information regarding functional groups present in the molecule like aldehyde ketone, carboxylic acid etc.

2. UV Spectroscopy

Organic chemists use UV-vis spectroscopy mainly for detecting the presence and elucidating the nature of the conjugated multiple bond are aromatic rings.

Eg: Benzene $CH_2 = CH_2$, $CH_2 = CH - CH = CH_2$

3. Proton Magnetic Resonance Spectroscopy (CPMR)

PMR spectroscopy involves nuclear magnetic resonances which depend on the magnetic property of nuclei. It gives information regarding number and type of magnetic nuclei (protons).

4. Electron Spin Resonance Spectroscopy (ESR)

It is given only by free radicals. It gives information regarding presence of free radicals.

5. Mass Spectroscopy

Mass spectrometry is not an absorption spectra because the molecule is not absorbing any energy from the EMR.

Mass spectroscopy gives information regarding mol. wt. mol. formula etc.

IR and Raman Spectra

Introduction

An instrumental method for detecting functional groups.

Infrared spectroscopy deals with the recording of absorption of radiations in the infrared region of the electromagnetic spectrum. The position of a given infrared absorption is expressed in microns (m) or more commonly in terms wave number (cm^{-1}). Since it is directly proportional to energy. The ordinary infrared region 2.5 – 15 (4000 – 66 cm^{-1}) is of practical use to organic chemists. The absorption of infrared radiation by a molecules occurs due to vibrational and rotational energy. When it is subjected to infrared irradiation. Thus, IR spectra are often called vibrational rotational spectra.

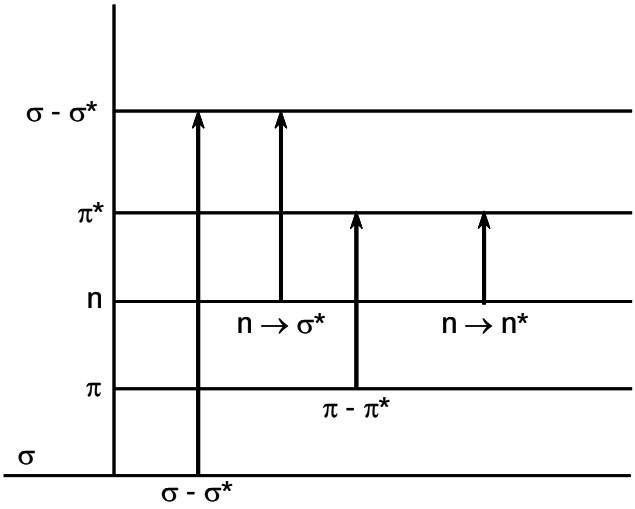
Q2. Explain the types of electronic transitions.

Ans :

According to molecular orbital theory when a molecule is excited by the absorption of energy (UV and visible light) its electrons are promoted from a bonding to an antibonding orbital. There are two types of electronic transitions.

(a) The transitions between bonding and antibonding orbitals.

(b) Transitions between non-bonding atomic orbitals and antibonding orbitals.



(a) Transitions b/w bonding and antibonding orbitals.

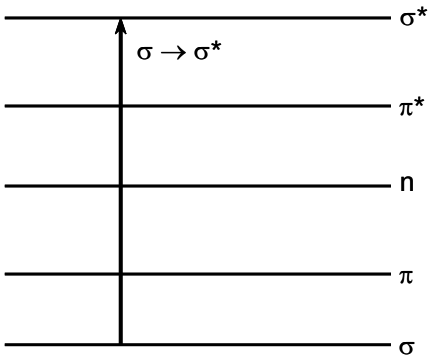
(i) σ - σ^* (bonding o to antibonding σ^*)

(ii) $\pi \rightarrow \pi^*$ (bonding *n* to antibonding π)

(i) $\sigma - \sigma^*$ Transition (120 - 400 nm)

Excitation between bonding sigma and antibonding sigma orbitals ($\sigma - \sigma^*$) requires large energies corresponding to the UV region. Thus these transitions in saturated hydrocarbons containing only σ bonds remain transparent in the near UV or visible region.

Example: Methane, Propane, cyclohexane

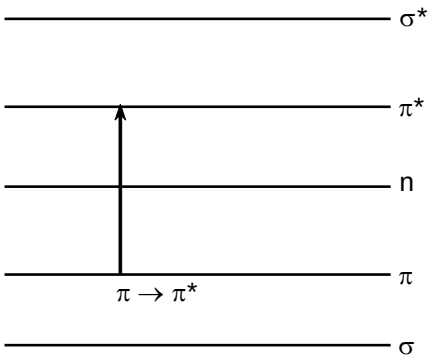


(ii) $\pi - \pi$ Transition

Transitions between bonding pi and antibonding pi are called $\pi - \pi^*$ transitions.

The transitions occur in compounds which contain double or triple bonds, aromatic rings and carbonyl group.

Ethylene absorbs at around 180 nm ig far or region where as butadiene absorbs at 217cm thus conjugated π electrons systems are readily excited $\left(\in x\ 1/\lambda\right)$.



(b) Transition between non bonding atomic orbitals to antibonding orbital.

(i) n – π* (non-bonding atomic orbital to antibonding n)

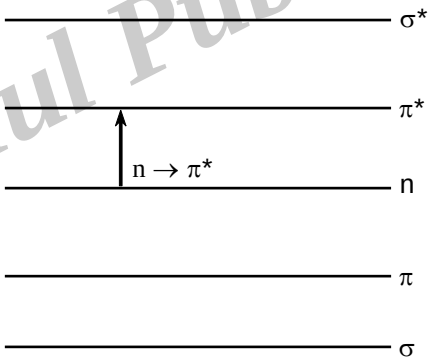
(ii) n – σ* (non bonding atomic orbital to antibonding σ)

(i) n - π* transition

Transition between non bonding atomic orbitals holding unshred pair of electrons and antibonding pi orbitals is known as n – π* transition.

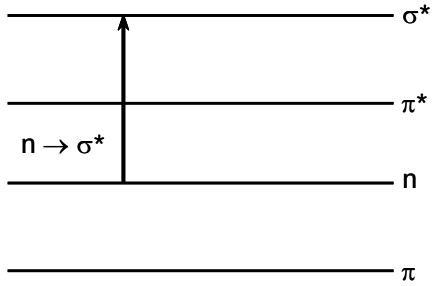
Non-bonding electrons are held loosely than s electrons and hence undergo transition at longer wavelength (because e = hv e = $\frac{hc}{\lambda}$ i.e., $\in \times \frac{1}{\lambda}$).

Example: Compounds containing double bonds involving hetro atom bearing unshared electrons. Aldehydes, ketones etc.



(iii) n – σ* Transition

Excitation of an electron in an unshared pair (non-bonding electrons) to an antibonding π* orbital is called n – σ* transition.

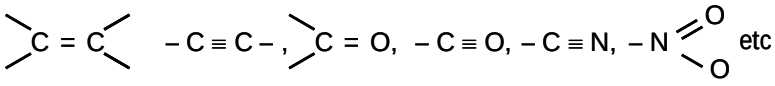


3. Define chromophores.

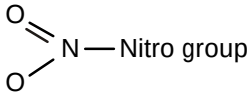
Ans :

It is defined as an unsaturated group covalently bonded unsaturated group that is responsible for absorption in the UV visible region.

Chromophore is responsible for imparting colour to the compound.

Example:  etc

Nitrocompounds are generally yellow in colour nitrogroup is chromophore which gives yellow colour to the compound.



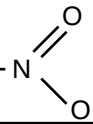
Chromophores which contains π electrons undergo $\pi - \pi^*$ transition.

Example: $H_2C = CH_2$; $HC \equiv CH$

Chromophore which contain both n and π electrons undergo two types of transitions.

$\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$

Example: Carboxyls  $C = O$, nitriles $C \equiv N$

Nitrocompounds ()

4. What is Auxochrome.

Ans:

Auxochrome is defined as the group which does not itself act as a chromophore but its presence brings shift of the absorption bond towards red end of the spectrum.

(or)

A convalently bonded saturated group which when attached to a chromophore changes both the wavelength and intensity of the absorption, maximum is known as auxochrome.

Example: –OH, –OR, –NH, –NHR, –SH etc.

5. Explain bathochromic and hypsochromic shifts.

Ans:

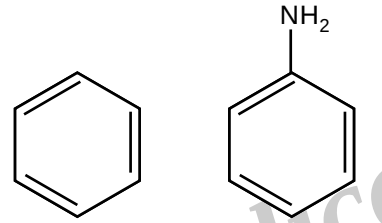
Absorption and Intensity Shifts

(i) Bathochromic Shift (or) Red Effect

Shift of an absorption maximum to a longer wave length due to the presence of an auxochrome (or) solvent effect is called as bathochromic shift (or) Red shift.

Eg: Ethylene

Benzene absorbs at 255 nm whereas aniline has absorption maximum at 280 nm (λ_{max}) Increase in λ_{max} in aniline is due to the presence of auxochrome group-NH_2.



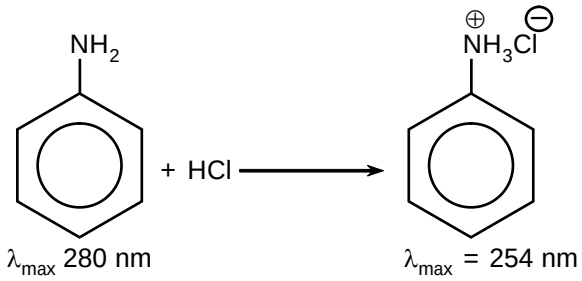
(ii) Hypsochromic Shift (OR) Blue Effect

The shift of an absorption maximum towards shorter wavelength is called hypsochromic or blue shift.

This is caused by the removal of conjugation or change in the solvent polarity.

Example: Aniline has λ_{max} at 280 nm whereas anilinium ion (acidic solution of aniline). Shown absorption maximum at 254 nm.

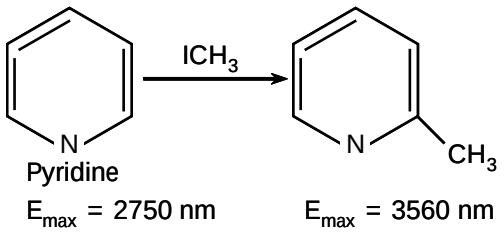
This is because of protonated aniline do not have lone pair of electrons for conjugation. Hence hypsochromic shift is due to the removal of n-π conjugation of pair of electrons of the nitrogen atom with π bonded electron system of the benzene ring on protenation.



(iii) Hyperchromic Shift

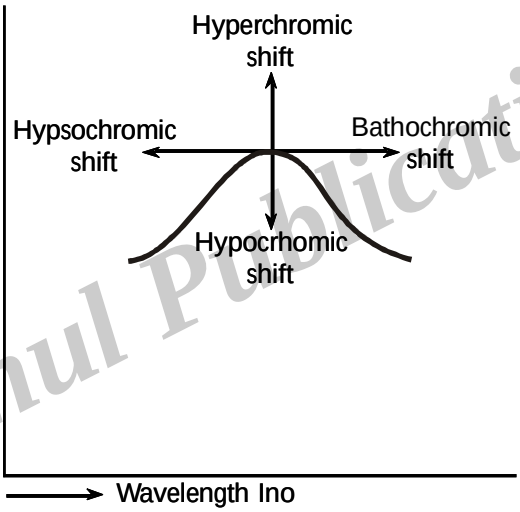
An effect which leads to an increase in absorption intensity E_{max} is called hyperchromic shift. Introduction of an auxochromes usually causes hyperchromic shift.

Example



(iv) Hyperchromic Shift

An effect which leads to a decrease in a absorption intensity E_{max} is called Hypochromic effect. This is caused by the introduction of a group which distorts the chromophore.



6. Discuss rotation spectra and selection rules of diatomic linear molecules.

Ans :

Selection Rules

For a rigid diatomic rotating molecule, the transition may take place only between those rotational energy levels for which the change in rotational quantum number is unity i.e.,

$$\Delta J = \pm 1$$

Thus according to this selection rule, transitions from rotational levels J = 0 $\rightarrow$ J = 1, J = 1 $\rightarrow$ J = 2, J = 2 $\rightarrow$ J = 3, etc. are possible, whereas transitions of the type J = 0 $\rightarrow$ J = 2 or J = 2 $\rightarrow$ J = 4, etc. are not possible. The transitions $\Delta J = +1$ and $\Delta J = -1$ correspond respectively to absorption and emission of radiation.

7. Write the selection rules of IR spectroscopy.

Ans :

Selection Rules

The simple selection rule for vibrational transition in a simple harmonic osicllator in terms of quantum number is

$$\Delta v = \pm 1$$

Thus, if any transition is taking place from any virbational level v to $v + 1$, then we have

$$\Delta E = E_{v+1} - E_v$$
$$= [(v + 1) + 1/2)]h\nu_e - [(v + 1/2)]h\nu_e$$
$$= h\nu_e \text{ (joules)}$$

or $$\Delta E / hc = \bar{\nu} = 1/2\,\bar{\nu}_e \ (cm^{-1})$$

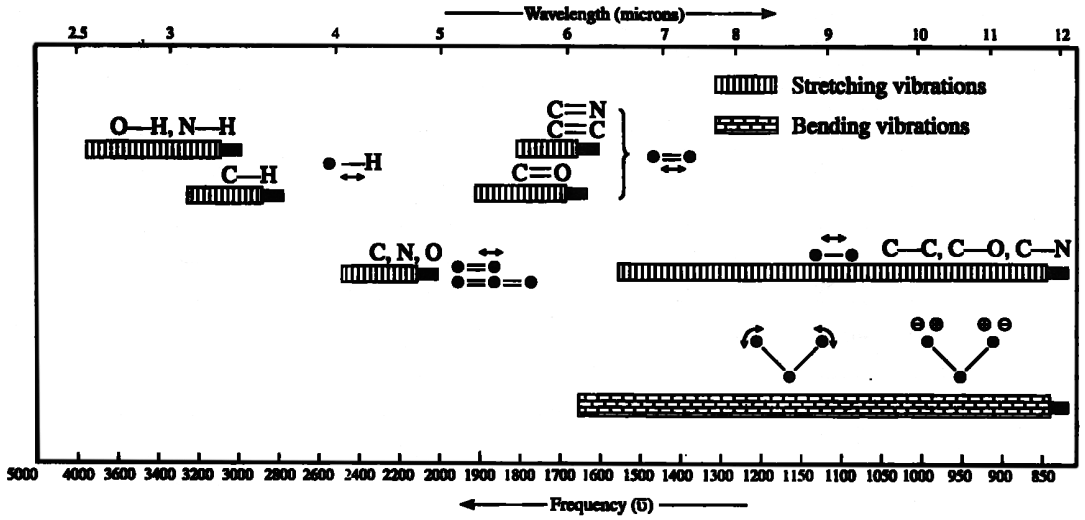
Therefore, only one spectral line will be obtained in the virbational spectrum of the diatomic molecule performing simple harmonic oscillations.

8. Give the fingerprint region of Infrared spectrum.

Ans :

Group Frequencies and Finger Print Region

The general regions of the infrared spectrum in which various kinds of vibrational bands are observed are outlined in figure. Note that the shaded sections above the dashed line refer to stretching vibrations, and below the line encompass bending vibrations. The complexity of infrared spectra in the 1450 to 600 cm^{-1} region makes it difficult to assign all the absorption bands, and because of the unique patterns found there, it is often called the fingerprint region. Absorption bands in the 4000 to 1450 cnr.[1] region are usually due to stretching vibrations of diatomic units, and this is sometimes called the group frequency region. Detailed information about the infrared absorptions observed for various bonded atoms and groups is usually presented. Since most organic compounds have C—H bonds, a useful rule is that absorption in the 2850 to 3000 cm^{-1} is due to sp^3 C—H stretching; whereas, absorption above 3000 cm^{-1} is from sp^2 C—-H stretching or sp C—H stretching if it is near 3300 cm^{-1}.



9. Define Terms Employed In Absorption Spectroscopy

Ans :

Transmittance (T)

Transmittance (T) is the ratio of the intensity of the light transmitted by the sample P to the intensity of the light incident on the sample P_0

$$T = P/P_{0,} \qquad \%T = \frac{P}{P_0} \times 100$$

The transmitted light is measured as % transmittance, which can have any value from 0-100 (100% transmittance means no absorption by the compound). From the value of the % transmittance, we can determine the amount of incident light energy absorbed. The amount of light energy absorbed by the sample solution actually depends on the concentration of the compound in the sample and this forms the basis for a quantitative analysis.

Absorbance (A)

The absorbance (A) of a solution is defined as the logarithm to the base 10 of the reciprocal of transmittance T, i.e., $A = \log_{10}\left(\frac{1}{T}\right)$

$$A = -\log T$$

as $T = \frac{P}{P_0}$

$$A = -\log\frac{P}{P_0} = \log\frac{P_0}{P}$$

Absorptivity or extinction coefficient (a)

The absorptivity a of a solution is defined as the ratio of the absorbance, A, to the product of concentration c, and path-length b

$$a = A/bc \quad \text{or} \quad A = abc,$$

where a is a proportionality constant called absorptivity, since, generally 6 is 1 cm and c is g/1, the absorptivity a has units of lg^{-1} cm^{-}.

Molar absorptivity or molar extinction coefficient ∈

When the concentration, c, in the equation A - abc is expressed in moles per litre and the path-length b, is in centimeters, the proportionality constant is called the 'molar absorptivity' or 'molar extinction coefficient' and given the symbol e.

Thus,

$$A = \varepsilon bc \quad \text{or} \quad \varepsilon = \frac{A}{bc}$$

where b is in cm and c is in mol/1, and e has the units 1 mol^{-1} cm^{-1}.

Choose the Correct Answers

1. Which of the following molecule is microwave active _____. [c]

   (a) CH_4 (b) CO_2

   (c) OCS (d) N_2

2. Which of the following molecule is microwave inactive _____. [b]

   (a) CO (b) CO_2

   (c) NO (d) NO_2

3. For symmetrical-top molecules, the moment of inertia along 3-axes is _____. [c]

   (a) $I_a \neq 0, 1_b = I_c$ (b) $I_a = I_b = I_c$

   (b) $I_a \neq 0, 1_b = I_c$ (d) $I_a \neq 0, 1_b \neq I_c$

4. Which of the following condition is not suitable for microwave spectra _____. [b]

   (a) $\Delta J = \pm 1$ (b) $\mu = 0$

   (c) $p \neq 0$ (d) Gaseous molecule

5. The region corresponding to infrared part of the electromagnetic spectrum covers the wevelength. [c]

   (a) < 4000 Å (b) < 8000 Å

   (c) > 8000 Å (d) > 2000 Å

6. The wave number's region corresponding to finger print region is _____. [b]

   (a) 1500 – 770 cm^{-1} (b) 4000 – 2000 cm^{-1}

   (c) 4000 – 6000 cm^{-1} (d) 2000 – 3000 Å

7. Which of the following molecules exhibits IR spectrum. [d]

   (a) C_2H_6 (b) C_2H_5OH

   (c) CH_3CHO (d) All

8. The carbonyl group exhibits an IR peak at 1730 cm^{-1}. The peak corresponding to 2nd overtone will be at _____. [b]

   (a) 865 cm^{-1} (b) 5190 cm^{-1}

   (c) 3460 cm^{-1} (d) 5920 cm^{-1}

9. Formation of inter molecular H-bond makes O—H bond frequency occurs at _____. [c]

(a) 3600 cm^{-1} (b) > 3600 cm^{-1}

(c) < 3600 cm^{-1} (d) 1800 cm^{-1}

10. The peaks of prominance observed in C_6H_5 CHO correspond to [d]

(a) C = C (b) C—C (aromatic)

(c) C = O (d) all

11. Why IR spectra of 1° alcohols are recorded in dilute solution in $CC1_4$. [b]

(a) Alcohols do dissociate (b) Alcohols do not form H-bonds in $CC1_4$

(c) Alcohols are not volatile (d) Alcohols are in soluble

12. The number of vibrational modes corresponding to linear a triatomic molecule is _____. [a]

(a) 4 (b) 2

(c) 3 (d) 6

13. Bending vibrations are named as _____. [d]

(a) twisting (b) wagging

(c) rocking (d) all

14. The intensity of IR peak depends on the change in ground state and excited sate of _____.[a]

(a) dipole moment (b) vibrational energy

(c) vibrational frequency (d) none

15. Ethylene and Benzene molecules undergo which of the following transition _____. [c]

(a) $\sigma \rightarrow \sigma^*$ (b) $n \rightarrow \sigma^*$

(c) $\pi \rightarrow \pi^*$ (d) $n \rightarrow \pi^*$

16. $n \rightarrow \sigma^*$ transition is found in which of the following molecule? [c]

(a) Methane (b) Ethane

(c) Ethanol (d) Propane

17. $n \rightarrow \pi^*$ transition is mainly found in which of following molecule? [d]

(a) Methane (b) Methanol

(c) Ethyl alcohol (d) Acetaldehyde

18. Which of following is a chromophore? [c]

(a) C—C (b) — OH

(c) $>C = 0$ (d) $— NH_2$

19. Which of following is an auxochrome? [c]

(a) $C = C$ (b) $C = O$

(c) — OH (d) $— N = N —$

Fill in the Blanks

1. Absorption of microwave radiation by polar gaseous molecules causes _____ motion.
2. The essential conditoion for the molecules, to become microwave active is _____.
3. The wave length of microwave region is _____.
4. For linear molecules. The moment of inertia along 3-axis is _____.
5. For spherical-top molecules, the moment of inertia along 3-axis is _____.
6. The region above 8000 cm^{-1} (wave number) in electromagnetic spectrum is _____ called region.
7. Generally the characterstic _____ frequency of the bond is used in Ir spectral identification.
8. An individual molecule can be identified by examining the vibrational bands in the _____ region.
9. The prominent vibrational band of aldehydes corresponds to _____ bond.
10. The second overtone band is due to vibrational transition from vibrational level zero to _____.
11. _____ type of molecules only absorb IR radiation.
12. The band vibrations are not generally used in IR spectral analysis.
13. The O—H bond band will be _____ in alcohols if there is intermolecular H-bond in alcohols.
14. The characteristic frequency of the OH group is at _____ cm^{-1}.
15. The characteristic frequency of the N—H band in primary amines is at _____.
16. The wave length of U.V-radiations used U.V-spectra is _____ nm.
17. U.V-visible radiation causes _____ in molecules.
18. The type of transition observed in alcohols and alkyl halides is _____.
19. The transition at λ_{max} = 252 nm, ε_{max} = 20,000 is called as _____.
20. The shift of the absorption maximum (λ_{max}) towards Shorterwave lengths is known as _____.

ANSWERS

1. Rotational
2. dipole moment (μ) not be zero
3. 10^2 m – 10^4 m (1cm – 100 μm)
4. $I_a = 0, I_b = I_c \neq 0$
5. $I_a = 0, I_b = I_c$
6. IR

7. Stretching
8. Finger Print
9. $>C = 0$
10. 3
11. Polar
12. C—C
13. Widened
14. 3600 cm^{-1}
15. 3500 cm^{-1}
16. 200 – 400 nm
17. Electronic transitions
18. $n \rightarrow \sigma^*$
19. Allowed transition
20. Hypsochromic shift

UNIT - II

(NMR and Mass Spectrometry)

S5-E-A-II: Proton Magnetic Resonance Spectroscopy

Principles of nuclear magnetic resonance, equivalent and non-equivalent protons, position of signals. Chemical shift, factors affecting chemical shifts, NMR splitting of signals - spin-spin coupling, representation of proton NMR spectrum - Integrations. 'H NMR spectrum of - ethyl bromide, acetaldehyde, 1,1,2-tribromo ethane, ethyl acetate and acetophenone.

Mass Spectrometry

Electron Impact Mass: Basic principles, Nitrogen rule, types of ions: Molecular ion and fragment ions. Representation of mass spectrum, types of peaks (molecular ion peak, base peak and isotopic ion peaks). Determination of molecular formula. Mass spectrum of ethyl chloride, ethyl bromide and acetophenone.

S5-E-A-II: PROTON MAGNETIC RESONANCE SPECTROSCOPY

Q1. Discuss the principles of NMR.

Ans : **(Imp.)**

Principles of NMR

1. Quantum Approach
2. Classical Approach

1. Quantum Approach

(a) Resonance: Matching of frequencies of radiation and frequency corresponding to energy gap of nuclear energy level is called Resonance.

(b) Flipping of Nuclei: Spin inversion of nuclei (or) change in direction of orientation of nuclei is called flipping.

Any sample with protons placed at room temperature, the protons arrangement/orientation is highly disordered. Energy of all nuclei are same. The condition is said to be degenerate, once sample is placed in external magnetic field. In this condition disordered orientation of nuclei disappears. Nuclei takes only two possible directions. Little excess nuclei orients parallel to applied magnetic field direction and little less number of nuclei orients antiparallel to direction of external magnetic field.

Parallel/aligned orientations are low energy orientations, anti parallel/opposed orientations are higher energy orientation.

The energy developed between these two orientations is known as nuclear energy gap.

Whenever sample irradiated with radio frequency radiation under resonance condition lower energy nuclei absorbs energy from radiation undergoes transitions with inversion of spin orientation and enter into higher energy levels.

Absorbed energy by nuclei will be converted into proper signals. These signals are represented in the form of a 2D-graph by taking intensity Vs frequency of radiation absorbed.

Selection rule for NMR $\Delta_{MI} = \pm 1$

MI = Nuclei magnetic spin quantum number. The number of possible spin orientation for nuclei in external magnetic field is equal to $2I+1$.

I = Spin quantum number for H = I = 1/2 $\Rightarrow 2 \times \frac{1}{2} + 1 = 2$.

NMR equation $\Delta E = \frac{\gamma h H_0}{2\pi}$

$\Delta E = gB_0 \mu_0$

γ = gyromagnetic rotation

γH = 26,753 × 10^7 radians /Tesla / Sec

g = Constant factor

μN = Nuclear magneton

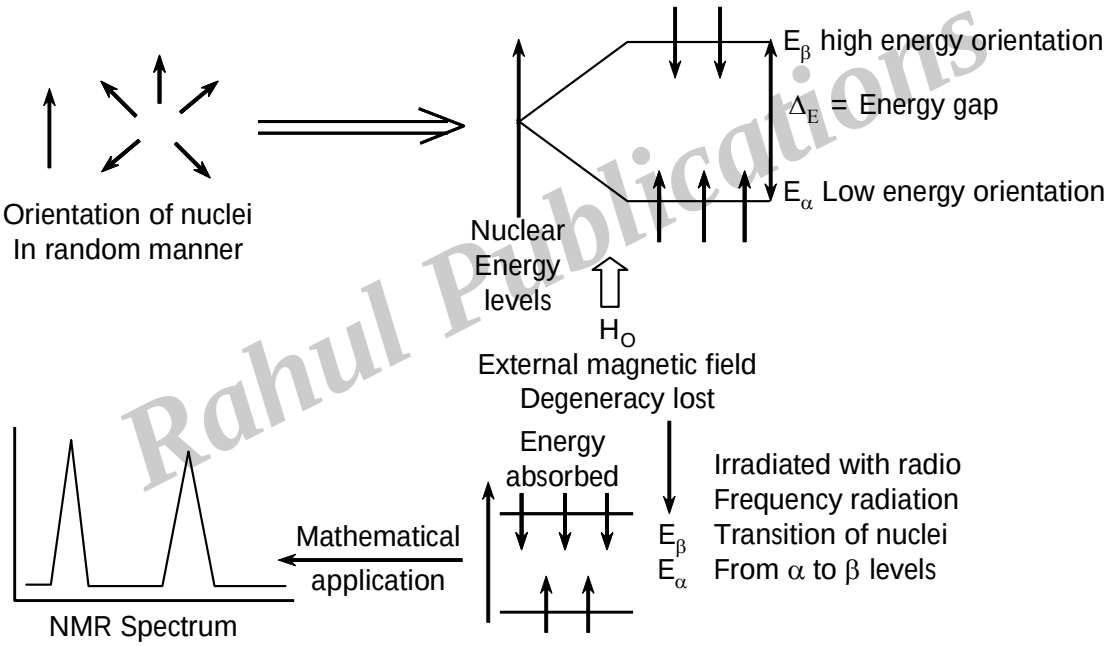
$$DE = \frac{\gamma h H_0}{2\pi}$$

But $\Delta E = h\nu$ [From plancks theory]

$$h\nu = \frac{\gamma h H_0}{2\pi} \Rightarrow \nu = \frac{\gamma H_0}{2\pi}$$

$\boxed{\Delta E \alpha H_0}$ Energy difference between two energy levels is directly proportional to strength of external magnetic field.

$\boxed{\nu \alpha H_0}$ Frequency of the nucleus directly proportional to the strength of external magnetic field.

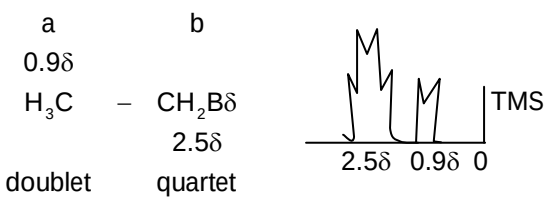


Q2. Represent the H-NMR signals and splitting pattern in the given complexes.

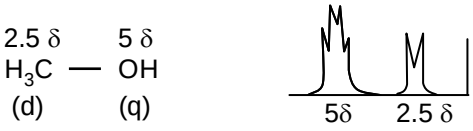
Ans : **(Imp.)**

Position of Signals

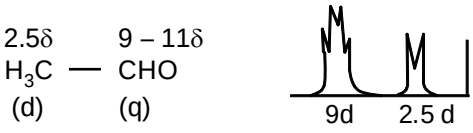
1. Ethylbromide



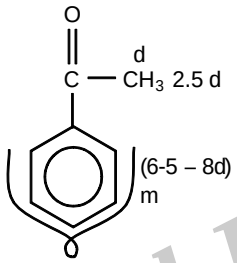
2. Ethanol



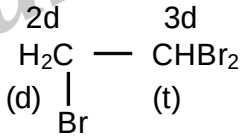
3. $H_3C - CHO$



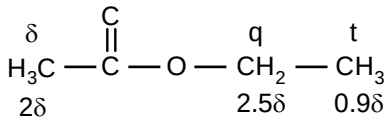
4. Acetophenone



5. 1, 1, 2, Tritromo Ethane

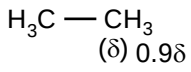


6.



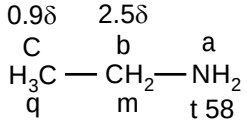
Ethylacetate

7.

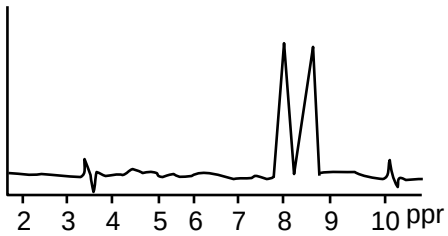


Ethane

8.



Ethylamine



Q3. Define chemical shift.

Ans : **(Imp.)**

Chemical Shift

Difference between absolute precessional frequencies of same protons and reference protons is called chemical shifts of proton.

- Chemical shift in Hz depends an applied external magnetic field, strength, frequency of instrument (ν_I).
- Reference protons frequencies are fixed at given field strength.

$$\text{Chemical Shift } (H_z) = \frac{\text{Precessional frequenceis of sample protons } (H_z)}{\text{Precessional frequenceis of reference protons } (H_z)}$$

$$\Delta\nu = \nu_{sample} - \nu_{reference}.$$

$$\delta = \frac{\text{Precessional frequencies of sample protons (Hz)} - \text{Precessional frequencies of reference protons (Hz)}}{\text{frequency of instrument in MHz}} \text{ PPM.}$$

Q4. Explain spin-spin splitting of the signals.

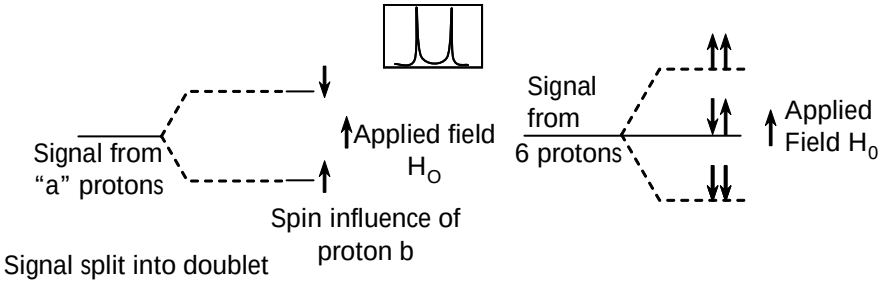
Ans : **(Imp.)**

Spin Splitting of the Signals

The splitting of a signal is due to the different environment of the absorbing proton not with respect to electrons but with respect to the near by proton's attached to the adjacent carbon atoms.

Example : 1, 1, 2 – Trichloro - ethane $\underset{(b)}{ClCH_2} - \underset{(a)}{CHCl_2}$

This compound has two types of protons. The mutual magnetic influence between the protons "a" and b is not transmitted through space but through the electrons in the intervening bonds. The nuclear spin of protons or first couples with the spin of C – H_a bonding $e^{\Theta}s$ and these in twin couple with C-C bonding $e^{\Theta}s$ and then with C–H_b bonding $e^{\Theta}s$. H_a signal appears as double because of two possible spin orientation of H_b. Upward orientation of H_B enhances Magnetic field at H_A, downward spin orientation of H_B decreases Magnetic field at H_A. Upward downward orientations equally probable because of difference fields at H_A shows two difference precessional frequencies comes into resonance twice and shows 2 line – NMR – signal. H_b proton will split into three preks under the influence of two equivalent protons a. In general a set of n equivalent protons split up a signal into a group of (n + 1) peaks (Due to neighbouring protons).



Q5. Give the factors effecting the chemical shift.

Ans : **(Imp.)**

Factors Influencing Chemical Shift

Following are the factors which influence the chemical shift:

(a) Inductive effect

(b) van der Waal's deshielding

(c) Anisotropic effects

(d) Hydrogen bonding

(a) Inductive Effect

A proton is said to be deshielded if it is attached with an electronegative atom or group. Greater the electronegativity of the atom, greater is the deshielding caused to the proton. If the deshielding is more for a proton, then its δ value will also be more. Consider the following compounds:

b a b a

(i) CH_3CH_2F (ii) $CH_3—CH_2—Cl$

Two signals are expected for each of the two compounds. Deshielding for protons 'a' in compound (i) is more than that for similar protons in compound (ii).

As the distance from the electronegative atom increases, the deshielding effect due to it diminishes. Protons are comparatively less deshielded and hence will resonate at comparatively lower value of δ.

(b) Van der Waal's deshielding

In overcrowded molecules, it is possible that some proton may be occupying sterically hindered position. Clearly, electron cloud of a bulky group (hindering group) will tend to repel the electron cloud surrounding the proton. Thus, such a proton will be deshielded and will resonate at slightly higher value of δ than expected in the absence of this effect.

(c) Anisotropic effects (Space effect)

The deshielding effect on protons attached to C = C is higher than that can be accounted for by the inductive effect alone. Aldehydic and aromatic protons are much more deshielded. Alkyne protons appear at relatively low value of δ. The values of δ (chemical shift) in each case can be justified by explaining the manner in which the π electrons circulate under the influence of the applied field. Consider an alkene. It is so oriented that the plane of the double bond is at right angles to the applied field. Induced circulation of π electrons generates induced magnetic field which is diamagnetic around carbon atom and paramagnetic in the region of the alkene protons. Thus the protons will feel greater field strength and hence resonance occurs at lower applied field.

Alkynes. In alkynes, electronic circulation around triple bond takes place in such a way that the protons experience diamagnetic shielding effect. When the axis of the alkyne group lies parallel to the direction of the applied field, the π electrons are induced to circulate around the axis in such a way that the induced field opposes the applied field. Thus, protons feel smaller field strength (shielding) and hence resonance occurs at higher applied field (low δ value).

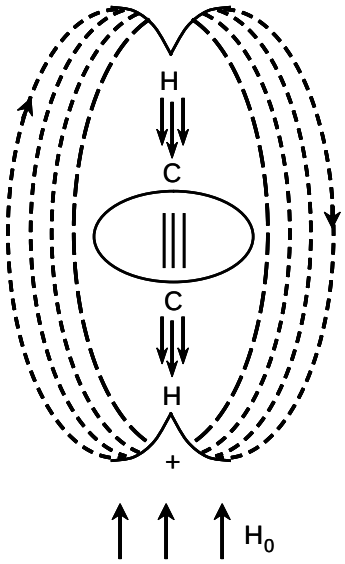
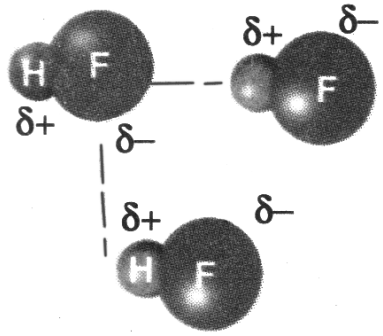


Fig.: Shielding of acetylene protons

(d) Hydrogen bonding: a hydrogen atom exhibiting property of ,hydrogen bonding in a compound absorbs at a low field. The hydrogen bonded proton being attached to a highly electronegative atom will have smaller electron density around it. Being less shielded, the field felt by such a proton will be more and hence resonance will occur downfield. The downfield shift depends upon the strength of hydrogen bonding. Intermolecular and intra-molecular hydrogen bonding can be easily distinguished.



In case of phenols, absorption occurs between – 2 to 6τ. But if the concentration is decreased, *i.e.,* if the volume of the solvent, say, carbon tetrachloride is increased, then the absorption for OH proton occurs upfield. In case the OH group on benzene is intramolecularly bonded with some other group in the ortho position, the absorption for OH proton may occur even at the negative tau value. For example, the OH proton in salicylic acid absorbs at – 0.6τ. Ends show strong intra-molecular hydrogen bonding which is further stabilised by resonance. Due to this, a great deshielding effect is caused and absorption for such a proton occurs at the negative tau value (10.5-128). It is due to the fact that acids exist as dimers as a result of hydrogen bonding. A signal for carboxylic acid proton in ethoxy acetic acid appears at –0.95τ (108).

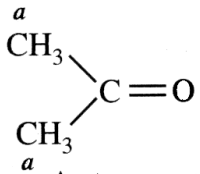
Amines show hydrogen bonding and thus, absorptions in them occur downfield. Since intermolecular hydrogen bonding is concentration dependent, the concerned proton absorption shifts upfield by decreasing the concentration. With decreasing concentration, the extent of hydrogen bonding falls the paramagnetic effect is also diminished.

Q6. What are equivalent and nonequivalent proton give examples.

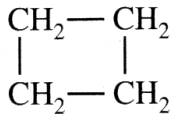
Ans :

Number of Signals

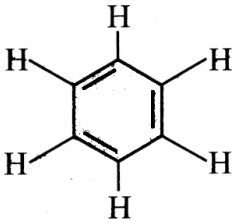
The number of signals in the nmr spectrum tell the number of different sets of equivalent protons in a molecule. Each signal corresponds to a set of equivalent protons magnetically equivalent protons are chemically equivalent protons.



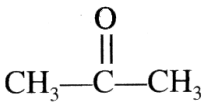
Acetone
(one signal)



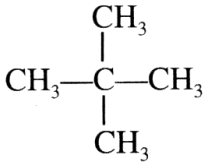
Cyclobutane
(one signal)



Benzene
(one signal)

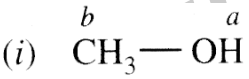


Acetone
(one signal)

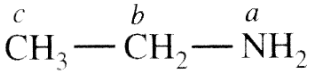


Tetramethyl methane
(one signal)

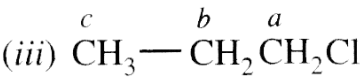
Some compounds showing more than one signal are as follows:

(*i*) 

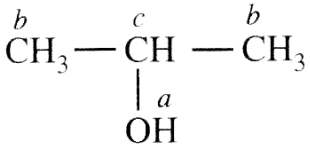
2 *nmr* signals

(*ii*) 

3 *nmr* signals

(*iii*) 

3 *nmr* signals

(*iv*) 

3 *nmr* signals

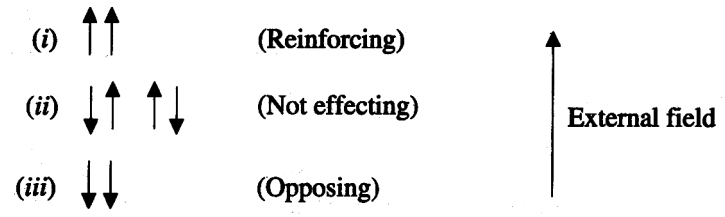
The protons may be chemically equivalent but magnetically non-equivalent. Magnetically equivalent protons have the same coupling constant to very other nucleus in the system.

Q7. Write about Spin-spin Coupling.

Ans : **(Imp.)**

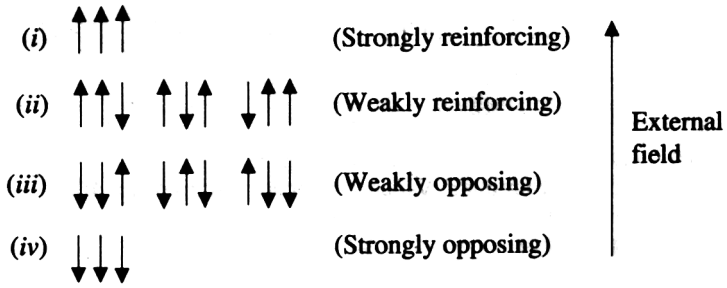
Spin-spin Coupling*

To understand it properly, consider a molecule of ethyl bromide (CH_3CH_2 Br). The spin of two protons (—CH_2—) can couple with the adjacent methyl group (— CH_3) in three different ways relative to the external field. The three different ways of alignments are:



Thus, a triplet of peaks results with the intensity ratio of 1 : 2 : 1 which corresponds to the distribution ratio of alignment.

Similarly, the spin of three protons (CH_3—) can couple with the adjacent methylene group (—CH_2—) in four different ways relative to the external field.



Thus, quartet of peaks results with an intensity ratio of 1 : 3 : 3 : 1 which corresponds to the distribution ratio of all the alignments. The relative intensities of the individual lines of a multiplet correspond to the numerical coefficient of the lines in the binomial expression:

$$(1 + x)^n = 1 + x \text{ if } n = 1$$

If n = 2, then $(1 + x)^2 = 1 + 2x + x$. Thus, the lines of the triplet have relative intensities 1:2:1.

If n = 3, then $(1 + x)^3 = 1 + 3x^2 + 3x + x^3$. Thus, the lines of the quartet formed due to the influence of three equivalent protons will have relative intensities 1 : 3 : 3 : 1.

Similarly, the lines of the pentet (quintet) formed will have relative intensities 1 : 4 : 6 : 4 : 1.

Hence, the splitting of a signal is due to the different environment of the absorbing proton not with respect to electrons but with respect to the nearby protons (Protons attached to the adjacent carbon atom). Let us consider the case of 1 : 1 : 2 trichloro-ethane $\overset{a}{Cl}CH_2-\overset{b}{C}HCl_2$.

This compound has two types of protons in it. The mutual magnetic influence between the protons 'a' and 'b' is not transmitted through space but through the electrons in the intervening bonds. The nuclear spin of protons 'a' first couples with the electron spin of C—H_a bonding electrons and these in turn couple with C—C bonding electrons and then with C—H_6 bonding electrons. Thus, the coupling is eventually transmitted to the spin of H_A nucleus. The magnetic field that the proton 'a' feels at a particular instant is slightly increased or decreased by the spin of the neighbouring proton 'b'. The field felt by proton 'a' is increased if the proton 'b' happens to be aligned at that instant with the applied field. If the proton 'b' is aligned against the applied field, then at that instant, the field felt by the proton 'a' will be slightly decreased. Thus, absorption by protons 'a' is shifted slightly downfield for half the molecules and slightly upfield for other half of the molecules. Thus, the signal for 'a' kind of protons is split into two peaks, i.e., into doublet with equal peak intensities.

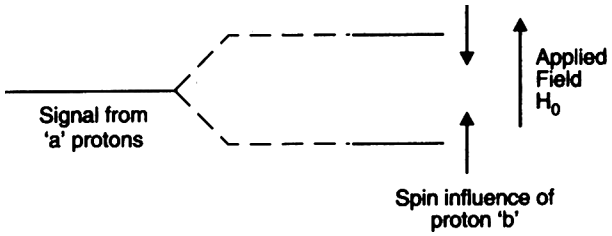


Fig.: SIngnal is split into a doublet

Thus, coupling with proton 'b' (one proton) gives 1 : 1 doublet.

Splitting by Proton 'a'

The signal from 'b' proton is effected by 'a' kind of proton (Two protons). These two protons can be aligned with the applied field in three different ways and will consequently influence the proton 'b'.

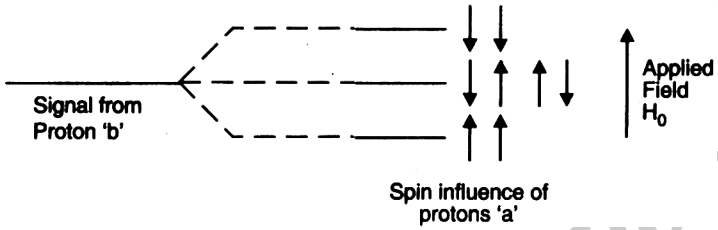


Fig.: Signal is split into a triplet

Thus, a signal for 'b' proton will be split up into three peaks (Triplet) which are equally spaced with peak intensities 1 : 2 : 1. It is called one proton triplet. Thus, in the above compound, we observe 1 : 1 doublet which corresponds to 'a' protons (—CH_2-—) and 1:2:1 triplet which represents 'b' proton. (—$CHC1_2$). The total area under the doublet is twice as large as the total area under the triplet which shows that the number of protons representing doublet is twice as many as the number of protons which represent a triplet. From this, we see that a single proton 'b' is split into a triplet (group of three peaks) under the influence of two equivalent protons 'a'. Similarly, a signal for two equivalent protons 'a' is split into a doublet under the influence of the neighbouring proton 'b'. Thus, in general, we say that a set of n equivalent protons splits up a signal (due to neighbouring protons) into a group of (n + 1) peaks (multiplet).

Q8. Can you predict whether the given compound is aromatic or not by PMR spectroscopy?

Ans :

Yes. The induced magnetic field of circulating aromatic electrons is larger than in the alkanes due to large effective ring of electrons. Due to this, the aromatic protons absorb downfield i.e. with higher δ-value (6.0 - 8.5 ppm).

Q9. What is spin-spin splitting?

Ans : **(Imp.)**

It is the interaction of the magnetic fields of two or more nuclei, both through their connecting bonds and space. Spin-spin splitting causes the PMR signals to split and thus, appear as multiplets, i.e doublets, triplets etc.

Q10. Define coupling constant (J).

Ans :

The distance between the centres of the two adjacent peaks in a multiplet is usually constant and is called the coupling constant.

Q11. What is meant by (n + 1) rule in spin-spin coupling?

Ans :

The protons on adjacent carbon atoms with different electronic environments cause spin- spin coupling. The signal that is being split by n equivalent protons appears as a multiplet with (n + 1) peaks. It is called (n + 1) rule. For example in CH_3—CH_2—Cl, the signal for CH_3 protons under the influence of —CH_2— equivalent protons appears as a triplet.

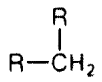
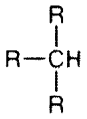
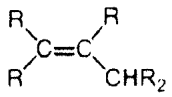
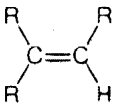
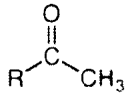
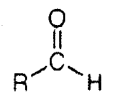
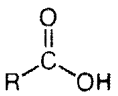
Q12. Do spin-spin coupling giving multiplets has any relation with coupling constants?

Ans :

Non-equivalent protons on adjacent carbon atoms usually show splitting and appears as multiplets. The presence of one split absorption necessitates the presence of another split signal in the spectrum. The coupling constants in these multiplets must be the same.

Q13. Give the chemical shift values of different organic compounds.

Ans : **(Imp.)**

Type of Proton	Chemical Shift (ppm)	Type of Proton	Chemical Shift (ppm)
$R-CH_3$	0.9 – 1.2	$X-CH_2R$ (X: Cl, Br, I)	3.1 – 3.8
	1.2 – 1.5	R—OH	variable, 1 – 5
	1.4 – 1.9	$R-NH_2$	variable, 1 – 5
	1.5 – 2.5		4.5 – 6.0
	2.0 – 2.6	Ar—H	6.0 – 8.5
$Ar-CH_3$	2.2 – 2.5		9.5 – 10.5
$R-C\equiv C-H$	2.5 – 3.0		10 – 13
$(H)R-O-CH_3$	3.3 – 4.0		

Q14. Define Mass spectrometry.

Ans :

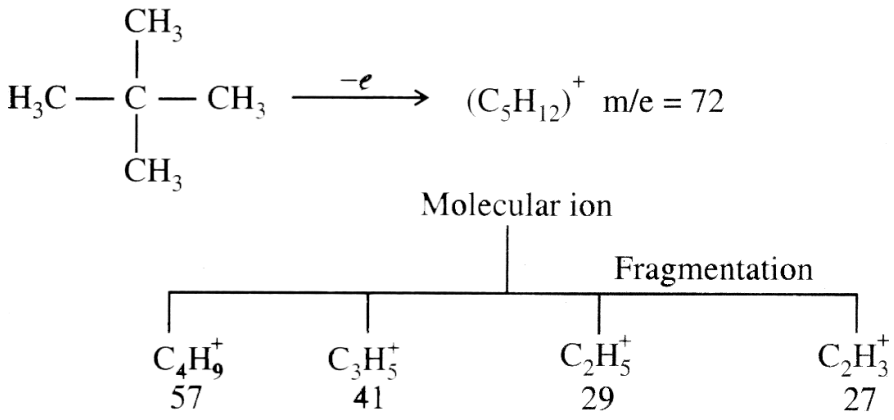
The most intense or the abundent peak in the mass spectrum of the compound is called the base peak.

Q15. Write the principle involved in mass-spectrometry elementary organic spectroscopy.

Ans :

Mass spectrometry is the most accurate method for determining the molecular mass of the compound and its elemental composition. In this technique, molecules are bombarded with a beam of energetic electrons. The molecules are ionised and broken up into many fragments, some of which are positive ions. Each kind of ion has a particular ratio of mass to charge, *i.e.* m/e ratio (value). For most ions, the charge is one and thus, m e ratio is simply the molecular mass of the ion.

Thus, for Neopentane



The molecular ion (here $C_s H_{12}^+$) is called parent ion and is usually designated as M^+. It is positively charged molecule with an unpaired electron.

The set of ions (fragment ions or daughter ions) are analysed in such a way that a signal is obtained for each value of m/e that is represented. The intensity of each signal represents the relative abundance of the ion producing the signal. The largest peak in the structure is called the base peak and its intensity is taken as 100. The intensities of other peaks are represented relative to the base, peak.

The molecular ion (parent ion) peak may not be confused with the base pcak.The base peak has 100% abundance. Mass spectrum of a compound is a plot which represents the intensities of the signals at various m/e values. It is highly characteristic of a compound. No two compounds can have exactly similar mass spectra. A single mass spectrum is equivalent to dozens of physical properties of that compound for revealing the structure. Mass spectra is used in two general ways:

(a) To prove the identity of two compounds.

(b) To establish the structure of a new compound.

The mass spectrum of a compound helps to establish the structure of a new compound in several different ways:

(a) It can give the exact molecular mass.

(b) It can give a molecular formula or it can reveal the presence of certain structural units in a molecule.

Q16. Represent the types of ions in mass spectrum.

Ans :

A parent ion results when one electron is removed from the parent molecule of the substance,

$$M(g) + e \rightarrow M^+(g) + 2e$$

The m/e value of the parent ion is equal to the molecular mass of the compound. In a few cases, the parent ion peak may be the base peak and can be easily recognized. In most of the cases, parent ion peak is not the base peak and is often of very small abundance. Many elements occur naturally as isotopes, out of these the lightest one greatly predominates. The mass spectrometer is designed to perform three basic functions. These are:

(i) To vapourise compounds of varying volatility.

(ii) To produce ions from the neutral compounds in the vapour phase.

(iii) To separate ions according to their mass over charge ratio and to record them. The plot of m/e values taken along abscissa and their relative intensities along the ordinate is called the mass spectrum.

$$M^+(g) \rightarrow m_1^+ + m_1^+ + m_4^2$$

or

$$m_1 + m_2$$

Neutral particles, produced in the process of fragmentation (whether neutral molecules or radicals) cannot be detected in the mass spectrometer.

The mass spectrum of Neopentane is shown as follows:

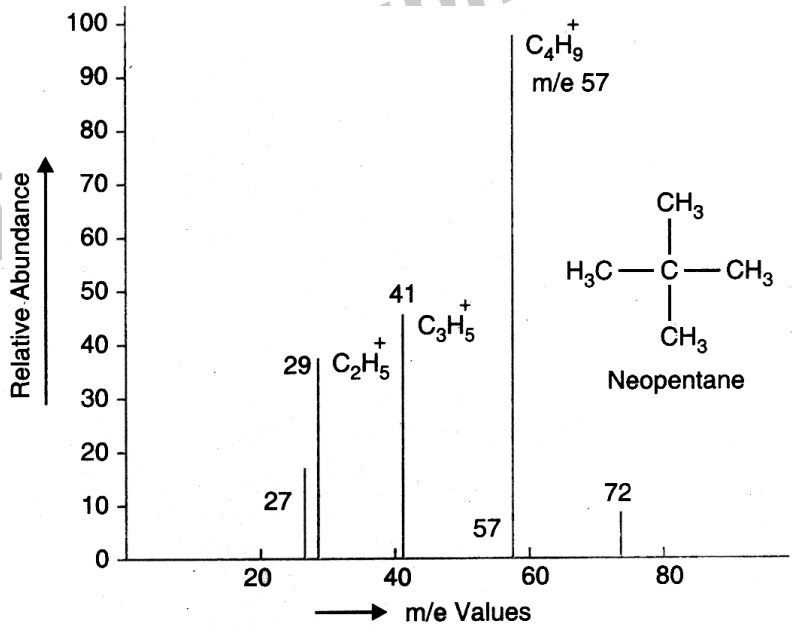


Fig.: Mass spectrum of Neopentane

The masses and the relative abundances of the molecular ion and the positively charged fragments formed from it by the electron bombardment. The m/e ratios are taken along the abscissa while the relative abundances are taken along the ordinate. Base peak is the highest peak or the most intense peak in the spectrum. Relative abundance of an ion means the percentage of the total ion current.

The molecular ion or parent ion

The electron bombardment with energy 10-15 eV usually removes one electron from the molecule of the organic compound in the vapour phase. It results in the formation of molecular ion. The highest occupied orbital of aromatic system and non-bonding electron orbitals on oxygen and nitrogen atoms readily lose one electron. An electron from double bond (two π-electrons) or triple bond (four π-electrons) is usually lost. In alkanes, the ionisation of C—C sigma bonds is easier than that of C—H bonds.

In case of chloro or bromo compounds, isotope peaks are also formed along with the molecular ion peak. In case of bromo compounds, M^+ and $(M^+ + 2)$ peaks are formed in the intensity ratio 1 : 1. In case of chloro compounds, M^+ and $(M^+ + 2)$ peaks are formed in the intensity ratio 1 : 3.

Q17. Determine the molecular formula in mass spectrometry.

Ans :

Determination of Molecular Formula

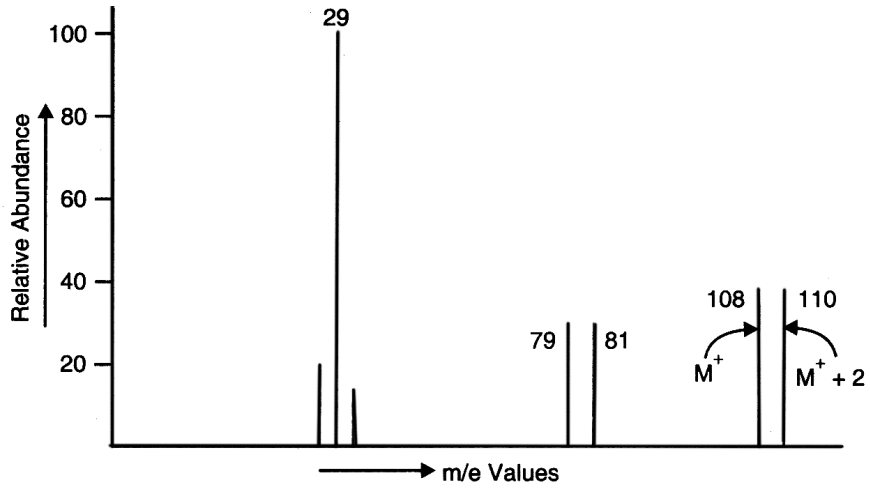
The mass spectrum is a plot representing the m/e values of the various ions (parent as well as fragment ions) against their corresponding relative abundances. The peak on the extreme right *(i.e.,* particle of highest mass) corresponds to the molecular mass of the original molecule. In case of straight chain hydrocarbons, the abundance of the parent ion peak is fair and it also gives $(M^+ + 1)$ peak which is of 9.9% abundance compared to the parent peak. Consider that a compound forms peaks at m/e values of 100, 85, 71, 57, 43 (100%) etc. Clearly, it is a straight chain alkane because fragment peaks are formed 14 units apart. In case of straight chain hydrocarbon, a peak due to $C_3H_7^+$ is most abundant *i.e.,* a base peak. Thus, a molecular formula of the compound can be obtained. In case an organic compound gives fragment as well as parent peaks in pairs which are two units apart, then

(a) If the pair of peaks are in the intensity ratio of 1 : 3, then it must be a chloro compound.

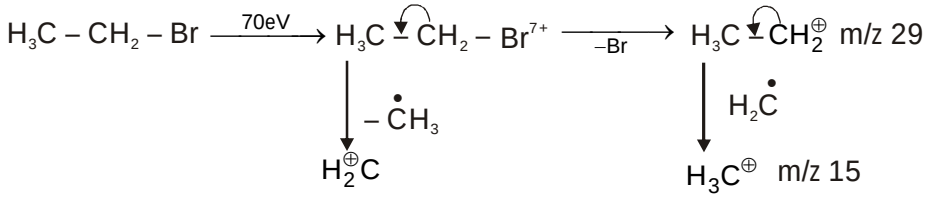
(b) If the pair of peaks appear in the intensity ratio of 1 : 1, then it must be a bromo compound.

Q18. Draw the mass spectrum of Ethyl bromide.

Ans : **(Imp.)**



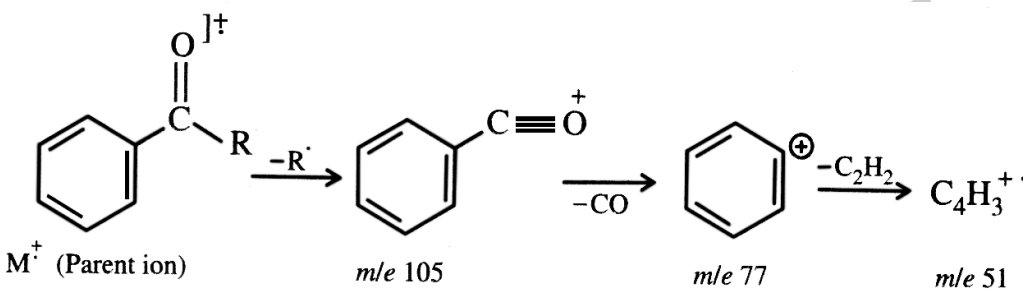
As the pair of peaks are of equal intensity, it is a bromo compound. The isotopes of bromine are $_{79}Br$ and $_{81}Br$. The pair on the extreme right corresponds to M^+ and $(M^+ + 2)$ peaks. The spectrum corresponds to the molecular formula C_2H_5Br.

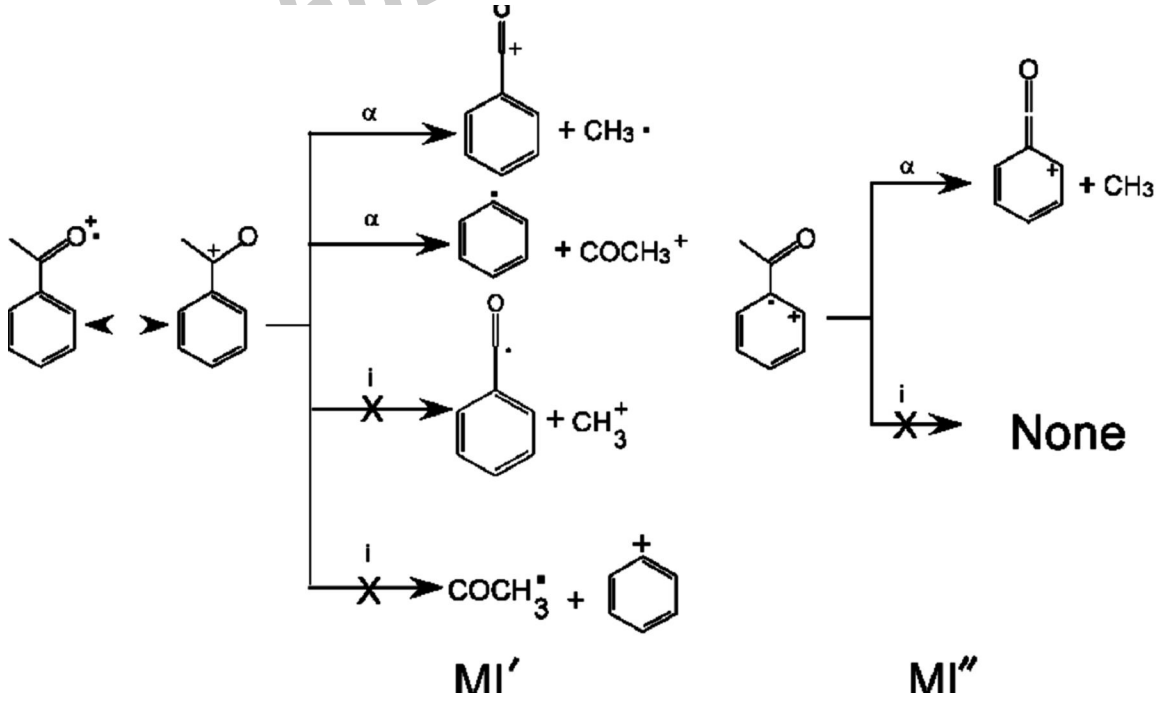


Q19. Represent the Mass Spectrum of Acetophenone.

Ans : **(Imp.)**

In ketones, the loss of larger group is preferred by an α-cleavage. Consider the fragmentation of alkyl phenyl ketone:





Q20. Define Nitrogen rule.

Ans :

A molecule of even numbered molecular mass must contain no nitrogen atom or an even number of nitrogen atoms. An odd numbered molecular mass requires an odd number of nitrogen atoms. This rule holds for all compounds containing carbon, hydrogen, oxygen, nitrogen, sulphur and halogens.

Q21. What do you mean by the base peak.

Ans :

In mass spectroscopy, the vapours of the substance are bombarded with energetic electrons. The molecular ion and fragment ions are formed which are separated according to their m/e ratio. Mass spectrum of a substance is a plot between m/e values of the ions versus relative abundance.

Q22. Determine the molecular formula by Mass Spectrometry.

Ans :

Molecular determination formula

Common elements - their isotopes and their natural Abundance

Element	Common	%M^+	Isotope-I	% M+1	Isotope-II	% M+2
Carbon	^{12}C	98.9	^{13}C	1.1	^{14}C	–
Nitrogen	$^{N}14$	100	^{15}N	0.38	–	–
Oxygen	^{16}O	100	^{17}Q	0.04	^{18}O	0.2
Sulphur	^{32}S	100	^{33}S	0.78	^{34}S	4.4
Chlorine	^{35}Cl	100	–		^{37}Cl	33%
Bromine	$^{79}B8$	100	–		$^{81}B8$	98%

Presence of M+2 peack indicates the presence of sulphur atom and bromine atom.

If M+2 peak is 4.4% indicates the presence of 'S'– atom.

- If M + 2 peak is 33% Cl M^+ : M + 2 is 3 : 1 ratio. If the M + 2 peak is equal to M^+ : M + 2 is 1 : 1 indicates the presence of Br - atom.
- M + 1 peak is useful to calculate the no.of carbon atom s.

Calculation of Molecular formula

1. Isotopic Abundance

	M^+	M + 1	M + 2
m/z	16	17	18
% RA	100	1.1	–

M + 2 peak is absent indicates the absence of S, Cl, or Br atoms.

M^+ indicates molecule of the compound = 16

M + 1 indicates the no. of carbon atoms.

No. of 'C' atoms = $\frac{\%\text{ of } M+1}{1.1} = \frac{1.1}{1.1}$ = 1 carbon

Remaining mass = Molar – wt abc

= 16 – 12 = 4

No. of Hydrogens = 4

∴ Molecular formula = CH_4

2. Isotopic Abundance

	M^+	M + 1	M + 2
m/z	50	51	52
% RA	100	1.1	33

M + 2 peak is present indicates the presence of Cl - atom M^+ : M + 2 3 : 1

No. of C – atoms = % of $\frac{M+1}{1.1} = \frac{1.1}{1.1}$ = 1 C atom

Mol.wt of 'C' atom = 1 × 12 = 12

'Cl' atom = 1 × 35 = $\frac{35}{47}$

Remaining mass = Mol wt – wt of 'C' and 'Cl' atom

= 50 – 47 = 4

No. of Hydrogen atoms = 3

∴ The molecular formula can be given as CH_3Cl

3. Isotopic Abundance

	M^+	M + 1	M + 2
m/z	72	73	74
% RA	100	4.52	0.27

No. of carbon atoms = $\frac{4.52}{1.1}$ = 4

wt of 'C' atom = 4 × 12 = 48

Remaining mass = 24

May be 'O' is present = 1 × 16 = 16

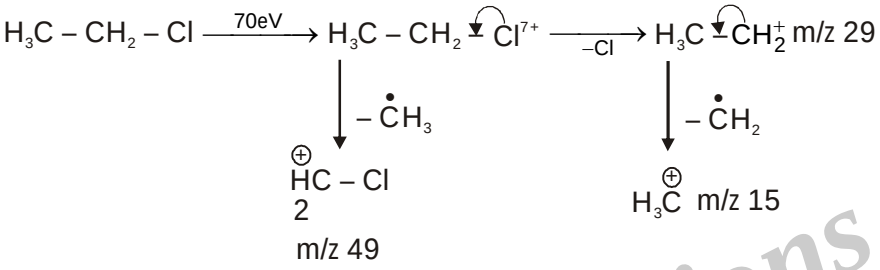
= 24 – 16 = 8 No. of H-atoms

∴ Molecular formula = C_4H_8O.

Q23. Represent the mass spectrum of Ethylchloride.

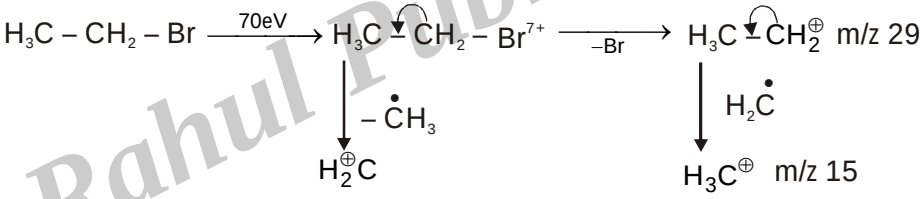
Ans : **(Imp.)**

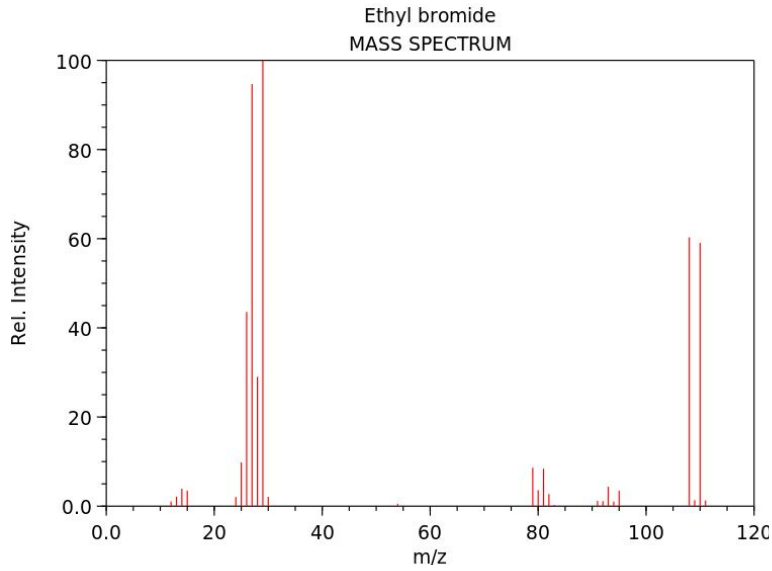
- Bond dissociation energy stability of neutral fragment are steric factors are some of the factors which determine formation of fragment ions
- E.g.: Ethyl chloride
- $CH_3 - CH_2 - Cl + e = CH_3 - CH_2 - Cl^- + 2e^-$
- $CH_3 - CH_2 - Cl^- = CH_3 - \overset{-}{C}H_2 + Cl$ or $CH_2 - \overset{-}{C}H_2 + HCl$ (Fragment ion)



Q24. Give the fragmentation pattern of Ethylbromide.

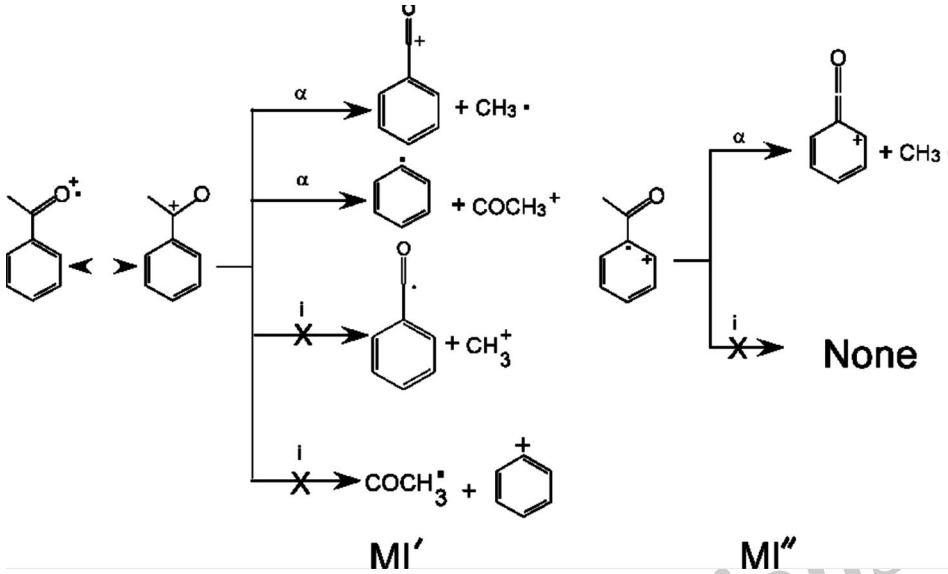
Ans : **(Imp.)**

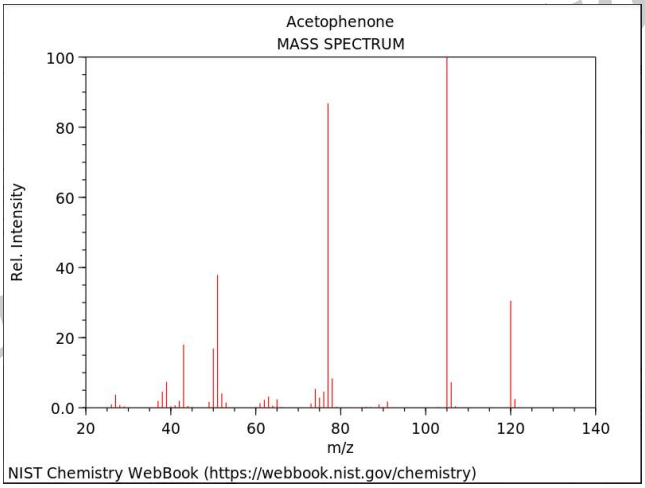


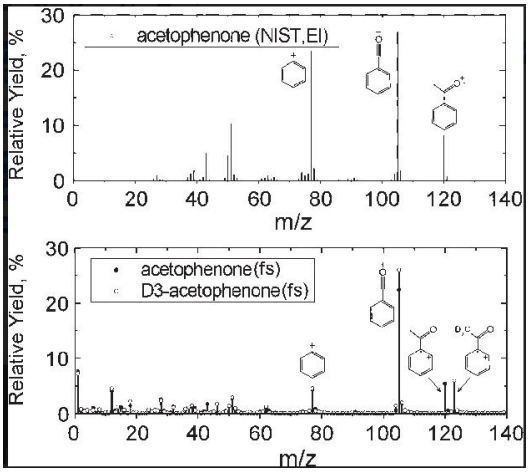


Q25. Show the fragmentation pattern in acetophenone.

Ans : **(Imp.)**







Short Question and Answers

1. Define chemical shift.

Ans :

Chemical Shift

Difference between absolute precessional frequencies of same protons and reference protons is called chemical shifts of proton.

- Chemical shift in Hz depends an applied external magnetic field, strength, frequency of instrument (ν_i).
- Reference protons frequencies are fixed at given field strength.

$$\text{Chemical Shift } (H_z) = \frac{\text{Precessional frequenceis of sample protons } (H_z)}{\text{Precessional frequenceis of reference protons } (H_z)}$$

$$\Delta\nu = \nu_{sample} - \nu_{reference}.$$

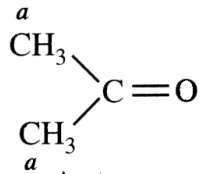
$$\delta = \frac{\text{Precessional frequencies of sample protons (Hz)} - \text{Precessional frequencies of reference protons (Hz)}}{\text{frequency of instrument in MHz}} \text{ PPM}$$

2. What are equivalent and nonequivalent proton give examples.

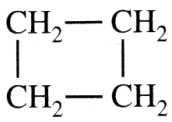
Ans :

Number of Signals

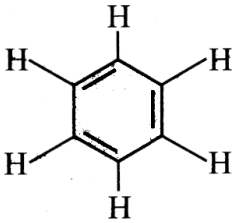
The number of signals in the nmr spectrum tell the number of different sets of equivalent protons in a molecule. Each signal corresponds to a set of equivalent protons magnetically equivalent protons are chemically equivalent protons.



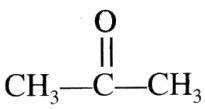
Acetone
(one signal)



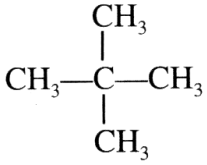
Cyclobutane
(one signal)



Benzene
(one signal)

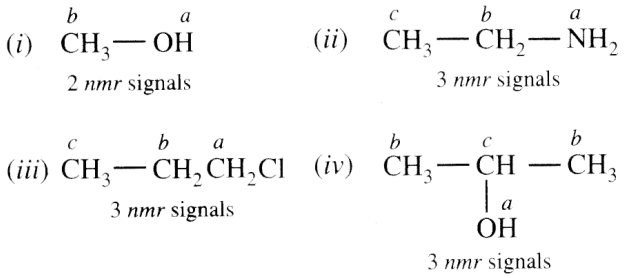


Acetone
(one signal)



Tetramethyl methane
(one signal)

Some compounds showing more than one signal are as follows:



The protons may be chemically equivalent but magnetically non-equivalent are non equivalent protons. Magnetically equivalent protons have the same coupling constant to very other nucleus in the system..

3. Can you predict whether the given compound is aromatic or not by PMR spectroscopy?

Ans :

Yes. The induced magnetic field of circulating aromatic electrons is larger than in the alkanes due to large effective ring of electrons. Due to this, the aromatic protons absorb downfield i.e. with higher δ-value (6.0 - 8.5 ppm).

4. What is spin-spin splitting?

Ans :

It is the interaction of the magnetic fields of two or more nuclei, both through their connecting bonds and space. Spin-spin splitting causes the PMR signals to split and thus, appear as multiplets, i.e doublets, triplets etc.

5. Define coupling constant (J).

Ans :

The distance between the centres of the two adjacent peaks in a multiplet is usually constant and is called the coupling constant.

6. What is meant by (n + 1) rule in spin-spin coupling?

Ans :

The protons on adjacent carbon atoms with different electronic environments cause spin- spin coupling. The signal that is being split by n equivalent protons appears as a multiplet with (n + 1) peaks. It is called (n + 1) rule. For example in CH_3—CH_2—Cl, the signal for CH_3 protons under the influence of —CH_2— equivalent protons appears as a triplet.

7. Do spin-spin coupling giving multiplets has any relation with coupling constants?

Ans :

Non-equivalent protons on adjacent carbon atoms usually show splitting and appears as multiplets. The presence of one split absorption necessitates the presence of another split signal in the spectrum. The coupling constants in these multiplets must be the same.

8. Define Mass spectrometry.

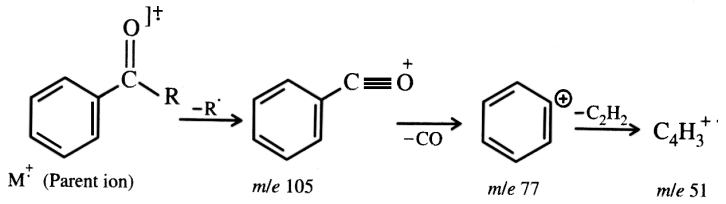
Ans :

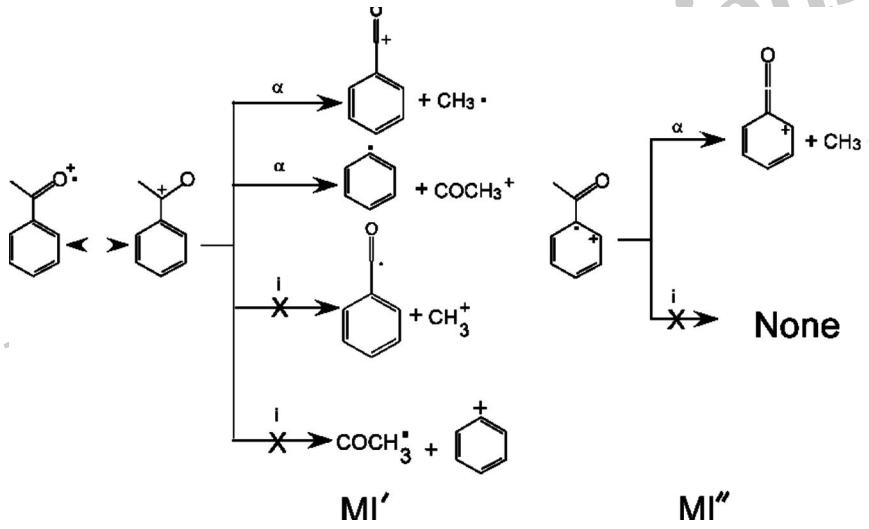
The most intense or the abundent peak in the mass spectrum of the compound is called the base peak.

9. Represent the Mass Spectrum of Acetophenone.

Ans :

In ketones, the loss of larger group is preferred by an α-cleavage. Consider the fragmentation of alkyl phenyl ketone:





10. Define Nitrogen rule.

Ans :

A molecule of even numbered molecular mass must contain no nitrogen atom or an even number of nitrogen atoms. An odd numbered molecular mass requires an odd number of nitrogen atoms. This rule holds for all compounds containing carbon, hydrogen, oxygen, nitrogen, sulphur and halogens.

11. What do you mean by the base peak.

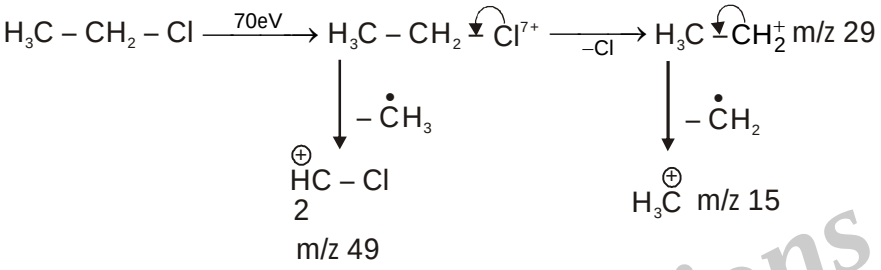
Ans :

In mass spectroscopy, the vapours of the substance are bombarded with energetic electrons. The molecular ion and fragment ions are formed which are separated according to their m/e ratio. Mass spectrum of a substance is a plot between m/e values of the ions versus relative abundance.

12. Represent the mass spectrum of Ethylchloride.

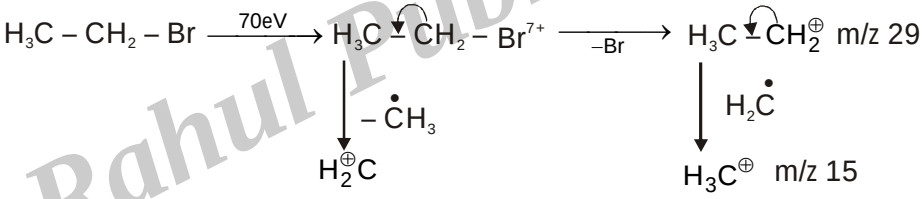
Ans :

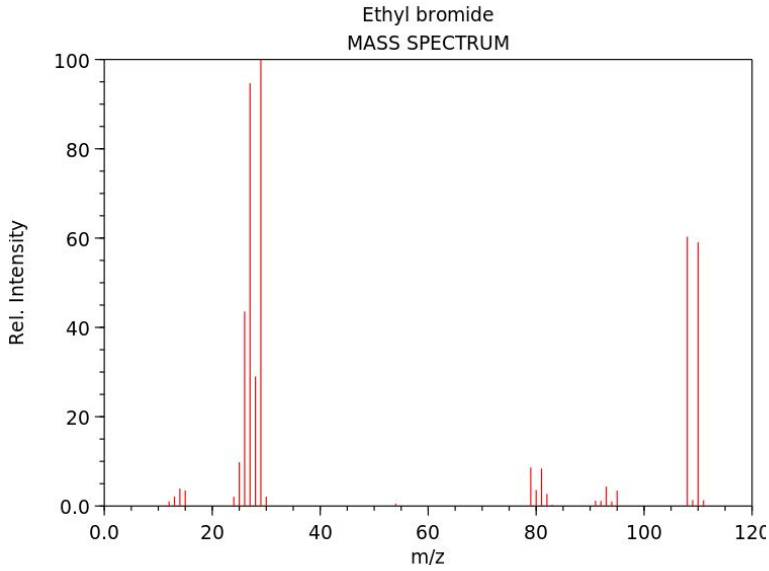
- Bond dissociation energy stability of neutral fragment are steric factors are some of the factors which determine formation of fragment ions
- E.g.: Ethyl chloride
- $CH_3 - CH_2 - Cl + e = CH_3 - CH_2 - Cl^- + 2e^-$
- $CH_3 - CH_2 - Cl^- = CH_3 - \overset{-}{C}H_2 + Cl$ or $CH_2 - \overset{-}{C}H_2 + HCl$ (Fragment ion)



13. Give the fragmentation pattern of Ethylbromide.

Ans :





Choose the Correct Answer

1. How many NMR signals are formed for 2-chloro propene. [b]

   (a) 2 (b) 3

   (c) 1 (d) none

2. Tell the number of NMR signals in case of 1, 2 dichloropropane. [c]

   (a) 2 (b) 3

   (c) 4 (d) 5

3. Write the multiplicity of the signals in $CH_3CH_2OCH_2CH_3$ in NMR spectrum [b]

   (a) Two triplets (b) A triplet and a quartet

   (c) Two singlets (d) Two singlets and two triplets.

4. Write the multiplicity of signals in CH_3CH-,OH in NMR spectroscopy. [a]

   (a) Singlet, triplet and quartet (b) Two triplets and a quinlet.

   (c) Three singlets (d) None of these.

5. In an organic compound, the proton linked to sp hybridised carbonation is more deshielded than that linked to [c]

   (a) sp hybridised carbon (b) sp^3 hybridised carbon

   (c) Both of these (d) None of these

6. In ethyl benzene ( $CH_2 - CH_3$) the tau value for CH_2 proton will be _____ than those of CH_3 protons. [a]

   (a) Lower (b) Higher

   (c) Much higher (d) Not sure

7. Out of the olefinic, aldehydic and aromatic protons, the decreasing deshielding has the order:[b]

   (a) Olefinic > aldehydic > aromatic

   (b) Aldelydic > olefinic > aromatic

   (c) Aromatic > olefinic > aldehydic

   (d) Olefinic > aromatic > aldehydic

8. Which of the following solvents cannot be used in NMR spectroscopy? [c]

   (a) $CC1_4$ (b) CS_2

   (c) $CHC1_3$ (d) $(CC1_3)_2C = 0$

9. The spin is an integer 1, 2, 3... for a nucleus having [c]

(a) Even number of protons and neutrons

(b) Odd mass number

(c) Even mass number and odd number of protons

10. NMR spectra are observed in ..... region [a]

(a) Radio frequency (b) Microwave

(c) uv/vis (d) X-ray

11. Write the number of signals and their multiplicities for the NMR spectrum of the compound, Cl F_2C-CH_2C1. [a]

(a) One, Triplet (b) Two, singlet

(c) Two, Triplets (d) None of these

12. For two sets of protons for CH_3-CH_2-CO-, part of an organic compound, the value of J for these two sets will be [b]

(a) Different (b) Same

(c) May be same or different (d) None of these

13. The signal (s) for a compound like A-CH_2-CH_2-B will be: [a]

(a) Two triplets (b) Two singlets

(c) One singlet (d) One triplet

14. Nuclear overhauser effect helps in predicting the _____. [a, b]

(a) Geometry of the molecule

(b) Two different protons in close proximity

(c) Protons on adjacent carbon atoms

(d) Olefinic protons

15. The pair of compounds which can be distinguished by NMR spectroscopy are: [d]

(a) CH_3CH_2OH and CH_3O-CH_3

(b) $CH_3CH_2CH_2OH$ and $CH_3CH(OH)CH_3$

(c) CH_3CH_2CHO and CH_3COCH_3

(d) All of these

16. The NMR spectroscopy is useful for the detection of _____. [d]

(a) Hydrogen bonding (b) Aromaticity

(c) Geometrical isomers (d) All of these

17. The molecular ion peak is usually intense for _____. [a, b]

(a) aromatic compounds (b) conjugated olefins

(c) alcohols (d) Neoalkanes

18. McLafferty rearrangement ion peak in mass spectrum is usually the basic peak for _____. [d]

(a) aldehydes (b) ketones

(c) acids (straight chain) (d) all of these

19. In the mass spectrum of straight chain hydrocarbon. [d]

(a) peaks are observed at 14 mass unit a part.

(b) peaks at 43 and 57 are most intense

(c) abundance of parent peak decreases with increase in molecular mass.

(d) all of these

20. In alkyl substituted hydrocarbons, the most abundant peak is observed at _____. [b]

(a) m/e equal to molecular mass

(b) 91 due to tropylium ion

(c) at 65 due to $C_5H_5^+$

(d) None of thes

21. In case of polynuclear hydrocarbons, the base peak appears [a]

(a) as parent ion peak (b) at 91 due to tropylium lor.

(c) at 77 due to phenylcation (d) None of these

22. In case of long chain primary alcohols, the mass spectrum consists of _____. [d]

(a) base peak of m/e 31

(b) molecular ion peak of much lower abundance

(c) peak at m/e 43 of about 60% abundance

(d) all of these

23. For an organic compound, the mass spectrum has the following m/e values: 124, 122 (low abundance), 43 (base peak), 107, 109, The organic compound is _____. [c]

(a) n-Propyl chloride (b) w-propylalcohol

(c) n-Propyl bromide (d) None

24. The m/e values for the mass spectrum of an organic compound appears at 106. $M^+ - 1$ appears 105. The other prominent peaks are at 77,51. The organic compound can be _____. [a]

(a) C_6H_5CHO (b) $CH_3(CH_2)_4CHO$

(c) $C_6H_5CH_2CH_3$ (d) None of these

25. Following peaks are obtained in the mass spectrum of an organic compound m/e values 88 73 60(MR ion), 45.

The organic compound should be _____. [c]

(a) $CH_3CH_2COOCH_3$ (b) $CH_3COOCH_2CH_3$

(c) $CH_3CH_2CH_2COOH$ (d) None

26. Following peaks are obtained in the mass spectrum of an organic compound, m/e values 102 (M^+ ion), 74 (MR ion), 59, 43.

The organic compound should be _____. [c]

(a) $CH_3CH_2CH_2CH_2COOH$ (b) $CH_3CH_2COOCH_2CH_3$

(c) $CH_3CH_2CH_2COOCH_3$ (d) None

Fill in the Blanks

1. The most intense peak in the mass spectrum of the compound is called _____
2. A molecule of even numbered molecular mass most contain _____ no. of nitrogen atoms.
3. The mass spectrum of primary alcohols contains base peak is _____
4. NMR spectra are absorved in _____ region.
5. The distance b/w the centres of the two adjacent peaks in a multiple is called _____
6. A nucleus with an _____ atomic and _____ mass number has a nuclear spin absorved in NMR spedrometer.
7. Reference compound in HNMR spectrosocpy is _____
8. Spin states for H nucleus are _____
9. Spin multiplicity in NMR spectroscopy followed by _____ rule.
10. _____ Peaks are called Isotope peaks in Mass spectromelry.

ANSWERS

1. Base peak
2. No nitrogen or even
3. m/e. 31
4. Radio frequency
5. Cayling constant
6 Odd, odd
7. Tectramethylsilane
8. 2
9. (n + 1)
10. M + 2

UNIT - III

(Separation Techniques - I)

S5-E-A-III: Solvent Extraction - Principle, Methods of extraction: Batch extraction, Continuous extraction and counter current extraction. Application - Determination of Iron (III).

Chromatography

Classification of chromatographic methods, principles of differential migration, adsorption phenomenon, nature of adsorbents, solvent systems.

Thin layer Chromatography (TLC):

Advantages, preparation of plates, Solid phase and mobile phase used in TLC, eluotoic series, development of the chromatogram, Detection of the spots, visualizing agents, factors effecting Rf values and applications of TLC.

Paper Chromatography

Principle, choice of paper and solvent systems, development of chromatogram - ascending, descending, radial and two dimensional chromatorgraphy, detection of spots, and applications of paper chromatography.

S5-E-A-III: SOLVENT EXTRACTION

Q1. What is meant by solvent extraction.

Ans :

The process of isolation or separation of a desired product(s) selectively from the reaction mixture in solution form, by extraction with another immiscible solvent, is termed 'solvent extraction'.

Q2. Write the principle involved in solvent extraction.

Ans : **(Imp.)**

Liquid-liquid Extraction

The most common method of separating organic compounds from mixtures is the liquid-liquid extraction technique. The extraction of butyric acid present in water. The aqueous phase is taken as the solvent phase 1, and is designated as S_1. Butyric acid is isolated from its aqueous solution with an immiscible, low-boiling organic solvent like diethyl ether or methylene chloride, which is taken as solvent phase 2 and designated as S_2. The S_1 (water containing butyric acid) and S_2 (ether) which is the extracting-solvent are taken into a separating funnel and shaken vigorously for a few minutes until the equilibration of butyric acid occurs between S_1 and S_2 due to distribution or partition. The concentration of butyric acid in the two solvent phases depends on the relative affinity or solubility of butyric acid in these solvents. The concentration of butyric acid in ether is more than that in the aqueous solution. The ether layer is separated out and concentrated by distillation in a rotary evaporator and poured into a petri dish to obtain butyric acid.

The transfer of a solute from one phase to another is called partition. When a solute distributes itself between two immiscible liquids, there is a definite relationship between the solute concentrations in the two phases at equilibrium. The Nernst distribution law or partition law or law of heterogeneous equilibrium states that, if a system of two immiscible liquid layers, a solute A is added which is soluble in both layers, then the A distributes itself between the two layers such that the ratio of the concentration of the solute in one solvent to its concentration in the other solvent remains constant at constant temperature, and the molecular state of the substance is the same in both solvents. This relation is expressed as,

$$\frac{\text{Concentration of solute in } S_1}{\text{Concentration of solute in } S_2} = \frac{[C_A]_1}{[C_A]_2} = K_D \text{ (constant)}$$

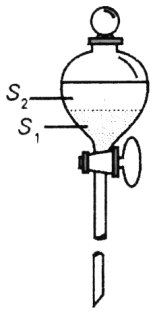


Fig.: Separating funnel with solute A distributed int two imiscible solvents S1 (water) and S2 (dietyl ether)

where S_1 and S_2 are two immiscible solvents (in this case, water and diethyl ether); A = solute (butyric acid); $[C_A]_1$ = concentration of A in S_2, i.e., diethyl ether; $[C_A]_2$ = concentration of A in S_1, i.e., water, and K_D is a constant known as the distribution or partition coefficient, and its value is constant at constant

temperature. Hence, the distribution coefficient of a compound A is equal to the ratio of its solubilities in the two solvents S_1 and S_2. Organic compounds are usually more soluble in organic solvents than in water. Hence, they can be easily extracted from aqueous solutions. For a compound A to dissolve completely in either S_1 or S_2, the value of K_D must be infinity or zero. Because A is partially present in both S_1 and S_2. If K_D is greater than 1.0 and the volume of S_1 and S_2 are equal, the compound A will be more soluble in S_1 than in S_2.

According to Nernst, there are various limitations to this law and it is applicable only when the following conditions are satisfied:

(1) The temperature should be maintained constant.

(2) It is applicable when dilute solutions are employed, because the ratio of $\frac{C_1}{C_2} = \frac{S_1}{S_2}$. will not remain constant if the concentrations are high. Therefore, the higher the concentrations in S_1 and S_2, the larger are the deviations.

(3) The Nernst equation is not applicable when the solute associates in S_2, and Eq. are modified as,

$$K_D = \frac{C_1}{n\sqrt{C_2}},$$

where n = number of molecules associating to give a larger molecule. Similarly, the Nernst equation is not applicable when the solute A dissociates in C_2. The law is modified to,

$$K_D = \frac{C_1}{C_2(1-x)}$$

Q3. Explain the Batch Extraction.

Ans : **(Imp.)**

Batch Extraction

This method is used when the distribution coefficient K_D of the compound that is to be isolated is very large in the chosen two-phase immiscible solvent system. To the solution containing the compound (with large K_D) taken in a separating funnel a solvent is added, shaken well and allowed to equilibrate. The compound is now predominantly present in the added solvent phase. The two layers can then be separated and the compound obtained. To get the remaining amount of compound from the solution, this process may be repeated two or three times with fresh solvent, Since the extraction is done in two or three batches of extraction operations, it is called batch extraction.

Batch Extraction

There are two common cases:

(1) Batch extraction of liquids, and

(2) Batch extraction of mixtures with active solvents. In these methods, the material to be extracted is brought in contact with the extracting solvent more than once, using small volumes of the extracting solvent. Hence, it is called batch extraction.

Batch Extraction of Liquids

Neutral organic compounds or neutral metal complexes (with large K_D value), dispersed or dissolved in water, can be easily separated by extracting (shaking) it with an immiscible volatile organic solvent such

as ethyl ether, $CHC1_3$, or benzene, using a separating funnel. Generally, the extracting solvent is used in three portions, shaken well each time, the separating funnel allowed to stand for some time, and the layer containing the compound is collected. After this process is repeated twice or thrice, the extracted solvent is collected and mixed together in a flask. It is dried with a drying agent such as anhydrous $CaCl_2$, or Na_2SO_4, and the solution is evaporated by distillation. The distilled solvent is collected while the residue remaining in the distillation flask contains the compound. This is a typical operation in several common organic syntheses. In the quantitative estimation of some metal ions, they are converted into neutral complexes by reaction with either 8-hydroxyquinoline or dithiazone. These complexes are generally coloured. The aqueous solution containing the complex is taken in a separating funnel and extracted with $CHC1_3$ or CH_2C1_2. The extraction is done once or twice. The coloured organic phase is analyzed spectrophotometrically, and the metal ion concentration is calculated using the Beer-Lambert Law.

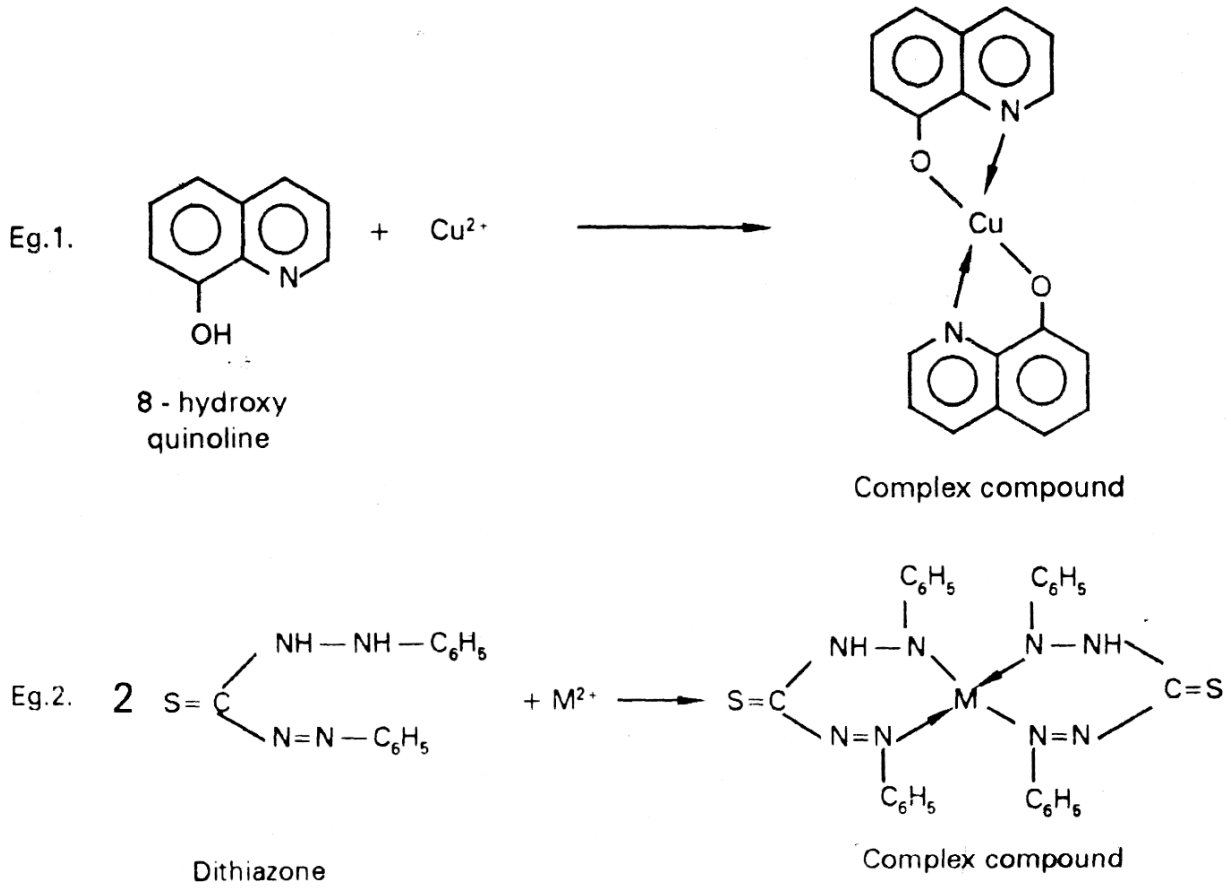


Fig.: Scheme of separation using neutal solvents or reagents

Batch Extraction of Mixtures with Active Solvents

An organic reaction product is generally a mixture of compounds. These compounds may be acidic, basic or neutral; and they may be solids or liquids. The selective separation of one or more of these compounds can be effected by the use of 'reactive solvents' such as aqueous NaOH, aqueous HC1, aqueous $NaHCO_3$, etc. Consider a mixture containing 2 g each of p-nitrobenzoic acid, p-nitroaniline and 1-nitronaphthalene. To effect their separation, this mixture is dissolved in about 200 ml of ether and taken into a separating funnel and shaken with 30 ml of 6 N HC1. The two layers are allowed to settle and the lower aqueous solution containing the p-nitroaniline as its hydrochloride salt is drained and collected in a beaker. This extraction is repeated with two more 30 ml portions of 6 N HC1. The entire amount of p-

nitroaniline is extracted as the hydrochloride salt. The neutralization of this salt solution with NH_3 leads to the precipitation of p-nitroaniline, which is collected by filtration using a Buchner funnel.

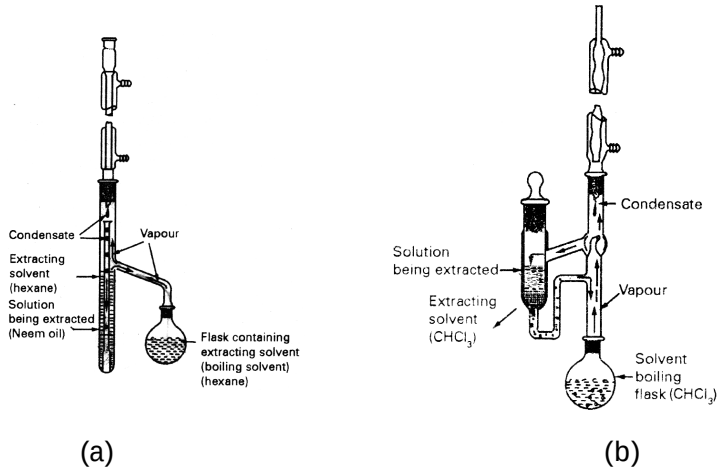
The ether solution in the separating funnel now contains p-nitrobenzoic acid and nitronaphthalene. To this, 30 ml of 6 N NaOH is added, shaken well, the layers allowed to separate, and the lower aqueous phase containing p-nitrobenzoic acids collected as its sodium salt in a beaker. The extraction is repeated with two more portions of 6 N NaOH and the aqueous solution is collected in the beaker. The entire p-nitrobenzoic acid is extracted as its sodium salt. The neutralization of this salt solution with cone. HC1 precipitates out the p-nitrobenzoic acid, which is filtered using a Buchner funnel and collected. The ether solution in the separating funnel now contains only 1-nitronaphthalene, This solution is drained out into a beaker, dried with a drying agent. The solution is taken into a china dish and warmed on a water-bath to remove the ether. The crystalline solid of p-Nitronaphthalene is obtained.

Q4. Discuss the technique of continuous extraction of liquids.

Ans : **(Imp.)**

Continuous Extraction of Liquids

This technique is generally used to isolate a desired compound from a natural oil (e.g., neem oil), and for isolating a compound more soluble in a water solvent than in the organic solvent. When the distribution coefficient of the compound between the organic phase and the aqueous solution/oil is small, we can efficiently extract the desired compound by continuous extraction, using a small quantity of the extracting (organic) solvent. Two types of extraction apparatus are used, depending on whether the extracting solvent is. lighter (benzene, hexane, ether) or heavier ($CHC1_3$, CH_2C1_2) than the extracted solution. When the extracting solvent is lighter, it moves upwards through the solution and thus the technique is known as extraction by upward displacement; and when the extracting solvent is heavier than the solution extracted, it moves downwards through the solution, and is known as extraction by downward displacement. In both these techniques, the extracting solvent, as it moves through the extracted solution, dissolves and extracts the solute. The solution of the extracting solvent and solute is continuously separated into a boiling flask. The solution is subjected to continuous distillation and the condensed distillate returns as fresh extracting solvent to the extraction vessel to be reused. In both these processes, the extracted compound builds up in an increasing concentration in the boiling flask. This is because more dilute solution is continuously draining into the flask, while, at the same time, the solvent is being distilled away.



(a) (b)

Fig.: Continuous extraction by upward and downward displacement

Extraction of Solids

The process of extraction of solids is generally used to obtain the active principle ingredients from the natural products, and from the dried tissues of plants, fungi, seaweed, mammals, etc. It is also very effective for the removal of non-steam-volatile compounds.

Batch process of extraction of sounds

This involves macerating the mature tissue with an appropriate solvent in a blender for a short time and filtering it to obtain the solvent extract. The residue is returned to the flask containing fresh solvent for further extraction. It is a crude and not very efficient method, and is considered as a preliminary extraction process.

Continuous solid-liquid extraction (Soxhlet extraction apparatus)

It is a process of continuous extraction of solids using the Soxhlet extraction apparatus. This method is used for the isolation of medicinal and other active compounds from dried plant tissues. The plant material to be extracted is taken in a porous (filter-paper or cloth) bag and placed in the main chamber of the Soxhlet apparatus. The Soxhlet is fitted with a round-bottomed flask at its lower end, containing the extracting solvent and a reflux condenser is fitted in the upper part. The solvent is heated to reflux and the distillate, as it drops from the condenser, collects in the chamber containing the plant material. This solvent is still warm and therefore effectively extracts the compounds present in the material. When the chamber fills to the level of the upper end of the siphon arm, the solution empties from this chamber into the boiling flask by a siphoning action. This constitutes one extraction cycle. The extraction cycles are continued automatically, without attendance, till the extraction of the compounds in the mixture is complete.

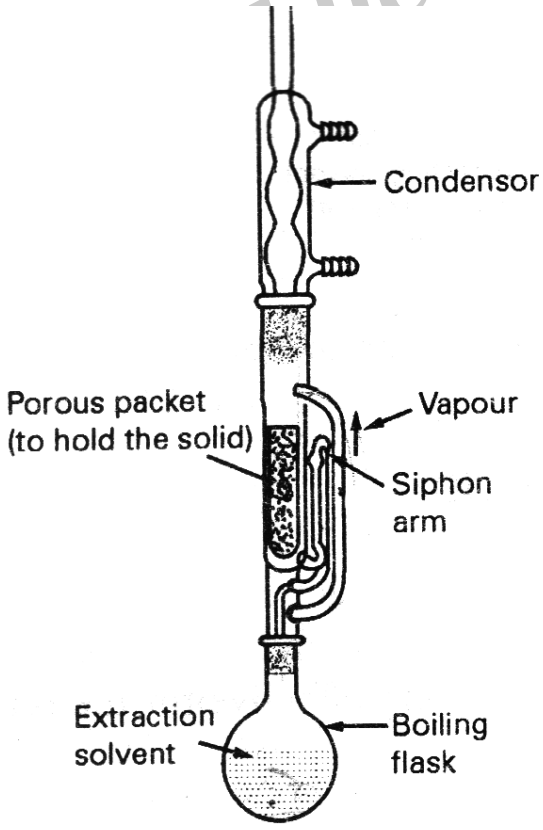


Fig.: Soxhlet extraction - continuous extraction of solids

The apparatus is disconnected and the solution in the flask is distilled to get the extracted compounds. This process of Soxhlet extraction is extensively used in research laboratories and in pilot-scale plants because of efficient separation of individual components from the mixture of compounds, and due to the simplicity of the operation. When the material to be extracted is not easily available or is available in minute quantities, this method is very useful. Depending upon the amount of material to be extracted, the size of the Soxhlet can be fabricated. Vincristin and Vimblastin are two anticancer drugs, which are extracted from the powdered leaves of the Vinca rosea plant by this procedure on a large scale. Large quantities of solvent are not required for extraction, because it is a cyclic process and the loss of solvent is low-barely 10%.

Q5. Explain the technique of counter current extraction.

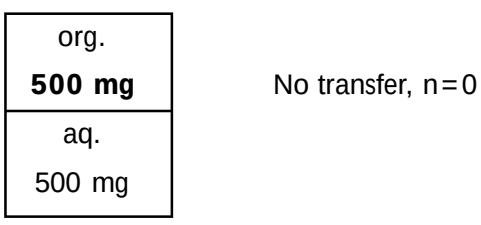
Ans : **(Imp.)**

Countercurrent Extraction

There are many cases - biological, plant extracts, reaction products - where the partition or distribution coefficients of the compounds in the mixture are of similar magnitude; that is, when the distribution coefficient of the compound desired to be separated is nearly equal to that of the other compounds in the mixture. In these cases, after solvent extraction, the relative concentration of the compounds in each of the two liquid phases may be nearly the same as those of the original mixture. Countercurrent extraction is frequently used for the separation of components in a mixture where the *differences* in the distribution coefficients are small. When the distribution coefficient is small, to get the maximum amount of solute, the total number of extractions may run from 100 to 1000, depending upon the nature of the solute. Since too large a number of manual operations running from 100 to 1000 with a separatory funnel is not practically possible, Craig has expounded the theory of the multiple extraction technique.

In such cases, the desired compound can be isolated by a solvent extraction technique called *countercurrent extraction.* It is a solvent extraction technique in which two immiscible liquids move in opposite directions in continuous contact with each other, with the resultant separation of solutes.

The basic concept of countercurrent extraction was proposed by L.C. Craig and the apparatus is named after him. Countercurrent extraction involves contact between the two phases in a large number of discrete steps. Consider 1000 mg of a solute dissolved in 100 ml of water. This is taken in a separating funnel, designated with a number 0. To this, 100 ml of an immiscible organic solvent which is lighter than water is added. In this technique, the lower, denser (aqueous) phase is designated as the stationary phase, while the upper, lighter (organic) phase is the mobile phase. Assume that the distribution coefficient of the solute in this immiscible two-phase liquid-liquid system is 1.0. The contents of the separating funnel 0 are shaken and allowed to equilibrate. After equilibrium is reached, there will be 500 mg of the solute in the lower aqueous phase and 500 mg in the upper organic phase. Next, take a second separating funnel, designated as number 1, and transfer the lighter, upper organic phase liquid from the first separating funnel (number 0) into the second separating funnel 1. Then add 100 ml of fresh aqueous solvent (water) to this organic phase in the second separating funnel and add 100 ml of the fresh organic phase to the first separating funnel 0. Now, shake both funnels until equilibrium is achieved. There will be 250 mg of solute in each layer, in each of the two funnels. This operation is designated as the first transfer.



Funnel 0	Funnel 1
org. 250 mg	org. 250 mg
aq. 250 mg	aq. 250 mg

At 1st transfer, n = l

Now, take a third separating funnel, designated as number 2, and transfer the organic phase from the second funnel (number 1) into the third funnel (number 2), and introduce 100 ml of fresh aqueous phase solvent (water) into it (third separating funnel). Now, transfer the organic phase in the first funnel (number 0) to the second funnel (number 1). Add fresh organic phase solvent (100 ml) to the 1st funnel (number 0). Then, the three funnels are shaken to equilibrium. This operation is designated as the second transfer, and the amount of solute in the three separating funnels is shown below.

Funnel 0	Funnel 1	Funnel 2
org. 125 mg	org. 250 mg	org 125 mg
aq. 125 mg	aq. 250 mg	aq. 125 mg

At 2nd transfer, n=2

It may be noted that the number of funnels is one more than the number of transfers, i.e., at the second transfer, the number of separating funnels is three. For this reason, the first funnel is labelled as 0. The number of transfers is designated as n; therefore, for the second transfer, n = 2.

From the first and second transfers, we know the general operation of this tech-nique. In the third transfer (n = 3), there are four separating funnels and the distribu-tion of the solute in these four funnels after equilibrium takes place is given below.

Funnel 0	Funnel 1	Funnel 2	Funnel 3
org. 62.5 mg	org. 187.5 mg	org 187.5 mg	org. 62.5 mg
aq. 62.5 mg	aq. 187.5 mg	aq. 187.5 mg	aq. 62.5 mg

At 3rd transfer, n=3

After the fourth transfer (n = 4), the distribution of the solute in the five funnels is given below.

Funnel 0	Funnel 1	Funnel 2	Funnel 3	Funnel 4
org. 31.25 mg	org. 125 mg	org 187.5 mg	org. 125 mg	org. 31.25 mg
aq. 31.25 mg	aq. 125 mg	aq. 187.5 mg	aq. 125 mg	aq. 31.25 mg

At 4th transfer, n=4

Doing this work manually is tedious, but using Craig's 'automated countercurrent extraction apparatus', up to 200 extractions (using 201 funnels) can be done in a relatively short time.

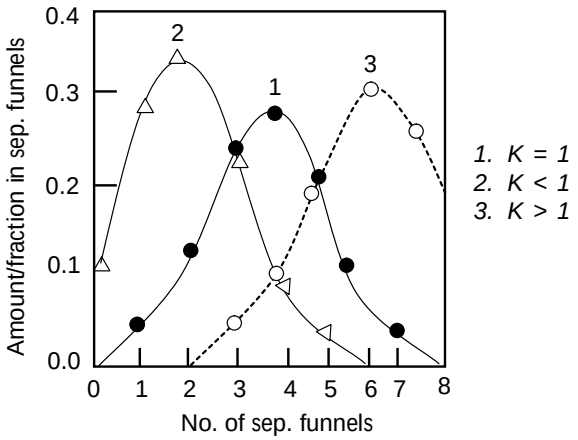


Fig.: Contercurrent extraction for different compounds whose KD = 1, KD<1, KD>1

As the number of transfers increases, the solute spreads out through more and more funnels, but it bunches up towards the centre (see the 4th transfer) and the fraction of the solute in the extreme funnels decreases. In general, the peak concentration of the solute appears in the central funnels when K_D = 1.0, as described above. For a different lute with a different distribution coefficient K_D < 1 favouring the aqueous phase, the peak concentration would not appear in the middle funnel, but rather towards the left. Likewise, a solute with K_D > 1, *i.e.*, favouring the organic phase, the peak concentration would be to the right of the centre (figure). Thus, for an aqueous solution containing several compounds with close K_D values, after several transfers, the compounds get concentrated in different funnels (left, middle or right) depending on their K_D values. Thus, countercurrent extraction is a very useful technique in the separation of complex mixtures, particularly biological molecules such as peptides, hormones, and other natural products.

Q6. How do you determine iron(III) by solvent extraction technique.

Ans : **(Imp.)**

Determination of Fe^{3+}

Based on the extracting capability, the compounds are broadly classified into two types. They are,

(i) Simple compounds

(ii) Ionic compounds.

(i) Simple Compounds

The compounds which are easily extractable from aqueous solution by using simple non-polar solvents such as aliphatic and aromatic hydrocarbons and carbon tetrachloride are considered as simple compounds. They are covalent in nature.

(ii) Ionic Compounds

The compounds which are not extractable into organic solvents from aqueous solution are called as ionic compounds. They can be extracted only by neutralizing the charges whose neutral compounds include chelate complexes, cationic complexs and non solvated salts.

Chelate System

The chelate system is used for the extraction of metal ions. Hence, its ligands with functional group, - OH and - SH are used such that the proton replaces metal ion and neutralize the charge. The chelating agents form coordinate linkages (by donating electron pair) by using coordinating atoms like N and O.

The chelating agents used in the extraction of Fe^{3+} ions are,

- Oxine (8-hydroxy quinoline)
- Cupferron (Ammonium salt of nitroso phenyl hydroxyl amine)
- DDTC (Dithiazone sodium di ethyl di thio carbonate) and
- PAN (1 - (2-phridyl azo) - 2 - napthol).

pH adjustment and concentration of chelating agents are responsible for complete extraction of chelate.

Ion Association System

In this system, halo metallic acids (formed by reaction of metal ions with halide ions in acid solution) and pseudo halo metallic acids (formed by reaction of metal ions with pseudohalide ions in acid solution) are employed in the extraction of the metal ions. Tributyl phosphate solvent is widely used for these systems.

Example

For $FeCl_3$ – HC1 – ether system, the extracted species are H^+(ether), $FeCl_4^-$.

Methods of Extraction

Fe^{3+} ion is extracted by the following two methods,

1. By Chloride Extraction

This method is used for the determination of iron in steel. Fe^{3+} i.e., Ferric solution (i.e., 99.9% of iron) can be extracted from concentrated HC1 (6M) using ether. Ether layer is separated and evaporated (by boiling). In acid solution, ferric iron is reduced to ferrous iron and then to (Fe(II)) solution which is then titrated with standard $K_2Cr_2O_7$ solution.

2. By Oxine (8-hydroxyquinoline)

In this method, 1% oxine in $CHC1_3$ of aqueous solution contains ferric iron. Initially 50 ml of iron solution and 10ml of 1% oxine solution are mixed to form a solution. Then, the separation of $CHC1_3$ extract gives the iron which is determined by calorimeter at 470nm. Blank Solvent (distilled water) is used such that the standard ferric iron solution is responsible to determine the Fe^{3+} ion based on the constructed graph in this process. This process is carried out only when pH ranges between 2 to 10.

S5-E-A-III: SOLVENT EXTRACTION

Q7. Define chromatography.

Ans :

Chromatography

Chromatography is a physical method of separating mixtures of Organic compounds biomolecules, organic and inorganic salts, etc., by distribution or partition between two phases. One of the phases is the stationary phase and the other is the mobile phase. These two phases are in contact with each other and the mobile phase moves through the stationary phase. The compounds in the mixture have different values of partition or distribution coefficients, and the separation is based on this difference in partition coefficients.

Q8. Classify the chromatography methods based on the

(i) Nature of the mobile phase

(ii) The Nature of the mobile phase

Ans : **(Imp.)**

Chromatography is a physical method of separating a mixture of compounds. The compounds or ions in the mixture do not undergo any chemical change during a chromatographic separation. Properties such as adsorption or partition of a compound between two phases form the basis for the separation of mixtures. Using chromatography, it is possible to separate a mixture of organic compounds, amino acids, sugars, cations and anions. Generally, it is possible to obtain all the compounds in a mixture in a pure form. A chromatography experiment is carried out in order to:

The common chromatographic techniques are:

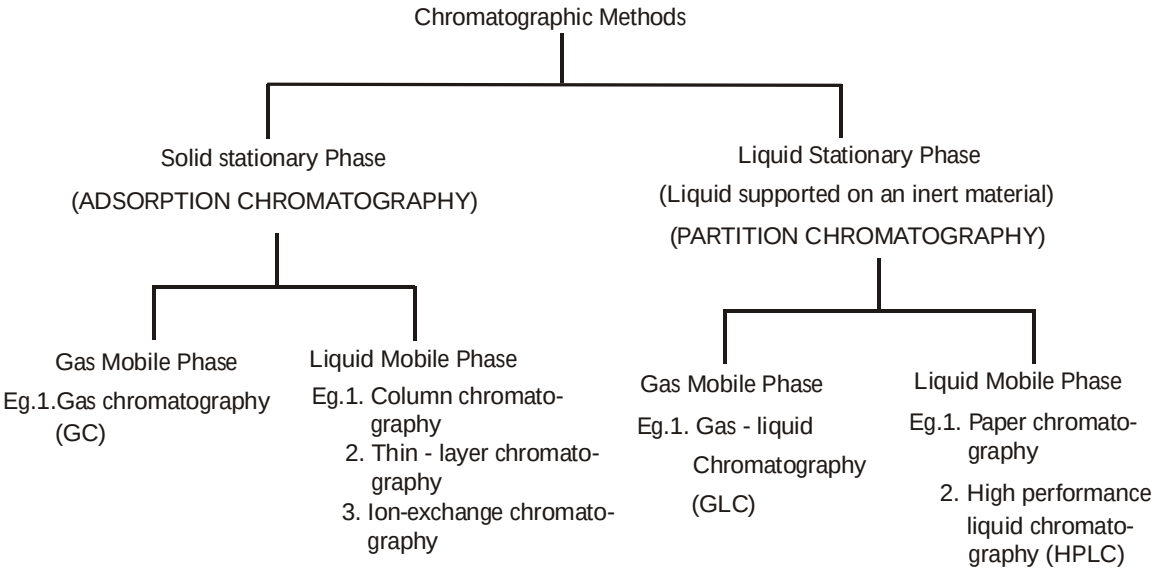
1) Column chromatography (CC)
2) Thin-layer chromatography (TLC)
3) Ion-exchange chromatography (IEC)
4) Gas chromatography (GC)
5) Paper chromatography (PC)
6) Gas-liquid chromatography (GLC) and
7) High-performance liquid chromatography (HPLC)

In all these chromatographic methods, two phases are necessary to effect the overall separation of the compounds in the mixture analyzed. These two phases are: (1) the stationary phase a phase that does not move. The stationary phase may be a solid or liquid, and (2) the mobile phase a phase that moves. A mobile phase may be a liquid or gas.

Classification of chromatographic Methods

Chromatographic methods are classified either on the basis of the stationary' phase used or the mobile phase used.

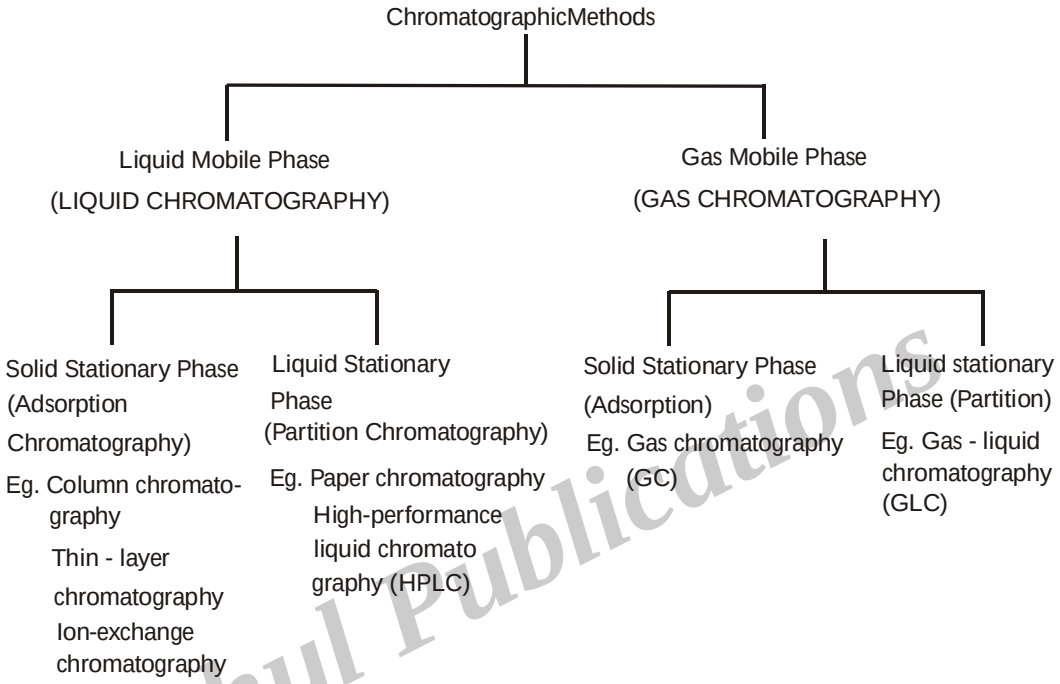
Classification based on the stationary phase



The techniques that use a solid as the stationary phase are called adsorption chromatography. Adsorption chromatography is based on the principle that different compounds in a mixture have different strengths of adsorption to the stationary phase.

The techniques that use a liquid as the stationary phase are called partition chromatography. Partition chromatography is based on the principle that different compounds in the mixture have different values of partition or distribution coefficients in a two-phase (liquid-liquid or liquid-gas) system.

Classification based on the mobile phase



The classification based on the stationary phase is generally followed in the study of chromatography.

Q9. Explain the Technique of thin layer chromatography (TLC).

Ans : **(Imp.)**

Principle of TLC

A solid is used as the stationary phase and a liquid as the mobile phase, the TLC is a solid-liquid chromatography. The physical principle involved in the separation of compounds in a mixture is adsorption. Thus, TLC is an adsorption chromatography. The separation of compounds is due to their different adsorption strengths to the adsorbent. The role of the mobile phase solvent is to desorb the compounds from their binding to the adsorbent, dissolve the compounds in themselves and carry the compounds upwards, in the direction of the movement of the solvent. The compounds move due to dynamic adsorption and desorption. Thus, in the case of the sample containing A and B, where B is more polar than A and the stationary phase is polar, A is partitioned or distributed between the stationary phase (adsorbent) and mobile phase (solvent). Similarly, B is partitioned between the adsorbent and the solvent.

$$\text{Partition coefficient of A} = \frac{\text{Conc. of A in the stationary phase}}{\text{Cone. of A in the mobile phase}} = k_A$$

$$\text{Partition coefficient of B} = \frac{\text{Conc. of B in the stationary phase}}{\text{Conc. of B in the mobile phase}} = k_B$$

The partition coefficient of B is greater than A, because B being more polar than A has greater affinity to the stationary phase than A. Compound A with a lower value of partition coefficient is present more in the mobile phase, the moving phase, and therefore moves faster than B. Thus, the compounds get separated due to differences in their adsorption strengths which results in different values of partition coefficients.

Q10. Describe the applications of TLC in a Organic Laboratory.

Ans : **(Imp.)**

TLC has several applications. It is used to:

1. Determine the number of compounds in a sample (purity determinations).
2. Monitor the progress of organic reactions.
3. Identify unknown compounds by a comparison with suspected references or standards.
4. Monitor the progress of column chromatographic separation or any other purification process such as crystallization, distillation, solvent extraction, etc.
5. Select the starting eluent for column chromatography.
6. Effect small-scale (10 – 100 mg) quantitative separation of mixtures. This technique is known as preparative TLC.

   A thin-layer chromatography experiment consists of: (1) preparation of TLC, plates (coating the stationary phase on a glass plate as a thin layer), (2) application of the sample solution as a spot, (3) development of the TLC plate using organic solvents (mobile phase), and (4) detection of the compounds.

11. Write the stationary phase materials or adsorbents in TLC.

Ans : **(Imp.)**

Stationary phase materials: Adsorbents : Preparation of TLC plates

In thin-layer chromatography, as in the case of column chromatography, the stationary phase materials are silica gel and alumina. These are the adsorbents for TLC. For TLC, a special grade of silica gel and alumina known as silica gel G and alumina G, where G stands for calcined gypsum $CaSO_4 \frac{1}{2} H_2O$, are used. Silica gel G and alumina G contain 10-13% by weight of gypsum which acts as a binder. The silica gel G or alumina G is finely-powdered and fiour-like. (The column chromatography-grade silica gel or alumina have 60-200 mesh size and do not contain gypsum). The silica gel G or alumina G is made into a slurry in $CHCl_3$:MeOH - 2:1 v/v or water and applied as a thin layer on a microscope slide or glass plate of 20 × 5 cm size. Due to the water/MeOH, gypsum becomes $CaSO_4.2H_2O$ and binds the adsorbent silica gel or alumina firmly to the glass plate. The water used for coating the adsorbent to the glass plate is removed by drying the TLC plate in a hot-air oven. When $CHCl_3$:MeOH is used, the TLC plate is dried just by exposing it to the air for 5 minutes. The thin layer of silica gel G or alumina G coated on the glass plate is the stationary phase for this chromatography, the glass plate is only acting as a support for this coating. This solid stationary phase is also called an adsorbent. In addition to silica gel G and alumina G, materials such as finely-powdered cellulose and polyamide are also used as adsorbents for TLC. The choice of a proper adsorbent depends on the type of compunds to be separated or analyzed. Silica gel G is widely used for routine TLC work.

Silica gel G	:	Majority of organic compounds, in particular, phenols, esters, acids
Alumina G	:	Basic compounds
Cellulose and Polyamide	:	Polyhydroxy and highly polar organic compounds

Q12. How do you prepare the TLC plates and spotting of sample on TLC.

Ans :

Sample application : Spotting

To know the number of compounds in a sample using the TLC method, a dilute solution of the sample (about 1 mg/1 ml) is made in a volatile organic solvent such as $CHCl_3$, CH_3COCH_3, MeOH, etc. Using a microcapillary tube, a few microlitres of the sample solution is applied as a spot on the adsorbent-coated TLC plate at a distance of about 1 cm from the end of the plate. In this process, a

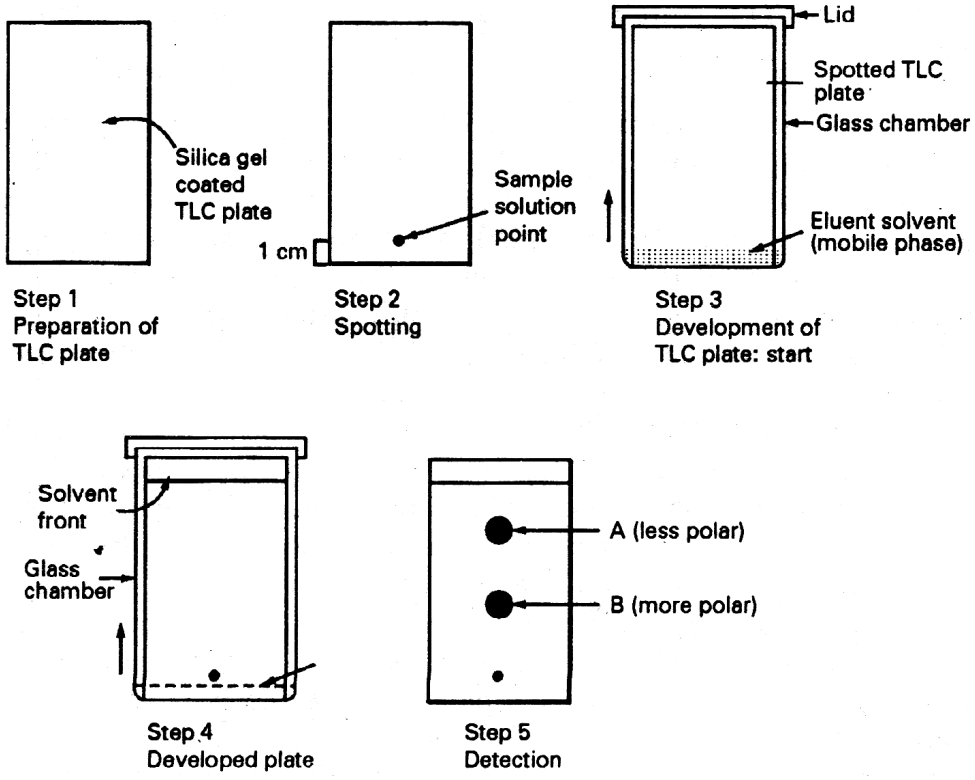


Fig:. Thin - loyer chromatography

few micrograms of the sample is delivered onto the adsorbent. This step is known as the spotting of the sample or sample application. The compounds in the sample are adsorbed onto the surface of the adsorbent particles. Different compounds in the sample are adsorbed with different adsorption strengths. The strength of adsorption or binding is due to a combination of van der Waals', dipole interactions, and H bonding. These adsorption forces depend on the molecular weight, size, shape and/or functional groups in the compound. No two compounds have the same strength of binding towards a given adsorbent. Assume that the sample solution spotted contains two compounds A and B, where B is more polar than A. The adsorbents, silica gel G and alumina G, are highly polar and are called polar stationary phases. Towards a polar stationary phase, the more polar B is strongly adsorbed relative to A. To a polar stationary phase, polar compounds bind strongly and less polar compounds bind weakly.

Q13. What are R_f values. How do you calculate and write there applications.

Ans : **(Imp.)**

R_f Values in TLC

R_f (retardation factor) values and its applications

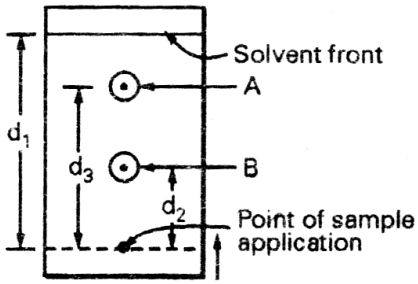


Fig:. R_f values in thin - layer chromatography

The thin-layer chromatography particulars of a compound are expressed in terms of retardation factor (R_f) values. The stationary phase "retards" the motion of the compounds by adsorption. Different compounds are retarded to different extents, depending on their adsorption strength. In TLC, the solvent moves ahead of the compounds and the edge of the solvent is called the solvent front. The ratio of the distance that the compound travels to the distance the solvent front travels is called the R_f value. The R_f value of a compound can be calculated by measuring the distances in centimetres, between the solvent front and the point of sample application (d_1) and the distance from the middle of the spot to the point of sample application (d_3, d_2) for compounds A and B.

$$R_f \text{ of compound A} = \frac{\text{distance moved by compound A } (d_3)}{\text{distance moved by solvent front } (d_1)}$$

$$R_f \text{ of compound B} = \frac{\text{distance moved by compound B } (d_2)}{\text{distance moved by solvent front } (d_1)}$$

The R_f value of a compound is always less than 1. The R_f value depends on the: (1) nature of the adsorbent (silica gel or alumina), (2) nature of the solvent system, and (3) thickness of the adsorbent coating. These parameters should be mentioned while reporting the R_f values. The R_f value of a compound remains the same whether it is in its pure form or if it is present in a mixture.

The R_f value is an important physical property of a compound, and is useful to identify an unknown compound by its TLC comparison with a suspected reference or standard. Solutions of the unknown and of the suspected reference compounds are spotted on the same TLC plate, developed and the spots detected.

Q14. Describe the technique of paper chromatography justify that it is a liquid - liquid partition chromatography.

Ans :

Principle of paper chromatography : Partition or distribution Coefficients

The mobile phase moves through the stationary phase, the compounds A and B get distributed or partitioned between the two phases. Note that cellulose-bound water is the stationary phase and the

organic solvent. + water is the mobile phase, and together they constitute a immiscible two-phase system. Now, compound A is distributed or partitioned between the stationary phase and the mobile phase. The ratio of the concentration of A in the stationary phase to the concentration in the mobile phase is known as the partition coefficient or distribution coefficient. Similarly, the compound B is partitioned between the stationary phase and the mobile phase and will have a value of partition coefficient, which is different from that of A. No two organic compounds, cations, anions, etc. have the sam'e value of partition coefficient in a given two-phase system.

Suppose that B is more soluble in the mobile phase than A. Its concentration is more in the mobile phase and less in the stationary phase and its partition coefficient value is less. A is less soluble in the mobile phase (relative to B), its concentration in the mobile phase is less and its concentration in the stationary phase is more.

$$\text{Partition Coefficient of Compound B} = \frac{\text{Conc. of B in sta. phase}}{\text{Conc. of B in mob. phase}}$$

$$\text{phase Coefficient of Compound A} = \frac{\text{Conc. of A in sta. phase}}{\text{Conc. of A in mob.phase}}$$

The partition coefficient of A is greater than that of B. For chromatographic separation it is necessary that the compounds in a mixture must have different partition coefficients. Now, compound B is more soluble in the mobile phase (which is the moving phase), its concentration is more in the mobile phase, and it moves faster relative to A. Compound A has more solubility (more affinity) in the stationary phase (a phase that does not move), its concentration is more in the stationary phase, and therefore it moves slowly relative to B. Thus, compounds A and B get separated on the paper.

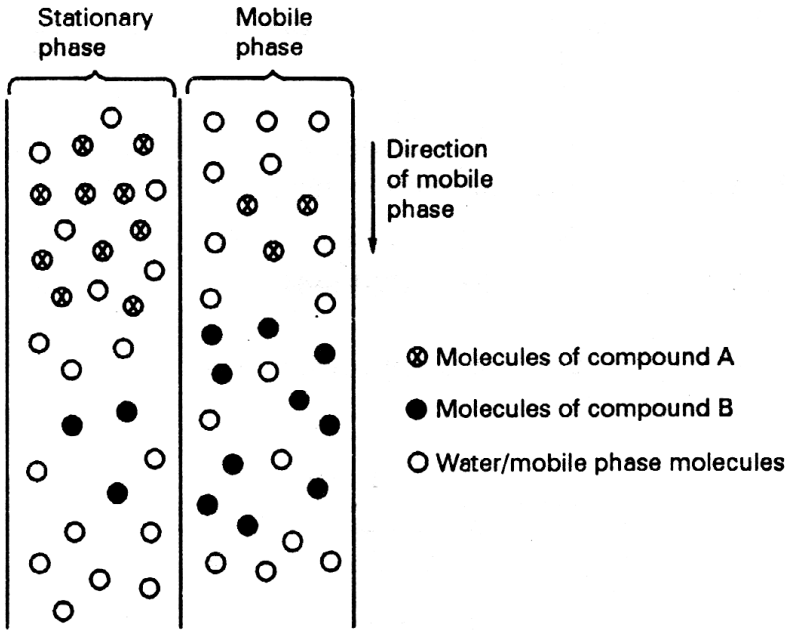
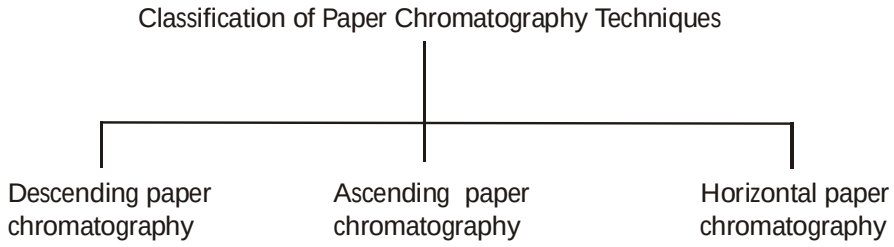


Fig:. Partition of A and B during paper chromatography (diagrammatic)

Q15. Describe the main features of (i) Descending (ii) Ascending (iii) Horizontal paper chromatography.

Ans : **(Imp.)**

Various modes of Development: Ascending, Descending and Horizontal



Descending paper chromatography

A rectangular tank with a well-fitting lid, (to prevent the escape of solvent vapours) is used. Near the top of the tank are supports for a trough in which the mobile phase solvent is placed. The spotted end of the paper is placed in the trough and held in position by keeping a glass rod over it. The paper lies hanging down. (While spotting the sample on the paper, care should be taken to apply the spots about 3-4 cm from the end of the paper.) The mobile phase percolates through the paper by capillary action and after travelling 1-2 cms downwards, moves through the sample spot. The mobile phase solvent now moves down (descends) by capillary action and gravity. For this reason, the flow of solvent is faster in this method, than in the ascending and horizontal paper chromatography methods. When the solvent front reaches to about 4/5 of the length of the paper, it is removed, the solvent front marked, the paper sprayed with a colour reagent and the compounds are visualized. The R_f values of the compounds are then calculated.

Ascending paper chromatography

A rectangular or cylindrical tank with a well-fitting lid, (to prevent the escape of solvent vapours), is used. The mobile phase solvent is placed at the bottom of the chamber. The spotted paper, from its non-spotted end, is suspended from the top of the tank by clipping it to a glass rod or hook. By this arrangement, the spotted end of the paper touches the mobile phase solvent. The solvent level in the chamber should be below the level of the spots on the paper. The solvent percolates and moves up (ascends) the paper by capillary action only. Relative to the descending method, the solvent moves slowly and a longer time is needed for the development. After the development is complete, i.e., when the solvent front has moved to about 4/5 of the length of the paper, it is removed, the solvent front is marked, the paper is dried and sprayed with a colour reagent and the compound spots are visualized. The R_f values of the compounds are then calculated.

Horizontal paper chromatography

In this method, the sample spot is placed at the centre of a circular-shaped paper. The mobile phase solvent is placed at the bottom of a circular chamber. The spotted paper is held horizontally on the chamber. The solvent for development is applied at the spot by a capillary tube or wick, (which is a cut portion of the circular paper) from which it spreads out radially. The compounds in the mixture spread into a series of concentric bands. In the descending and ascending paper chromatography methods, the compounds in the mixture appear as circular or oval spots after separation.

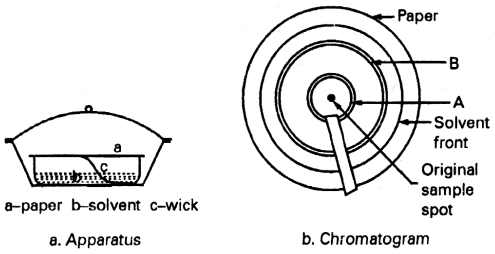


Fig:. Horizontal paper chromatography

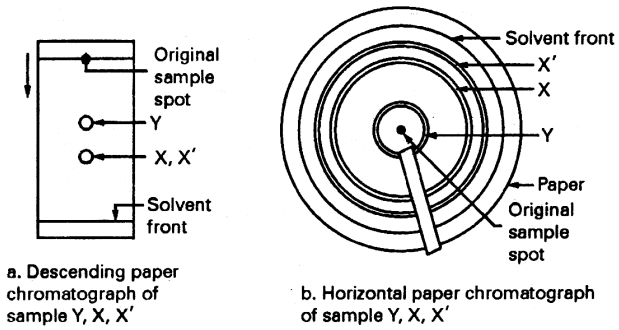


Fig:. Greater resoultion in horizontal paper chromatography

Q16. Explain the technique & applications of two dimensional paper chromatography.

Ans : **(Imp.)**

Two-dimensional paper chromatography

In the descending, ascending and horizontal paper chromatography, the solvent moves only once on the paper and in one direction only. These techniques may therefore be called one-dimensional paper chromatography.

In the case of mixtures which are complex, i.e., a larger number of compounds with close R_f values (such as the mixture of amino acids that results when a protein is hydrolyzed) the one-dimensional descending, ascending and horizontal chromatographic methods do not give good separations. For the separation of complex mixtures with close R_f values, two-dimensional paper chromatography is used

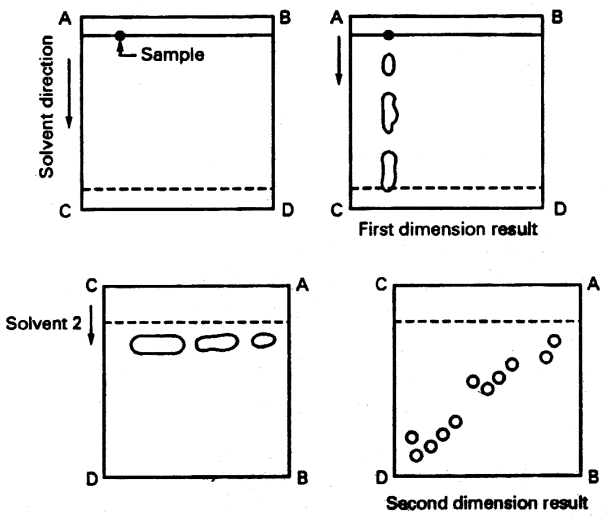


Fig:. Two-dimensional paper chromatography of a complex a mino acid mixture

Procedure A square sheet of Whatmann No.l paper, marked ABCD at its four corners, is used and the sample containing amino acids is spotted at one corner (A corner). The development of the paper with solvent 1 (n-butanol:acetic acid:water 12:3:5 v/v), with the edge of the paper AB immersed in the solvent trough and carrying out descending paper chromatography, gives partial separation as shown in fig. Three broad spots are seen. The sheet is removed and dried (chemical reagent spraying should not be done at this stage to visualize the spots). This constitutes first dimension chromatography. The paper is turned by 90° now, placed in another paper chromatography chamber with the CA end of the paper fixed into the trough containing solvent 2 (phenol water 500:125 g) and descending paper chromatography is done. This constitutes second dimension chromatography. After the development is complete, the paper is dried and sprayed with ninhydrin. The separation is complete and clear, and all the component amino acids show up individually (11 different amino acids). The spot pattern is compared with the similar 2sp paper chromatography of known standards and mixtures, by the use of R_f values.

Short Question and Answers

1. What is meant by solvent extraction.

Ans :

The process of isolation or separation of a desired product(s) selectively from the reaction mixture in solution form, by extraction with another immiscible solvent, is termed 'solvent extraction'.

2. Write the principle involved in solvent extraction.

Ans :

Liquid-liquid Extraction

The most common method of separating organic compounds from mixtures is the liquid-liquid extraction technique. The extraction of butyric acid present in water. The aqueous phase is taken as the solvent phase 1, and is designated as S_1. Butyric acid is isolated from its aqueous solution with an immiscible, low-boiling organic solvent like diethyl ether or methylene chloride, which is taken as solvent phase 2 and designated as S_2. The S_1 (water containing butyric acid) and S_2 (ether) which is the extracting-solvent are taken into a separating funnel and shaken vigorously for a few minutes until the equilibration of butyric acid occurs between S_1 and S_2 due to distribution or partition. The concentration of butyric acid in the two solvent phases depends on the relative affinity or solubility of butyric acid in these solvents. The concentration of butyric acid in ether is more than that in the aqueous solution. The ether layer is separated out and concentrated by distillation in a rotary evaporator and poured into a petri dish to obtain butyric acid.

The transfer of a solute from one phase to another is called partition. When a solute distributes itself between two immiscible liquids, there is a definite relationship between the solute concentrations in the two phases at equilibrium. The Nernst distribution law or partition law or law of heterogeneous equilibrium states that, if a system of two immiscible liquid layers, a solute A is added which is soluble in both layers, then the A distributes itself between the two layers such that the ratio of the concentration of the solute in one solvent to its concentration in the other solvent remains constant at constant temperature, and the molecular state of the substance is the same in both solvents. This relation is expressed as,

$$\frac{\text{Concentration of solute in } S_1}{\text{Concentration of solute in } S_2} = \frac{[C_A]_1}{[C_A]_2} = K_D \text{ (constant)}$$

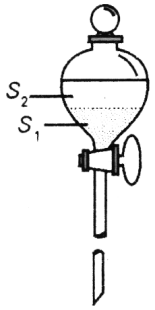


Fig.: Separating funnel with solute A distributed int two imiscible solvents S1 (water) and S2 (dietyl ether)

where S_1 and S_2 are two immiscible solvents (in this case, water and diethyl ether); A = solute (butyric acid); $[C_A]_1$ = concentration of A in S_2, i.e., diethyl ether; $[C_A]_2$ = concentration of A in S_1, i.e., water, and K_D is a constant known as the distribution or partition coefficient, and its value is constant at constant

temperature. Hence, the distribution coefficient of a compound A is equal to the ratio of its solubilities in the two solvents S_1 and S_2. Organic compounds are usually more soluble in organic solvents than in water. Hence, they can be easily extracted from aqueous solutions. For a compound A to dissolve completely in either S_1 or S_2, the value of K_D must be infinity or zero. Because A is partially present in both S_1 and S_2. If K_D is greater than 1.0 and the volume of S_1 and S_2 are equal, the compound A will be more soluble in S_1 than in S_2.

According to Nernst, there are various limitations to this law and it is applicable only when the following conditions are satisfied:

(1) The temperature should be maintained constant.

(2) It is applicable when dilute solutions are employed, because the ratio of $\frac{C_1}{C_2} = \frac{S_1}{S_2}$. will not remain constant if the concentrations are high. Therefore, the higher the concentrations in S_1 and S_2, the larger are the deviations.

(3) The Nernst equation is not applicable when the solute associates in S_2, and Eq. are modified as,

$$K_D = \frac{C_1}{n\sqrt{C_2}},$$

where n = number of molecules associating to give a larger molecule. Similarly, the Nernst equation is not applicable when the solute A dissociates in C_2. The law is modified to,

$$K_D = \frac{C_1}{C_2(1-x)}$$

3. Explain the Batch Extraction.

Ans :

Batch Extraction

This method is used when the distribution coefficient K_D of the compound that is to be isolated is very large in the chosen two-phase immiscible solvent system. To the solution containing the compound (with large K_D) taken in a separating funnel a solvent is added, shaken well and allowed to equilibrate. The compound is now predominantly present in the added solvent phase. The two layers can then be separated and the compound obtained. To get the remaining amount of compound from the solution, this process may be repeated two or three times with fresh solvent, Since the extraction is done in two or three batches of extraction operations, it is called batch extraction.

Batch Extraction

There are two common cases:

(1) Batch extraction of liquids, and

(2) Batch extraction of mixtures with active solvents. In these methods, the material to be extracted is brought in contact with the extracting solvent more than once, using small volumes of the extracting solvent. Hence, it is called batch extraction.

Batch Extraction of Liquids

Neutral organic compounds or neutral metal complexes (with large K_D value), dispersed or dissolved in water, can be easily separated by extracting (shaking) it with an immiscible volatile organic solvent such

as ethyl ether, $CHC1_3$, or benzene, using a separating funnel. Generally, the extracting solvent is used in three portions, shaken well each time, the separating funnel allowed to stand for some time, and the layer containing the compound is collected. After this process is repeated twice or thrice, the extracted solvent is collected and mixed together in a flask. It is dried with a drying agent such as anhydrous $CaCl_2$, or Na_2SO_4, and the solution is evaporated by distillation. The distilled solvent is collected while the residue remaining in the distillation flask contains the compound. This is a typical operation in several common organic syntheses. In the quantitative estimation of some metal ions, they are converted into neutral complexes by reaction with either 8-hydroxyquinoline or dithiazone. These complexes are generally coloured. The aqueous solution containing the complex is taken in a separating funnel and extracted with $CHC1_3$ or CH_2C1_2. The extraction is done once or twice. The coloured organic phase is analyzed spectrophotometrically, and the metal ion concentration is calculated using the Beer-Lambert Law.

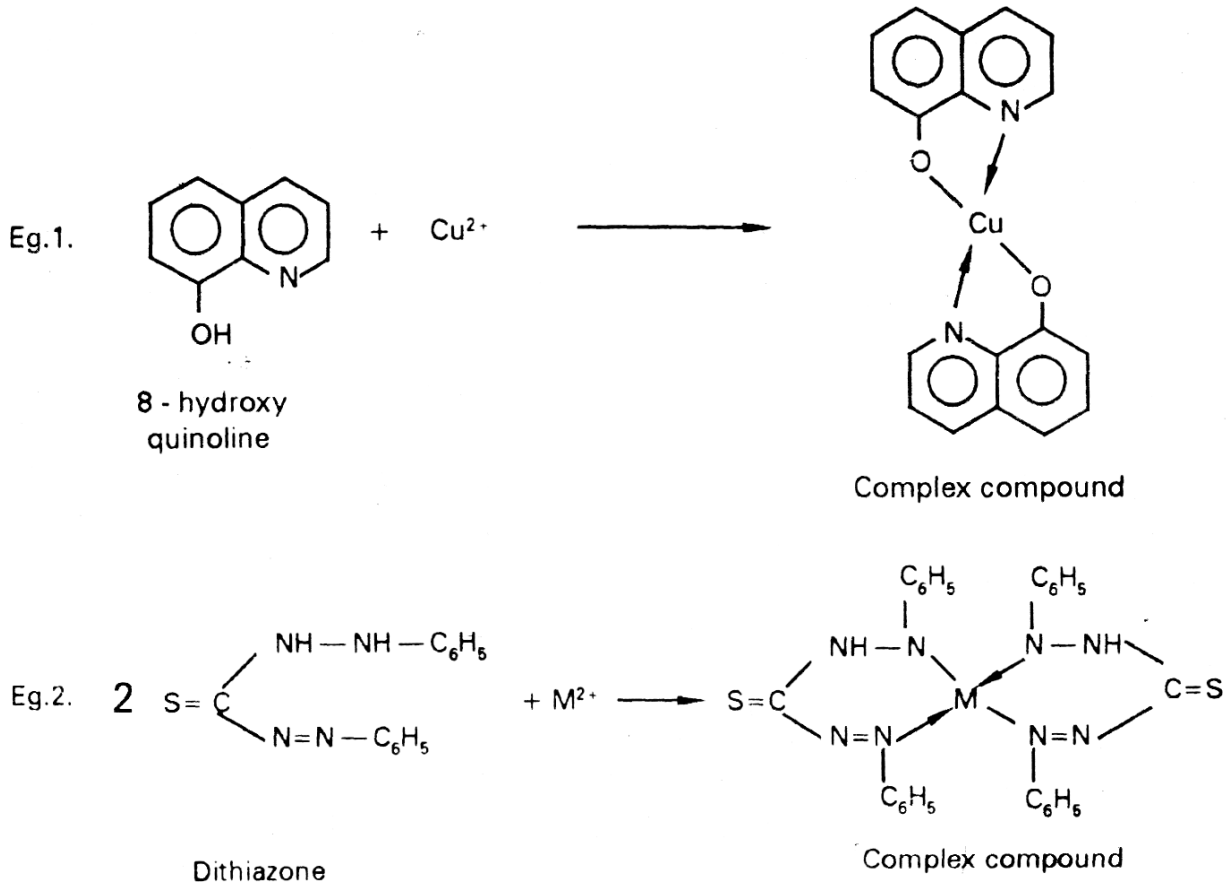


Fig.: Scheme of separation using neutal solvents or reagents

Batch Extraction of Mixtures with Active Solvents

An organic reaction product is generally a mixture of compounds. These compounds may be acidic, basic or neutral; and they may be solids or liquids. The selective separation of one or more of these compounds can be effected by the use of 'reactive solvents' such as aqueous NaOH, aqueous HC1, aqueous $NaHCO_3$, etc. Consider a mixture containing 2 g each of p-nitrobenzoic acid, p-nitroaniline and 1-nitronaphthalene. To effect their separation, this mixture is dissolved in about 200 ml of ether and taken into a separating funnel and shaken with 30 ml of 6 N HC1. The two layers are allowed to settle and the lower aqueous solution containing the p-nitroaniline as its hydrochloride salt is drained and collected in a beaker. This extraction is repeated with two more 30 ml portions of 6 N HC1. The entire amount of p-

nitroaniline is extracted as the hydrochloride salt. The neutralization of this salt solution with NH_3 leads to the precipitation of p-nitroaniline, which is collected by filtration using a Buchner funnel.

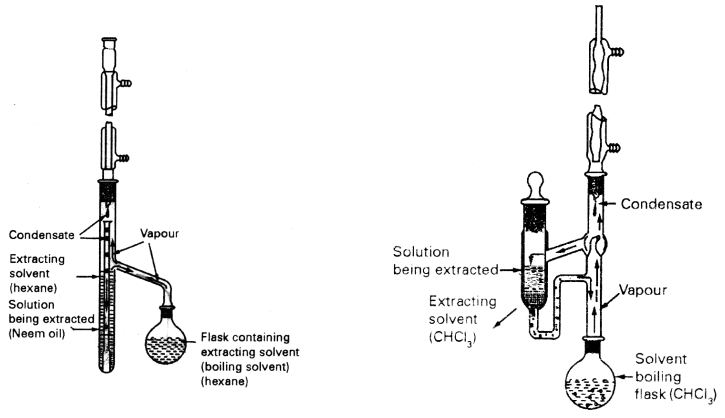
The ether solution in the separating funnel now contains p-nitrobenzoic acid and nitronaphthalene. To this, 30 ml of 6 N NaOH is added, shaken well, the layers allowed to separate, and the lower aqueous phase containing p-nitrobenzoic acids collected as its sodium salt in a beaker. The extraction is repeated with two more portions of 6 N NaOH and the aqueous solution is collected in the beaker. The entire p-nitrobenzoic acid is extracted as its sodium salt. The neutralization of this salt solution with cone. HC1 precipitates out the p-nitrobenzoic acid, which is filtered using a Buchner funnel and collected. The ether solution in the separating funnel now contains only 1-nitronaphthalene, This solution is drained out into a beaker, dried with a drying agent. The solution is taken into a china dish and warmed on a water-bath to remove the ether. The crystalline solid of p-Nitronaphthalene is obtained.

4. Discuss the technique of continuous extraction of liquids.

Ans :

Continuous Extraction of Liquids

This technique is generally used to isolate a desired compound from a natural oil (e.g., neem oil), and for isolating a compound more soluble in a water solvent than in the organic solvent. When the distribution coefficient of the compound between the organic phase and the aqueous solution/oil is small, we can efficiently extract the desired compound by continuous extraction, using a small quantity of the extracting (organic) solvent. Two types of extraction apparatus are used, depending on whether the extracting solvent is. lighter (benzene, hexane, ether) or heavier ($CHC1_3$, CH_2C1_2) than the extracted solution. When the extracting solvent is lighter, it moves upwards through the solution and thus the technique is known as extraction by upward displacement; and when the extracting solvent is heavier than the solution extracted, it moves downwards through the solution, and is known as extraction by downward displacement. In both these techniques, the extracting solvent, as it moves through the extracted solution, dissolves and extracts the solute. The solution of the extracting solvent and solute is continuously separated into a boiling flask. The solution is subjected to continuous distillation and the condensed distillate returns as fresh extracting solvent to the extraction vessel to be reused. In both these processes, the extracted compound builds up in an increasing concentration in the boiling flask. This is because more dilute solution is continuously draining into the flask, while, at the same time, the solvent is being distilled away.



(a) (b)

Fig.: Continuous extraction by upward and downward displacement

Extraction of Solids

The process of extraction of solids is generally used to obtain the active principle ingredients from the natural products, and from the dried tissues of plants, fungi, seaweed, mammals, etc. It is also very effective for the removal of non-steam-volatile compounds.

Batch process of extraction of sounds

This involves macerating the mature tissue with an appropriate solvent in a blender for a short time and filtering it to obtain the solvent extract. The residue is returned to the flask containing fresh solvent for further extraction. It is a crude and not very efficient method, and is considered as a preliminary extraction process.

Continuous solid-liquid extraction (Soxhlet extraction apparatus)

It is a process of continuous extraction of solids using the Soxhlet extraction apparatus. This method is used for the isolation of medicinal and other active compounds from dried plant tissues. The plant material to be extracted is taken in a porous (filter-paper or cloth) bag and placed in the main chamber of the Soxhlet apparatus. The Soxhlet is fitted with a round-bottomed flask at its lower end, containing the extracting solvent and a reflux condenser is fitted in the upper part. The solvent is heated to reflux and the distillate, as it drops from the condenser, collects in the chamber containing the plant material. This solvent is still warm and therefore effectively extracts the compounds present in the material. When the chamber fills to the level of the upper end of the siphon arm, the solution empties from this chamber into the boiling flask by a siphoning action. This constitutes one extraction cycle. The extraction cycles are continued automatically, without attendance, till the extraction of the compounds in the mixture is complete.

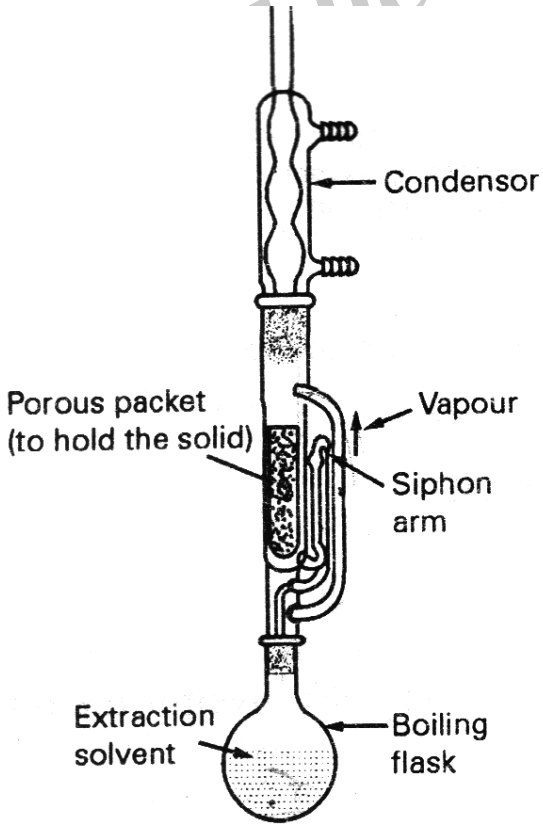


Fig.: Soxhlet extraction - continuous extraction of solids

The apparatus is disconnected and the solution in the flask is distilled to get the extracted compounds. This process of Soxhlet extraction is extensively used in research laboratories and in pilot-scale plants because of efficient separation of individual components from the mixture of compounds, and due to the simplicity of the operation. When the material to be extracted is not easily available or is available in minute quantities, this method is very useful. Depending upon the amount of material to be extracted, the size of the Soxhlet can be fabricated. Vincristin and Vimblastin are two anticancer drugs, which are extracted from the powdered leaves of the Vinca rosea plant by this procedure on a large scale. Large quantities of solvent are not required for extraction, because it is a cyclic process and the loss of solvent is low-barely 10%.

5. How do you determine iron(III) by solvent extraction technique.

Ans :

Determination of Fe^{3+}

Based on the extracting capability, the compounds are broadly classified into two types. They are,

(i) Simple compounds

(ii) Ionic compounds.

(i) Simple Compounds

The compounds which are easily extractable from aqueous solution by using simple non-polar solvents such as aliphatic and aromatic hydrocarbons and carbon tetrachloride are considered as simple compounds. They are covalent in nature.

(ii) Ionic Compounds

The compounds which are not extractable into organic solvents from aqueous solution are called as ionic compounds. They can be extracted only by neutralizing the charges whose neutral compounds include chelate complexes, cationic complexs and non solvated salts.

Chelate System

The chelate system is used for the extraction of metal ions. Hence, its ligands with functional group, - OH and - SH are used such that the proton replaces metal ion and neutralize the charge. The chelating agents form coordinate linkages (by donating electron pair) by using coordinating atoms like N and O.

The chelating agents used in the extraction of Fe^{3+} ions are,

- Oxine (8-hydroxy quinoline)
- Cupferron (Ammonium salt of nitroso phenyl hydroxyl amine)
- DDTC (Dithiazone sodium di ethyl di thio carbonate) and
- PAN (1 - (2-phridyl azo) - 2 - napthol).

pH adjustment and concentration of chelating agents are responsible for complete extraction of chelate.

Ion Association System

In this system, halo metallic acids (formed by reaction of metal ions with halide ions in acid solution) and pseudo halo metallic acids (formed by reaction of metal ions with pseudohalide ions in acid solution) are employed in the extraction of the metal ions. Tributyl phosphate solvent is widely used for these systems.

Example

For $FeCl_3$ – HC1 – ether system, the extracted species are H^+(ether), $FeCl_4^-$.

Methods of Extraction

Fe^{3+} ion is extracted by the following two methods,

1. By Chloride Extraction

This method is used for the determination of iron in steel. Fe^{3+} i.e., Ferric solution (i.e., 99.9% of iron) can be extracted from concentrated HC1 (6M) using ether. Ether layer is separated and evaporated (by boiling). In acid solution, ferric iron is reduced to ferrous iron and then to (Fe(II)) solution which is then titrated with standard $K_2Cr_2O_7$ solution.

2. By Oxine (8-hydroxyquinoline)

In this method, 1% oxine in $CHC1_3$ of aqueous solution contains ferric iron. Initially 50 ml of iron solution and 10ml of 1% oxine solution are mixed to form a solution. Then, the separation of $CHC1_3$ extract gives the iron which is determined by calorimeter at 470nm. Blank Solvent (distilled water) is used such that the standard ferric iron solution is responsible to determine the Fe^{3+} ion based on the constructed graph in this process. This process is carried out only when pH ranges between 2 to 10.

6. Define chromatography.

Ans :

Chromatography

Chromatography is a physical method of separating mixtures of Organic compounds biomolecules, organic and inorganic salts, etc., by distribution or partition between two phases. One of the phases is the stationary phase and the other is the mobile phase. These two phases are in contact with each other and the mobile phase moves through the stationary phase. The compounds in the mixture have different values of partition or distribution coefficients, and the separation is based on this difference in partition coefficients.

7. Explain the Technique of thin layer chromatography (TLC).

Ans :

Principle of TLC

A solid is used as the stationary phase and a liquid as the mobile phase, the TLC is a solid-liquid chromatography. The physical principle involved in the separation of compounds in a mixture is adsorption. Thus, TLC is an adsorption chromatography. The separation of compounds is due to their different adsorption strengths to the adsorbent. The role of the mobile phase solvent is to desorb the compounds from their binding to the adsorbent, dissolve the compounds in themselves and carry the compounds upwards, in the direction of the movement of the solvent. The compounds move due to dynamic adsorption and desorption. Thus, in the case of the sample containing A and B, where B is more polar than A and the stationary phase is polar, A is partitioned or distributed between the stationary phase (adsorbent) and mobile phase (solvent). Similarly, B is partitioned between the adsorbent and the solvent.

$$\text{Partition coefficient of A} = \frac{\text{Conc. of A in the stationary phase}}{\text{Cone. of A in the mobile phase}} = k_A$$

$$\text{Partition coefficient of B} = \frac{\text{Conc. of B in the stationary phase}}{\text{Conc. of B in the mobile phase}} = k_B$$

The partition coefficient of B is greater than A, because B being more polar than A has greater affinity to the stationary phase than A. Compound A with a lower value of partition coefficient is present more in the mobile phase, the moving phase, and therefore moves faster than B. Thus, the compounds get separated due to differences in their adsorption strengths which results in different values of partition coefficients.

8. Describe the applications of TLC in a Organic Laboratory.

Ans :

TLC has several applications. It is used to:

1. Determine the number of compounds in a sample (purity determinations).
2. Monitor the progress of organic reactions.
3. Identify unknown compounds by a comparison with suspected references or standards.
4. Monitor the progress of column chromatographic separation or any other purification process such as crystallization, distillation, solvent extraction, etc.
5. Select the starting eluent for column chromatography.
6. Effect small-scale (10 – 100 mg) quantitative separation of mixtures. This technique is known as preparative TLC.

   A thin-layer chromatography experiment consists of: (1) preparation of TLC, plates (coating the stationary phase on a glass plate as a thin layer), (2) application of the sample solution as a spot, (3) development of the TLC plate using organic solvents (mobile phase), and (4) detection of the compounds.

9. Write the stationary phase materials or adsorbents in TLC.

Ans :

Stationary phase materials: Adsorbents : Preparation of TLC plates

In thin-layer chromatography, as in the case of column chromatography, the stationary phase materials are silica gel and alumina. These are the adsorbents for TLC. For TLC, a special grade of silica gel and alumina known as silica gel G and alumina G, where G stands for calcined gypsum $CaSO_4 \frac{1}{2} H_2O$, are used. Silica gel G and alumina G contain 10-13% by weight of gypsum which acts as a binder. The silica gel G or alumina G is finely-powdered and fiour-like. (The column chromatography-grade silica gel or alumina have 60-200 mesh size and do not contain gypsum). The silica gel G or alumina G is made into a slurry in $CHCl_3$:MeOH - 2:1 v/v or water and applied as a thin layer on a microscope slide or glass plate of 20 × 5 cm size. Due to the water/MeOH, gypsum becomes $CaSO_4.2H_2O$ and binds the adsorbent silica gel or alumina firmly to the glass plate. The water used for coating the adsorbent to the glass plate is removed by drying the TLC plate in a hot-air oven. When $CHCl_3$:MeOH is used, the TLC plate is dried just by exposing it to the air for 5 minutes. The thin layer of silica gel G or alumina G coated on the glass plate is the stationary phase for this chromatography, the glass plate is only acting as a support for this coating. This solid stationary phase is also called an adsorbent. In addition to silica gel G and alumina G,

materials such as finely-powdered cellulose and polyamide are also used as adsorbents for TLC. The choice of a proper adsorbent depends on the type of compunds to be separated or analyzed. Silica gel G is widely used for routine TLC work.

Silica gel G	:	Majority of organic compounds, in particular, phenols, esters, acids
Alumina G	:	Basic compounds
Cellulose and Polyamide	:	Polyhydroxy and highly polar organic compounds

10. How do you prepare the TLC plates and spotting of sample on TLC.

Ans :

Sample application : Spotting

To know the number of compounds in a sample using the TLC method, a dilute solution of the sample (about 1 mg/1 ml) is made in a volatile organic solvent such as $CHCl_3$, CH_3COCH_3, MeOH, etc. Using a microcapillary tube, a few microlitres of the sample solution is applied as a spot on the adsorbent-coated TLC plate at a distance of about 1 cm from the end of the plate. In this process, a

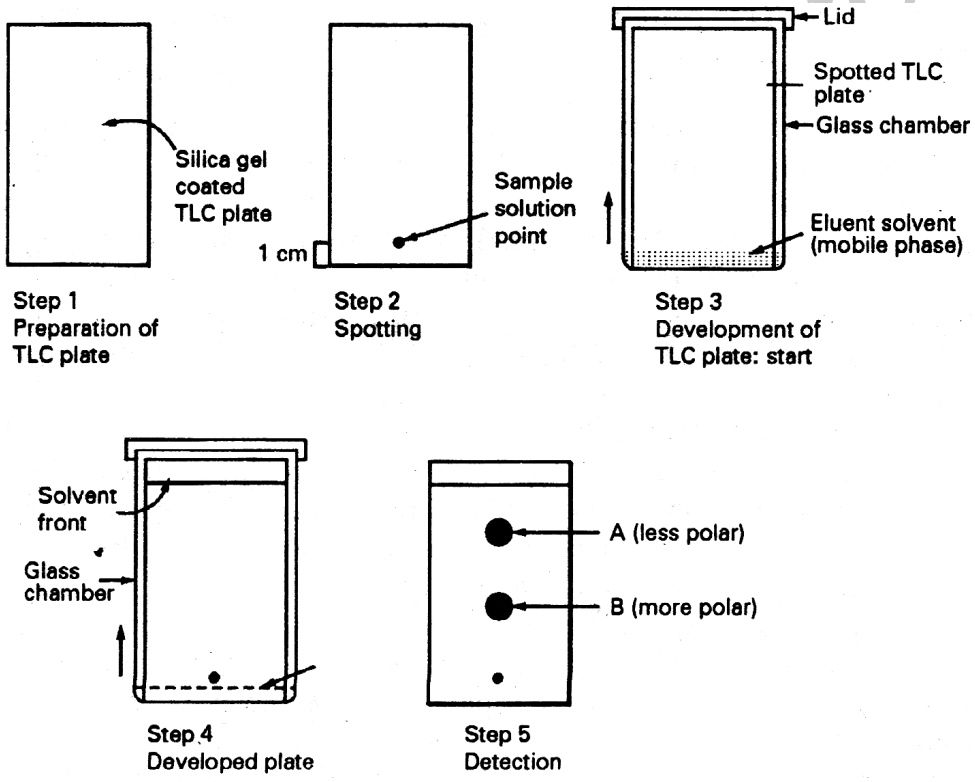


Fig:. Thin - loyer chromatography

few micrograms of the sample is delivered onto the adsorbent. This step is known as the spotting of the sample or sample application. The compounds in the sample are adsorbed onto the surface of the adsorbent particles. Different compounds in the sample are adsorbed with different adsorption strengths. The strength of adsorption or binding is due to a combination of van der Waals', dipole interactions, and H bonding. These adsorption forces depend on the molecular weight, size, shape and/or functional groups in the

compound. No two compounds have the same strength of binding towards a given adsorbent. Assume that the sample solution spotted contains two compounds A and B, where B is more polar than A. The adsorbents, silica gel G and alumina G, are highly polar and are called polar stationary phases. Towards a polar stationary phase, the more polar B is strongly adsorbed relative to A. To a polar stationary phase, polar compounds bind strongly and less polar compounds bind weakly.

11. What are R_f values. How do you calculate and write there applications.

Ans :

R_f Values in TLC

R_f (retardation factor) values and its applications

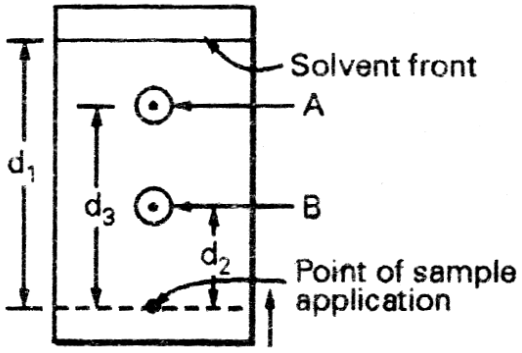


Fig:. R_f values in thin - layer chromatography

The thin-layer chromatography particulars of a compound are expressed in terms of retardation factor (R_f) values. The stationary phase "retards" the motion of the compounds by adsorption. Different compounds are retarded to different extents, depending on their adsorption strength. In TLC, the solvent moves ahead of the compounds and the edge of the solvent is called the solvent front. The ratio of the distance that the compound travels to the distance the solvent front travels is called the R_f value. The R_f value of a compound can be calculated by measuring the distances in centimetres, between the solvent front and the point of sample application (d_1) and the distance from the middle of the spot to the point of sample application (d_3, d_2) for compounds A and B.

$$R_f \text{ of compound A} = \frac{\text{distance moved by compound A } (d_3)}{\text{distance moved by solvent front } (d_1)}$$

$$R_f \text{ of compound B} = \frac{\text{distance moved by compound B } (d_2)}{\text{distance moved by solvent front } (d_1)}$$

The R_f value of a compound is always less than 1. The R_f value depends on the: (1) nature of the adsorbent (silica gel or alumina), (2) nature of the solvent system, and (3) thickness of the adsorbent coating. These parameters should be mentioned while reporting the R_f values. The R_f value of a compound remains the same whether it is in its pure form or if it is present in a mixture.

The R_f value is an important physical property of a compound, and is useful to identify an unknown compound by its TLC comparison with a suspected reference or standard. Solutions of the unknown and of the suspected reference compounds are spotted on the same TLC plate, developed and the spots detected.

12. Explain the technique & applications of two dimensional paper chromatography.

Ans :

Two-dimensional paper chromatography

In the descending, ascending and horizontal paper chromatography, the solvent moves only once on the paper and in one direction only. These techniques may therefore be called one-dimensional paper chromatography.

In the case of mixtures which are complex, i.e., a larger number of compounds with close R_f values (such as the mixture of amino acids that results when a protein is hydrolyzed) the one-dimensional descending, ascending and horizontal chromatographic methods do not give good separations. For the separation of complex mixtures with close R_f values, two-dimensional paper chromatography is used

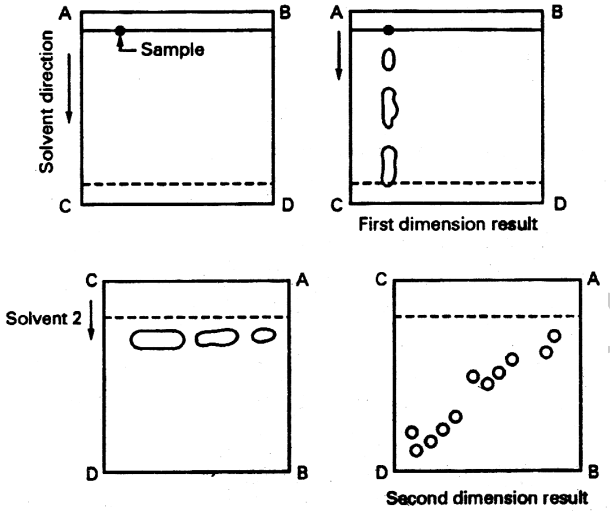


Fig:. Two-dimensional paper chromatography of a complex amino acid mixture

Procedure A square sheet of Whatmann No.l paper, marked ABCD at its four corners, is used and the sample containing amino acids is spotted at one corner (A corner). The development of the paper with solvent 1 (n-butanol:acetic acid:water 12:3:5 v/v), with the edge of the paper AB immersed in the solvent trough and carrying out descending paper chromatography, gives partial separation as shown in fig. Three broad spots are seen. The sheet is removed and dried (chemical reagent spraying should not be done at this stage to visualize the spots). This constitutes first dimension chromatography. The paper is turned by 90° now, placed in another paper chromatography chamber with the CA end of the paper fixed into the trough containing solvent 2 (phenol water 500:125 g) and descending paper chromatography is done. This constitutes second dimension chromatography. After the development is complete, the paper is dried and sprayed with ninhydrin. The separation is complete and clear, and all the component amino acids show up individually (11 different amino acids). The spot pattern is compared with the similar 2sp paper chromatography of known standards and mixtures, by the use of R_f values.

Choose the Correct Answer

1. Separation of individual components from a multi-component mixture can best be carried out by [d]

   (a) Solvent extraction (b) Chromatography

   (c) Fractional distillation (d) All the three methods.

2. The distribution of the solute in two immiscible solvent phases depends on the [a]

   (a) Relative solubility in the two solvents (b) Physical constants

   (c) Immiscibility of the solvents (d) Polarity of the solvents.

3. When the distribution coefficient K_D is small, the solvent extraction technique used is [a]

   (a) Continuous extraction (b) Batch extraction

   (c) Soxhlet extraction (d) Craig extraction.

4. When the amount of solvent for extraction is very limited, the most efficient technique is [c]

   (a) Batch extraction (b) Multiple extraction

   (c) Continuous extraction (d) Extraction with active solvent. [d]

5. The active principles from plant products are obtained by [a]

   (a) Soxhlet extraction (d) Craig extraction

   (c) Chromatography (d) All the three methods.

6. Extraction by a number of successive partial separations until we eventually achieve the desired degree of purity is the method of [a]

   (a) Multiple extractions

   (b) Countercurrent extraction

   (c) Craig extraction technique

   (d) Repeated extraction by successive portions of an organic solvent

7. The extraction technique in which both phases move continually in contact with each other and in opposite directions is a [a]

   (a) Countercurrent process (b) Fractional distillation process

   (c) Soxhlet extraction technique (d) Successive partial separation.

8. When the distribution coefficient of a solute is unity, then the solute distributes itself [a]

   (a) Equally in both solvents (b) Unequally in both solvents

   (c) Only in one solvent (d) None of the above.

Fill in the Blanks

1. The most common method of separating organic compounds from mixtures is _____
2. In a solven extraction technique two immiscible liquids more in opposite directions is called _____ extraction method.
3. When the distribution coefficient k_D of the compound is very large in _____ extraction.
4. Distribution coefficient K_D _____
5. When the distribution coefficient k_D of the compound is very small in _____ extraction.
6. When the dirtribution coefficient k_D of the compound is nearly equal the used extraction technique is called _____
7. Paper chromotography is an example of _____ chromotography.
8. The Rf value of a compound is always _____
9. Detection of Aminoacids checked in TLC by _____
10. Preparative TLC is a _____

ANSWERS

1. Liquid liquid extraction
2. Counter current
3. Batch
4. $K_D = \frac{\text{Concentration of solute in } S_1}{\text{Concentration of solute in } S_2}$
5. Continuous
6. Counter current extraction
7. Liquid - liquid
8. Less than 1
9. Ninlydrin
10. Separation Technique

UNIT - IV

Separation techniques - II

S5-E-A-IV: Column Chromatography-Principle, Types of stationary phases, Column packing Wet packing technique, Dry packing technique. Selection criteria of mobile phase solvents for eluting polar, non-polar compounds and its applications.

Ion exchange chromatography:

Principle, cation and anion exchange resins, its application in separation of ions, de-ionized water.

Gas Chromatography

Principle, theory and instrumentation (Block Diagram), Types of stationary phases and carrier gases (mobile phase), applications of GC.

High performance liquid chromatography:

Principle, theory and instrumentation, stationary phases and mobile phases. Applications of HPLC, Analysis of paracetamol.

S5-E-A-IV: SEPARATION TECHNIQUES

Q1. Describe the technique used in column chromatography.

Ans : **(Imp.)**

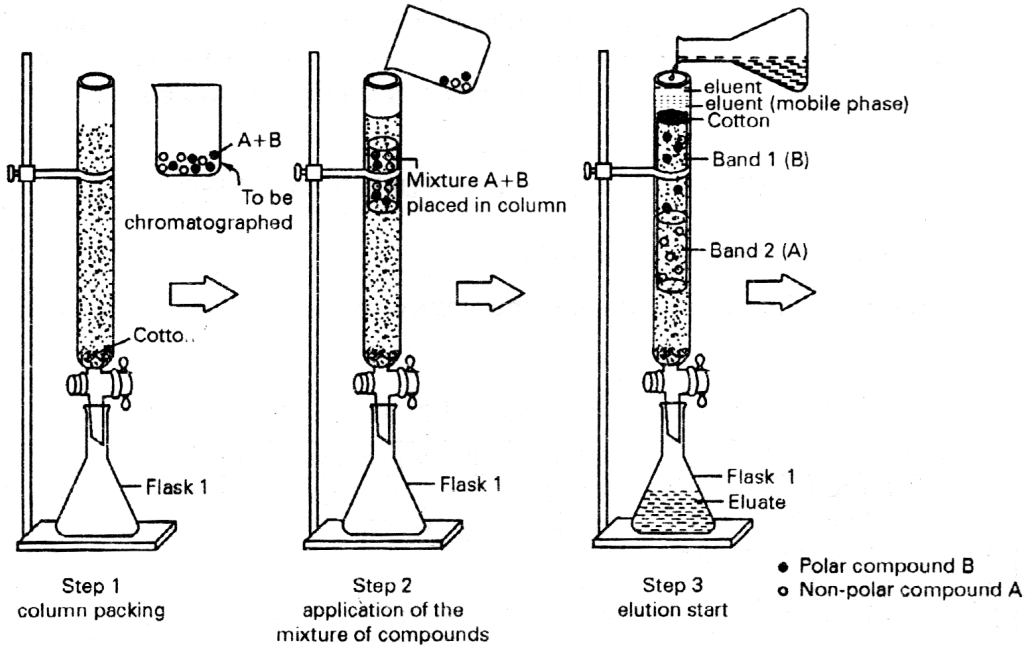
Column Chromatography

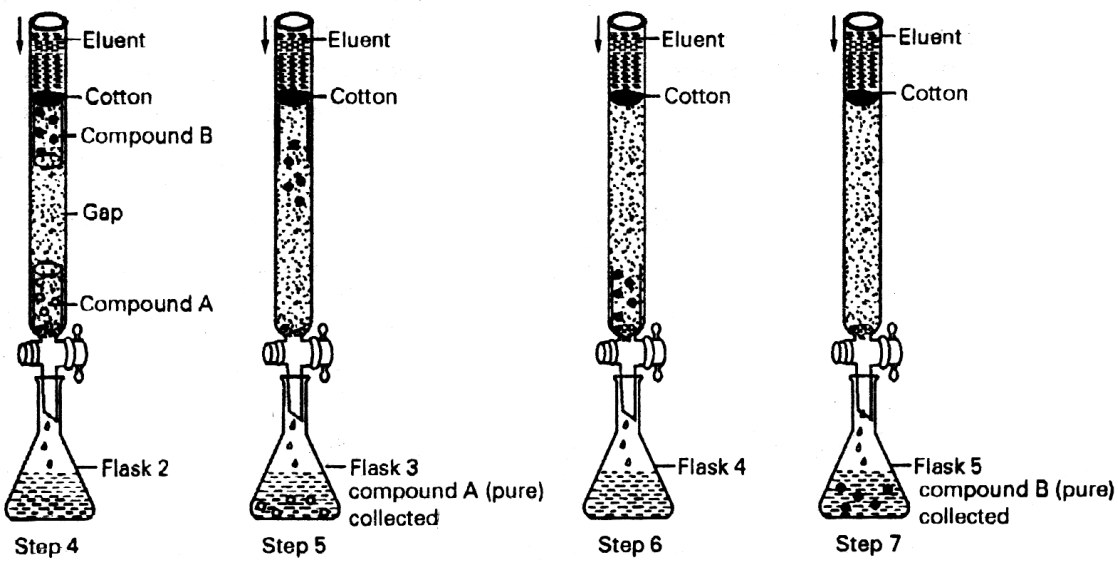
Column chromatography is a widely-used technique for the quantitative separation of complex mixtures of organic compounds in the gm-kg scale. It is mainly used in organic research laboratories for the separation and purification of reaction products. In the drug industry, two anti-cancer drugs - taxol and vincaleucoblastin are isolated in a pure form from their plant extracts by column chromatography.

In this type of chromatography, finely-powdered, porous solids such as silica gel or alumina, which constitute the stationary phase, are packed into a burette-like glass tube, commonly called the column. The column is clamped vertically. The mixture of compounds to be separated are dissolved in a very small volume of organic solvent and applied as a narrow band at the front end of the column. A liquid which is generally an organic solvent such as n-hexane, benzene, chloroform, constituting the mobile phase, is allowed to flow through the stationary phase by gravity. The compounds in the mixture have different adsorption strengths towards the material of the stationary phase. Therefore, the compounds in the mixture, when allowed to move down the column, move with different speeds. Weakly- adsorbed compounds move faster than the strongly adsorbed ones. The different speeds of movement of compounds in the column is known as the differential migration.

In chromatography techniques that use solid material as the stationary phase, e.g., column chromatography and thin-layer chromatography, the compounds in the mixture get separated due to their differences in adsorption strength towards the stationary phase. These techniques are therefore called adsorption chromatography and the stationary phase materials (silica gel, alumina) are called adsorbents.

The chromatography techniques that use. liquids as the mobile phase, e.g., column, thin-layer, ion-exchange, paper and high-performance liquid chromatography are all called liquid chromatography.





The solvent (mobile phase) introduced at the front end of the column stationary phase is called the eluent and that which leaves the column with or without the separated compounds, collected in conical flasks, is called eluate or column fraction. The process by which the compounds are carried through the stationary phase by the mobile phase is called the development or elution of the column.

Q2. Write about adsorbents used in column chromatography.

Ans :

Nature of Adsorbents

Stationary phase materials: Adsorbents

Table. Gives the list of adsorbents and the nature of their active sites used in column chromatography.

Sl.No.	Adsorbent	Nature of the surface active site
1.	Silica Gel	Acidic
2.	Alumina	Acidic and basic sites
3.	Magnesium Silicate	Acidic
4.	Kieselguhr	Neutral
5.	Charcoal	Neutral and Acidic
6.	Sucrose	Neutral
7.	Starch	Neutral

These adsorbents are finely-divided, porous particles with large surface areas ~ 50m^2/'g. The adsorbent may be directly taken into the column as the dry powder (dry-packing method), or it may be made into a slurry in an organic solvent and then poured into the column and the solvent drained off (wet-packing method). Silica gel and alumina are the two most common chromatographic adsorbents in use. They are cheap and readily available commercially.

Silica gel Adsorbents with the general formula $SiO_{2x}H_2O$ are called silica, silica gel, or silicic acid. The surface of the SiO_2 particle is covered by hydroxyl groups.

Free hydraxyl group Bound and reactive hydrocxl group

Fig:. The surface of a silica gel particle

It is the presence of these surface hydroxyl groups that is responsible for the selective adsorption properties of silica gel. Silica gel is therefore a highly polar, solid, stationary phase. The silica gel surface is weakly acidic (pH - 3 5).

Alumina, acidic ($A1_2O_3$) On heating hydrated alumina $Al_2O_3xH_2O$ to 300-400°C, most of the adsorbed water is drawn off, with the remainder of the water reacting with the surface $A1_2O_3$ to form hydroxyl groups. This type of alumina, used for column chromatography, is known as activated alumina. The surface of the particle of Al_2O_3 has hydroxy groups, which are acidic in nature (pH-4), and hence it is known as acidic alumina. Like silica gel, acidic alumina is also a highly polar, solid stationary phase.

Alumina, basic (Al_2O_3) Heating hydrated alumina to $Al_2O_3xH_2O$ to 800-1000°C removes the water molecules totally to give hydroxyl-free $A1_2O_3$, the oxide ions now give the basic properties to alumina. Basic alumina is also a polar, solid stationary phase.

Q3. Write the solvent systems used in column chromatography.

Ans :

Solvent Systems

Mobile phase solvent systems elutotropic series. The compounds in the mixture have different adsorption strengths towards the stationary phase. Their actual separation can be done only when a mobile phase moves through the stationary phase. The mobile phase is a pure organic solvent or a solvent mixture and is called the eluent. The common organic solvents, arranged in the increasing order of their polarity (dielectric constant), used as the mobile phase.

This arrangement of solvents in the increasing order of polarity, as shown is known as the elutotropic series. Any of these solvents can be used as an eluent. The elution of the column is started with the lower polarity solvent and gradually the solvent polarity is increased. For example, to begin with, benzene may be used as the eluent and later benzene : ethyl acetate 9 : 1 v/v can be used as the eluent. When a less polar solvent is used as the eluent, the less polar compound/s of the mixture are eluted first, as these are less strongly adsorbed to the stationary phase. That is, the less polar solvent displaces or desorbs the less polar compounds from their adsorption. The more polar compounds of the mixture are more strongly adsorbed, and more polar solvents are needed to desorb them from their adsorption and they elute later with a relatively more polar solvent. Thus, in column chromatography where the stationary phase is polar, the starting eluent is less polar and the eluent polarity is gradually increased. The less polar compounds are eluted first, followed by the more polar compounds.

Table. Solvent Systems for Column Chromatography Elutotropic Series

Hexane	Solvents arranged
Cyclohexane	in the increasing order
Benzene	of polarity, or
CH_2C1_2	increasing solvent power
$CHCl_3$	to desorb or dissolve
EtOAc	the compounds adsorbed
Acetone	to the stationary phase
EtOH	
MeOH	
Water	
AcOH	

Choice of the starting eluent for column chromatography When the mixture of compounds to be chromatographed have different polarity, one should strictly follow the elutotropic series for the eluents. The starting eluent is n-hexane. After the collection of some fractions of the eluate, the eluent polarity is increased. Benzene is the next eluent and still later the eluent can be more polar such as benzene : ethyl acetate 9 : 1 v/v. This increase in polarity of the eluent is continued till all the compounds in the mixture are eluted. Non-polar solvents elute non-polar compounds; polar solvents elute polar, compounds. The elution sequence for typical organic compounds is shown in Table.

Before beginning the column chromatographic separation of a mixture, one "should first study the TLC of this mixture. The TLC indicates which solvent gives the best separation of the compounds in the mixture, as well as the number of compounds in the mixture. The solvent which gives the best separation in the TLC can be used as the eluent (mobile phase) for column chromatography.

Table. Gives the adsorbents and the nature of their active sites used in column chromatography

Strong bases	Slowest (need a polar solvent for elution)
Acids	
Amines	
Alcohols	
Esters	
Aldehydes	
Ketones	
Aromatics	
Halocarbons	
Ethers	fastest (will elute with a non - polar solvent)
Olefins	
Hydrocarbons	

Q4. Explain the chromatography separation of a mixture of compounds based on the differences in there adsorption strengths and partition co-efficients.

Ans :

Principle of column chromatographic separation: Partition or distribution coefficients: Differential migration.

In all types of chromatography separations,, including column chromatography, two phases - the stationary phase and the mobile phase are needed. In column chromatography, the stationary phase is a finely-divided, porous solid, while the mobile phase is an organic solvent or solvent mixture. First, the mixture to be separated into its constituents is applied as a small band at the front end of the column stationary phase. The compounds in the mixture get adsorbed to the stationary phase particle surface, and the different compounds adsorb with differing strengths. Now, the mobile phase is introduced into the column which moves through the stationary phase and is in contact with it. The mixture to be separated is or two different compounds A and B. B is more polar than A, the molecules of B are strongly adsorbed to the stationary phase than A. The molecules of B get distributed or partitioned between the stationary phase and mobile phase, i.e., some molecules of B are adsorbed on the stationary phase and some of B are 'soluble' in the mobile phase. Similarly, the molecules of A are partitioned between the stationary phase and mobile phase. B being relatively more polar than A, prefers the polar stationary phase to the mobile phase, while A prefers the polar stationary phase less than B. The concentration of B is more than A in the stationary phase. The ratio of the concentration of A in the stationary phase to that in the mobile phase is the partition coefficient of A. Similarly, the partition coefficient of the B is the ratio of the concentration of B in the stationary phase to that in the mobile phase, its value is independent of A. This is the operating principle in column, thin-layer, and ion-exchange chromatography as well as in normal phase HPLC. In all these types of chromatography, a polar solid acts as the stationary phase.

$$\text{Partition coefficient of A} = \frac{\text{Conc. of A in the stationary phase}}{\text{Conc. of A in the mobile phase}} = k_A$$

$$\text{Partition coefficient of B} = \frac{\text{Conc. of B in the stationary phase}}{\text{Conc. of B in the mobile phase}} = k_B$$

The partition coefficients are also known as distribution coefficients and are represented by k_p or k_D. The partition coefficients of A and B are different. No two organic compounds will have the same value of partition coefficients in a two - phase system the concentration of A is less in the stationary phase than that of B, the partition coefficient of A is less than that of B. The concentration of A is more (relative to B) in the mobile phase, which is the moving phase, therefore A moves faster than B, and A is eluted first. Thus, in a mixture of compounds chromatographed, the compound with the lowest value of partition coefficient elutes first (moves faster), followed by compounds of increasing value of partition coefficient. Thus, in this chromatography (and also in thin-layer and ion- exchange chromatography in which the solid is the stationary phase), the difference in the adsorption strengths of the compounds in the mixture to the stationary phase results in their different partition coefficients which leads to their different rates of movement (differential migration), resulting in their separation.

Q5. Explain the column packing Techniques.

Ans : **(Imp.)**

Wet -packing technique

The chromatography column (thoroughly cleaned with chromic acid, washed with water, rinsed with methanol and dried) whose stopcock is without lubricating grease is clamped vertically, using two clamps. Using a glass rod the bottom end of the column is loosely plugged with a small piece of cotton and the column is filled with benzene to half level. 75 gm of silica gel is made into a slurry in about 400 ml of benzene in a 500 ml conical flask. It is shaken well to obtain a uniform slurry. This slurry is carefully transferred into the column with the stopcock slightly open. The slurry slowly settles down. Any excess benzene is drained out. When all the benzene is drained out and the slurry level in the column is unchanged, close the stopcock. The slurry height is about 2/3 of the length of the column. In a beaker, dissolve the mixture of o-nitroaniline and p-nitroaniline in a minimum volume of benzene (about 5 ml is needed for this). Using a droper, carefully transfer this solution onto the top of the silica gel slurry in the column and slightly open the stopcock. The' compound solution percolates and spreads as a small band at the top. Close the stopcock and insert a piece of cotton above the slurry, fill the column with benzene (mobile phase) and immediately open the stopcock. Adjust the eluate flow-rate to 10 ml/min. Feed the benzene continuously from the top. Collect the column eluate in 10 sequentially-labelled 250 ml conical flasks. In each conical flask, collect about 150 ml of eluate. Initially, an orange band is seen at the top of the column. After about 30 minutes one observes two bands, one a clear bright orange band moving faster than the pale yellow band, with a white gap in between them. The eluate in the flasks labelled 1, 2, 3 is colourless and is only pure benzene. The flasks 4, 5, 6 contain the orange compound (o-nitroaniline). The eluate in flask 7 and 8 is colourless and is pure benzene. The eluate in flasks 9 and 10 is pale yellow and contains the compound p-nitroaniline. The solutions in all the flasks are concentrated to a small volume (10-15 ml) and their TLC is examined. TLC gives information about the purity of the compounds in the 10 flasks. From the TLC information, the solutions in flasks 4, 5, 6 are combined, and the solvent is evaporated to give pure o-nitroaniline ~ 90 mg. Similarly, the solutions in flasks 9 and 10 are concentrated and the solvent is evaporated to give pure p-nitroaniline ~ 90 mg.

Dry - packing technique

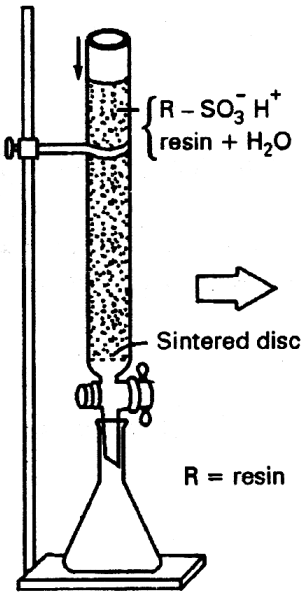
The above column chromatographic separation of o-and p-nitroanilines can also be performed using the dry-packing technique. In this method, fill the column with silica gel directly to about 3/4 level of the column (no solvent is used for this purpose). Now, disconnect the column, and withdraw about 1/5 of the silica gel from the column and transfer it into a beaker containing the mixture of o- and p- nitroanilines and a few millilitres of $CHC1_3$. With continuous stirring on a hot-water bath, evaporate the $CHC1_3$. The mixture of compounds, o- and p-nitroanilines are now adsorbed to the silica gel. It appears as a yellow powder. Now, clamp the column vertically and carefully pour this yellow powder at the top, place a piece of cotton, open the stopper and fill the column with benzene. Adjust the eluate flow rate to 10 ml/min. Feed benzene continuously from the top. Collect the column eluate in 10 serially-labelled 250 ml conical flasks. Concentrate the eluate fractions on a water bath and check the TLC. As in the wet-packing technique, the 1, 2, 3 fractions do not contain any compound, 4, 5, 6 fractions contain orange-yellow o-nitroaniline (~ 90 mg), and fractions 9 and 10 contain pale yellow p-nitroaniline (~ 90 mg).

Q6. Explain the principle involved in cation exchange chromatography.

Ans : **(Imp.)**

Principle of cation-exchange chromatography(Cation-exchange, adsorption, partition coefficients)

A strongly acidic cation-exchange resin (Resin $-SO_3^- H+$) is taken in a beaker containing distilled water. Water molecules percolate the resin matrix, and the resin swells. This resin slurry is poured into a chromatography column which has a sintered disc at the bottom and a stopcock. The resin settles, and the water layer above the resin is drained off. The resin is the stationary phase and its sulfonic acid groups are in the fully ionized form. A solution of Na^+ Cl^- and K^+ Cl^- is applied as a small band at the top end of the resin. The cations K^+ and Na^+ are exchanged by the H^+ cation of the resin. Due to the differences in their sizes, the cations K^+ and Na^+ are bound or adsorbed to the resin with different strengths. The hydrated K^+ ion has a radius which is smaller than the hydrated Na^+. Thus, K^+ is bound or adsorbed stronger than Na^+ to the resin.



Step a

Column packing
resin + water

$$\text{Resin } -SO_3^-H^+ + K^+Cl^- + Na^+Cl^- \longrightarrow \text{Resin } -SO_3^-K^+ \text{ Resin } -SO_3^-Na^+ + HCl$$

The column is now eluted with distilled water (water is the eluent, mobile phase). The K^+ and Na^+ ion remain adsorbed to the resin and remain at the front end of the column only. The column eluate, collected in a conical flask, contains H^+ Cl^-. The column is now eluted or developed with a strong H^+ Cl^- solution or an acidic buffer solution. The cation-exchange takes place again and the cations K^+ and Na^+ are desorbed.

$$\begin{matrix}\text{Resin} - SO_3^-K^+ \\ \text{Resin} - SO_3^-Na^+\end{matrix} + H^+Cl^- \longrightarrow \text{Resin} - SO_3^-H^+ + Na^+Cl^- + K^+Cl^-$$

As the mobile phase solution $H+Cl^-$ moves down; the cations K^+ and Na^+ also move down; Na^+ moves faster than K^+. This is because $K+$ has greater affinity or adsorption than Na^+ to the stationary phase resin. The column eluate is collected in small volumes in several conical flasks, which are serially numbered. As K^+ and Na^+ ions move down, they get separated. The initial column eluate contains pure Na^+ as Na^+Cl^- while the latter column eluate contains pure K^+C1^-. At the completion of elution of K^+ and Na^+, the cation-exchange resin is regenerated.

The rate at which the different cations move depend on: (1) the partition coefficient of the cations, and (2) the pH of the eluent.

Partition or distribution coefficients of the cations

The rate at which different cations ($K+$, Na^+) move on the column is determined by their distribution or partition coefficients.

Adsorbed to the Resin	K^+	K^+	in the Eluent
(Stationary phase)	Na^+	Na^+	(Mobile Phase)
Resin...	...	...	Mobile phase

The cation K^+ is more strongly bound or adsorbed to the resin than $Na+$, i.e., the amount of K^+ in the stationary phase (resin) is more than in the mobile phase. For $Na+$, which is weakly bound to the resin, its amount is relatively less in the stationary phase and more in the mobile phase (moving phase).

$$\text{Partition coefficient of } K^+ = \frac{\text{Conc. of } K^+ \text{ in the stationary phase}}{\text{Conc. of } K^+ \text{ in the mobile phase}}$$

$$\text{Partition coefficient of } Na^+ = \frac{\text{Conc. of } Na^+ \text{ in the stationary phase}}{\text{Conc. of } Na^+ \text{ in the mobible phase}}$$

The partition coefficient of Na^+ is less than that of K^+. The cation which has a lower partition coefficient moves faster and is eluted first. Thus, the order of elution of cations from the column is that the cation with lowest value of partition coefficient elutes first followed by cations with increasing values of partition coefficients. The differences in partition coefficients of the cations is due to their differences in adsorption strength towards the stationary phase. In view of this, cation (ion) exchange chromatography is an adsorption chromatography.

Strength of binding or adsorption of cations (1) The strength with which different, charged cations are held (adsorbed by the resin) decrease in the following order: $M^{+4} > M^{-3} > M^{+2} > M^{+1}$, i.e., Th^{+4} is firmly held by the resin than K^+, e.g., $Th^{+4} > Al^{+3} > Ca^{+2} > K^+$. (2) Within a particular series of ions (cations) carrying the same charge, ions with smaller hydrated ionic radius are held more strongly by the resin than those with a larger hydrated ionic radius. Thus, for M^{+2} cations, the decreasing order of binding is $Ba^{+2} > Sr^{+2} > Ca^{+2} > Mg^{+2} > Be+^2$, ($Ba^{+2}$ is more strongly than Be^{+2}). For M^{+1} cations, the decreasing order of binding to the resin is $Ag^+ > Cs^+ > Rb^+ > N^+H_4 > K^+ > Na^+ > H^+ > Li^+$($Cs^+$ is more strongly held than Li^+). The radius of Li^+ (radius in the solid state, from X-ray analysis) is 0.68 Å, its hydrated radius is 10 Å. For Cs^+ the radius is 1.65 Å and its hydrated radius is 5.05 Å.

pH of the eluent The pH of the eluent also determines the rate of movement of the cations. At a lower concentration of H^+ ions in the eluent the exchange of the cations, e.g., A^+ and B^+ by the H^+ of the eluent, is slower and may selectively exchange one of the cations A or B. At higher concentrations of H^+ in the eluent both A and B cations may be rapidly exchanged by the H^+, both move faster, however at different speeds.

Based on these same principles, using an anion-exchange resin, it is possible to separate a mixture of anions from their solutions. For the anions the affinity (adsorption) to the resin decreases as the radius of the hydrated anion increases

$$NCS^- > I^- > NO_3^- > Br^- > CN^- > HSO_3^- > NO_3^- > Cl^- \ HCO_3^- > CH_3COO^- > OH^- > F^-$$

(i.e., I^- is held stronger than Cl^-).

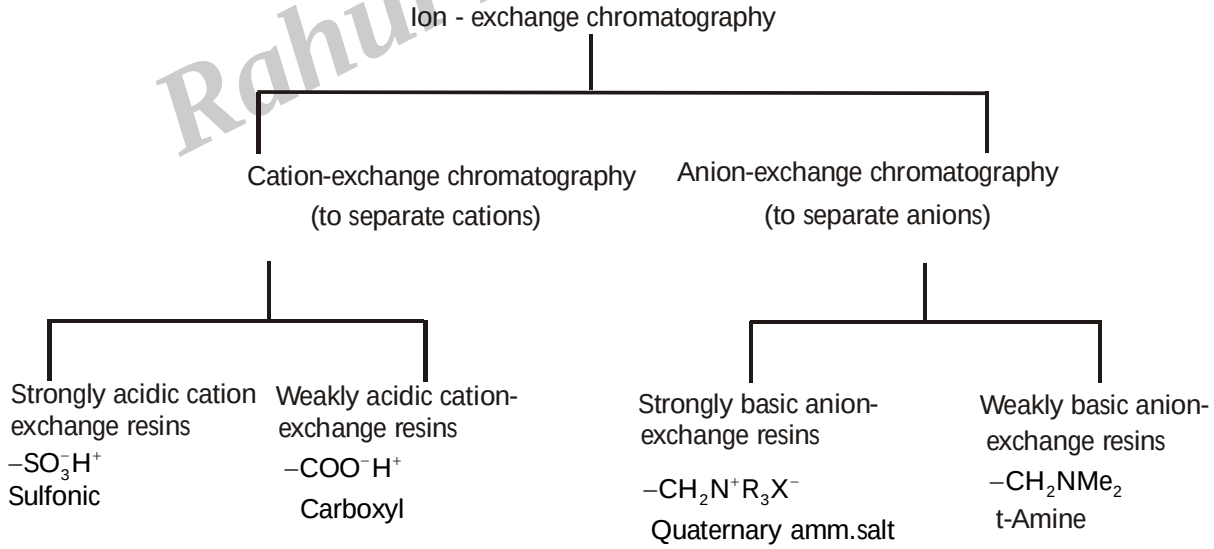
Q7. Write the priciple invvolved in Ion exchange chromatography.

Ans : **(Imp.)**

Ion-exchange chromatography is a technique for separating mixtures of charged compounds, such as cations (K^+, Na^+, Ca^{++}, Cu^{++}, Lanthanides), anions (Cl^-, Br^-, I^-), amino acids (ala, asp, orn), proteins or neutral molecules that can develop a charge in acidic or basic media such as carboxylic acids and amines.

Ion-exchange chromatographic separations are done by using porous resin beads (granules) to which are. bonded acidic groups such as $-SO_3H^+$ or $-COO^-H^+$, or basic groups such as $-CH_2N^+$, R_3X^- or $-CH_2NR_2$.

Depending on the type of bonded group used for ion-exchange chromatography, this technique is classified into two classes: (1) cation - exchange chromatography, and (2) anion - exchange chromatography.



Cation-exchange resins are of two types: (1) strongly acidic cation-exchange resins, and (2) weakly acidic cation-exchange resins. Anion-exchange resins are of two types: (1) strongly basic anion-exchange resins, and (2) weakly basic anion-exchange resins. These resins are used as stationary phases in ion-exchange chromatography.

Q8. Write about cation exchange Resins with chemical structures.

Ans :

(a) Strongly acidic cation-exchange resin

Styrene and divinyl benzene in the ratio 100: 4 -10 are copolymerized in an aqueous emulsion to give styrene-divinyl benzene copolymer in the form of small spherical beads or granules. The cross-linking of the styrene polymer with 4 -10% by weight of divinyl benzene gives a copolymer with good porosity. The resin beads are sulfonated to give sulfonated resin beads. In this sulfonated resin, the negatively charged sulfonated ions are firmly attached to the network and these are balanced by H^+ ions which move freely and can be exchanged. The H^+ ions are known as active or mobile cations.

Cation-exchange Strongly acidic cation-exchange resins contain free cations which can be exchanged for different free cations in the solution.

Eg. 1

$$Resin-SO_3^-H^+ + Na^+Cl^- = = = = => Resin-SO_3^-\ Na^+ + H^+Cl^-$$

(in water) (in solution) (in solution)

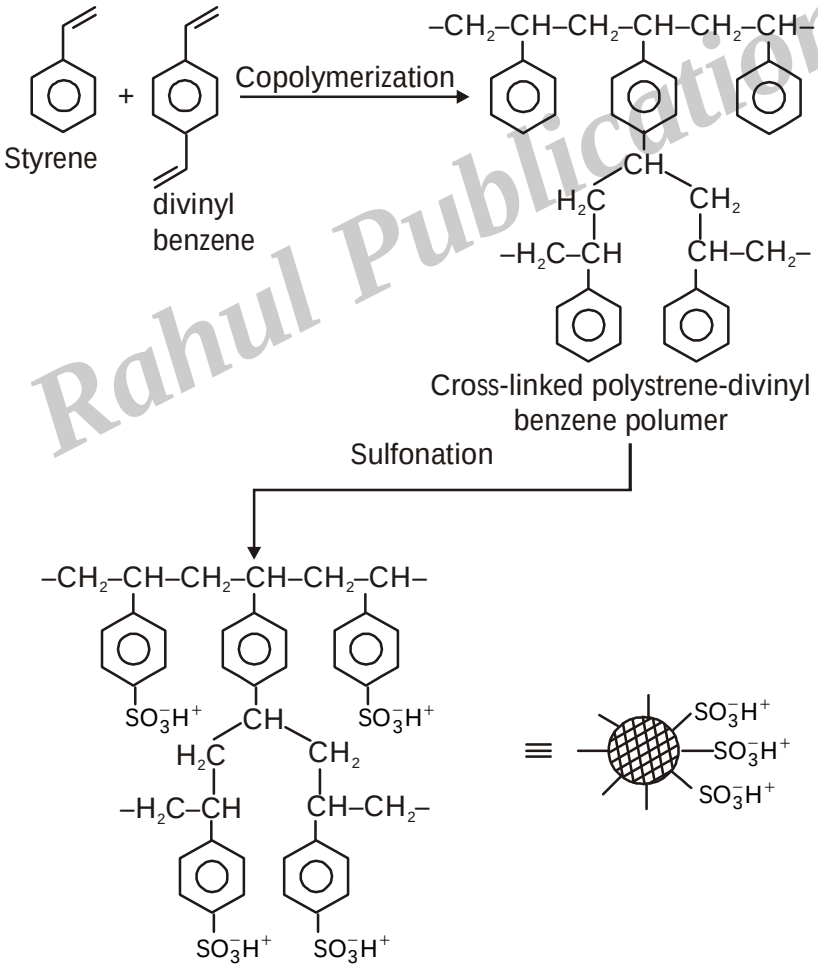


Fig:. Synthesis of strongly acidic cation-exchange resin

The H^+ cation of the resin is exchanged by the Na^+ cation from the solution. Due to this cation-exchange, an equivalent amount of H^+ Cl^- is liberated.

Eg. 2

$$Resin-SO_3^-H^+Ca^{++}2Cl^- = = = = = \Rightarrow (Resin-SO_3^-)_2Ca^{++} + 2H^+2Cl^-$$

The cation-exchange is stoichometric. One doubly-charged ion from the solution (e.g., Ca^{++}) will displace two singly-charged ions from the exchanger. In these two examples, H^+ (a cation) of the resin is exchanged by Na^+ or Ca^{++} cations. Such a process is known as a cation-exchange. The resin in its Resin $-SO_3^-$ H^+ form is known as the hydrogen ion form, while the resin in Resin $-SO_3^-$ Na^+ is known as the sodium ion form of the resin. Cation-exchange resins are marketed in the H ion form or Na ion form.

Eg. 3

$$\begin{matrix} Resin-SO_3^-\ Na^+ \\ or \\ (Resin-SO_3^-)_2Ca^{++} \end{matrix} \xrightarrow[\text{cation exchange}]{\text{strong } H^+\ Cl^-} Resin-SO_3^-\ H^+ + Na^+\ Cl^- or\ Ca^{++}\ 2Cl^- \quad (Resin)$$

A cation-exchange is reversible and the resin is regenerated. In the case of strongly acidic cation-exchange resins, the exchange capacity or ease of exchange is independent of the pH of the solution.

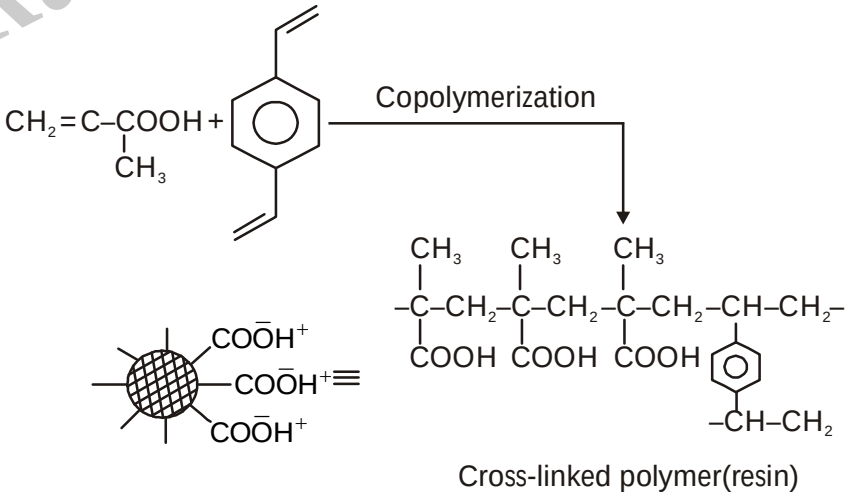
(b) Weakly acidic cation-exchange resin

It is prepared by the copolymerization of methacrylic acid and a small amount of divinyl benzene to give porous beads.

The resin beads contain free COOH groups, and have a weakly acidic property. For these resins, ionization occurs to an appreciable extent only in an alkaline solution. Therefore, these resins have no action below pH 7.

Resin – COOH + Na^+ OH^- $\rightarrow$ Resin – COO^- Na^+ + H_2O (Cation-exchange)

Resin – COOH + Na^+ Cl^- $\rightarrow$ No cation-exchange



Cross-linked polymer(resin)

These resins are marketed in the H ion form. Before being used, they have to be converted into Na ion form by washing with 1M NaOH. Some of the commercial brands of weakly acidic cation-exchange resins are Zeolite 226, and Amberlite 50.

Q9. Write about anion exchange Resins with chemical structure.

Ans :

(a) Strongly basic anion-exchange resin

It is prepared by the copolymerization of styrene with a small amount of divinyl benzene, followed by chloromethylation at the free para position of the phenyls. The reaction with trimethylamine gives quarternary ammonium salt groupings. The quarternary ammonium groups are an integral part of the polymer lattice and there are an equal number of anions (Cl^-), which are the mobile anions. These anions can be exchanged with other different anions in the solution.

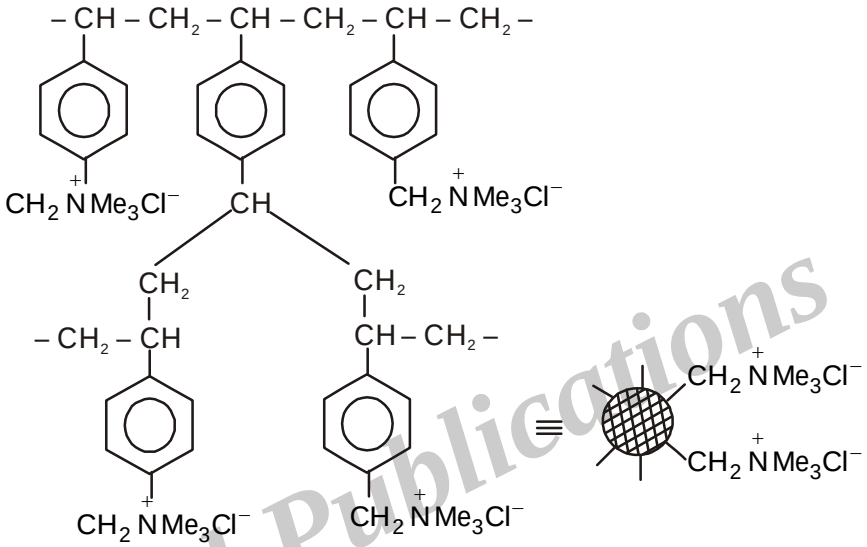


Fig:. The structure of a strongly basic anion - exchange resin

Eg.1

$$Resin - CH_2 - N^+Me_3Cl^- + Na^+OH^- \xrightarrow{\text{anion exchange}} Resin - CH_2 - N^+\ Me_3OH^- + Na^+Cl^-$$

Chloride form Hydroxide form

The resin – CH_2–N^+ Me_3Cl^- is known as the chloride form, and the resin –CH_2–N^+Me_3 OH^- is known as the hydroxide form. Both of them are strongly basic anion - exchange resins and they are largely ionized.

Eg. 2

$$2\ Resin - CH_2N^+Me_3Cl^- + SO_4^{-2} \rightarrow (Resin - CH_2N^+Me_3)_2SO_4^{-2} + 2Cl^-$$

In these two examples, Cl^-, an anion, is exchanged by OH^- or SO_4^{-2} anions, stoichiometrically. This process is known as an anion exchange.

Eg. 3

$$Resin - CH_2 - N^+Me_3OH^- + H^+Cl^- \xrightarrow{\text{anion exchange}} Resin - CH_2 - N^+\ Me_3Cl^- + H_2O$$

(Resin regenerated)

Some of the commercial brands of strongly basic anion-exchange resins are Zeolite FF and Amberlite – 400. They are supplied in the chloride form as they are stable in this form. To convert this resin into hydroxide form, it must be washed with NaOH solution. For these strongly basic anion-exchange resins, their exchange capacity is independent of the pH of the solution.

(b) Weakly basic anion-exchange resin

It is prepared by the copolymerization of styrene with a small amount of divinyl benzene. The resin is chloromethylated, and these groups are converted to t-amine groupings by a reaction with dimethylamine.

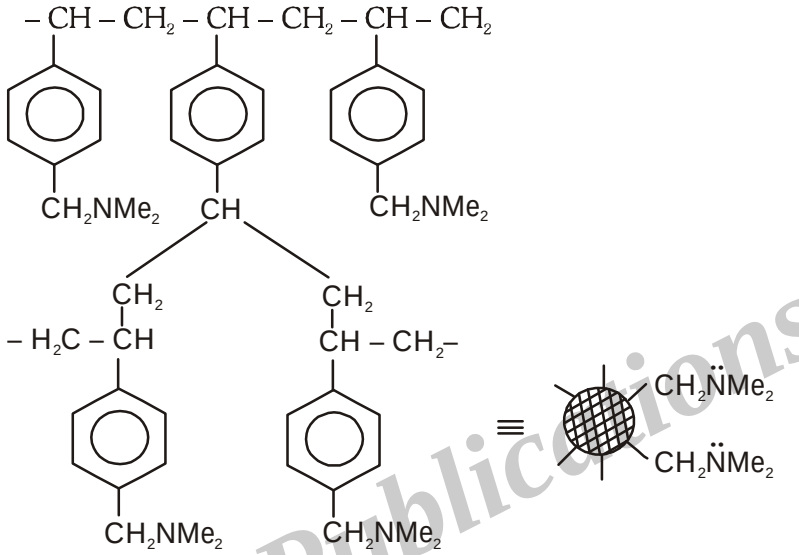


Fig:. The structure of a weakly basic anion-exchange resin

The weakly basic anion-exchange resins contain very little of the hydroxide form in basic solutions as well as in neutral solutions.

$$\underset{\text{(amino form)}}{\text{Resin} - CH_2NMe_2} + H_2O \rightleftharpoons \text{Resin} - \underset{\displaystyle H}{\overset{\displaystyle |}{CH_2 - N^+ Me_2}}\ OH^- \text{(hydroxide form)(less)}$$

The equilibrium is mainly to the left and the resin is largely in the amino form. However, in an acid medium (pH < 7), these resins behave as a strongly basic anion-exchange resin (like the Resin $-CH_2N^+Me_3Cl^-$).

$$\underset{\text{(amino form)}}{\text{Resin} - CH_2N\ Me_2} + H^+Cl^- \longrightarrow \text{Resin} - \underset{\displaystyle H}{\overset{\displaystyle |}{CH_2 - N^+ Me_2}}\ Cl^- \text{(hydrochloride form)}$$

This hydrochloride form of the resin can now be used in an acid solution for the exchange of anions.

$$\underset{\displaystyle H}{\overset{\displaystyle |}{\text{Resin} - CH_2N^+Me_2Cl^-}} + \underset{\text{in solution}}{NO_3^-} \xrightarrow{\text{anion exchange}} \text{Resin} - \underset{\displaystyle H}{\overset{\displaystyle |}{CH_2 - N^+Me_2NO_3^-}} + Cl^-$$

Q10. Write the principle involved in Gas chromotography.

Ans : **(Imp.)**

Principles

- The principle of separation in GC is "partition.
- The mixture of component to be separated is converted to vapour and mixed with gaseous mobile phase.
- The component which is more soluble in stationary phase travel slower and eluted later. The component which is less soluble in stationary phase travels faster and eluted out first.
- No two components has same partition coefficient conditions. So the components are separated according to their partition coefficient.
- Partition coefficient is "the ratio of solubility of a substance distributed between two immiscible liquids at a constant temperature.'

  Two major types:

Gas-solid chromatography

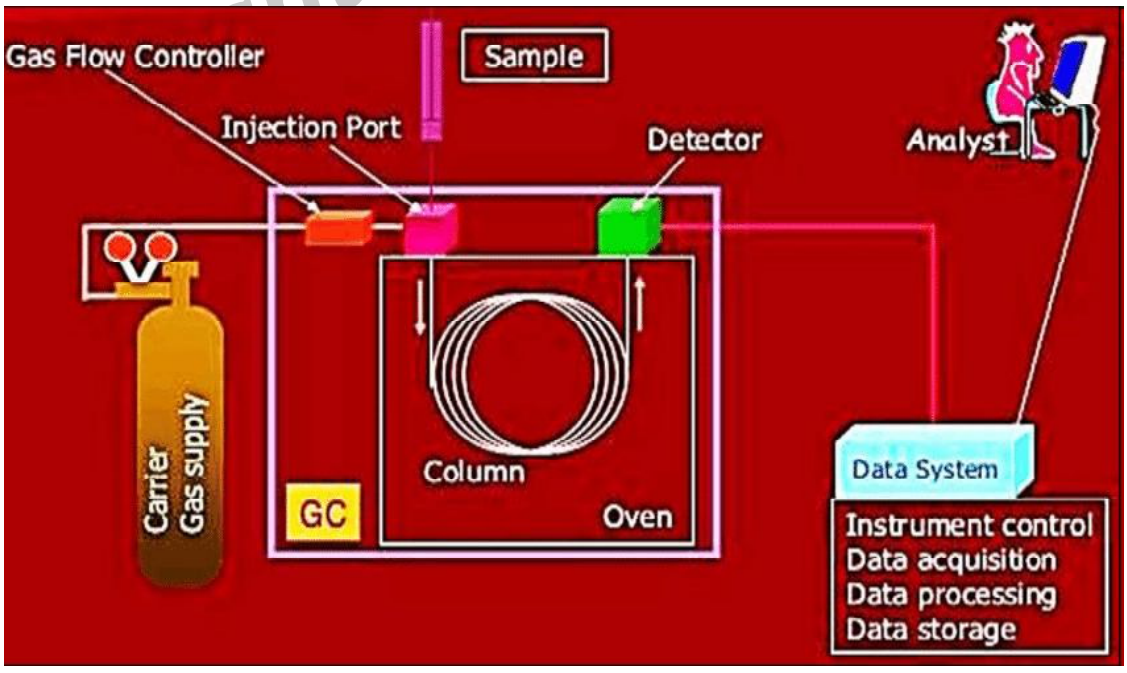
- Here, the mobile phase is a gas while the stationary phase is a solid.
- Used for separation of low molecular gases, e.g., air components, H_2S, CS_2 ,CO_2 ,rare gases, CO and oxides of nitrogen.

Gas-liquid chromatography

- The mobile phase is a gas while the stationary phase is a liquid retained on the surface as an inert solid by adsorption or chemical bonding.

Q11. Draw the block diagram of Gas chromatography.

Ans :



Q12. Explain the instrumentation in gas chromatography.

Ans : **(Imp.)**

Instrumentation

- Carrier gas
  - He (common), N_2, H_2, Argon
- Sample injection port
  - Micro syringe
- Columns
- Detectors
  - Thermal conductivity (TCD)
  - Electron capture detector(ECD)
  - Flame Ionization detector (FID)
  - Flame photometric (FPD) detector

Carrier gas

(i) The cylinder/ gas tank is fitted with a pressure controller to control the pressure of gas, a pressure gauge that indicates the pressure, a molecular sieve to transfer filtered dry gas and a flow regulator to ensure a constant rate of flow of mobile phase to the column.

(ii) It should meet the following criteria:

- Should be chemically inert
- Should be cheap and readily available
- Should be of high quality and not cause any fire accidents
- Should give best possible results
- Should be suitable for the sample to be analyzed and for the detector
- Hydrogen, helium, nitrogen and carbon dioxide are commonly used.
- Hydrogen has low density and better thermal conductivity. However, it reacts with unsaturated compounds and is inflammable and explosive in nature.
- Nitrogen is inexpensive but it gives reduced sensitivity.
- Helium is the most preferred gas.
- Inlet pressure ranges from: 10-50 psi
  - Flow rate : 25-150 mL/min for packed columns
  - Flow rate: 2-25 mL/min for open tubular column

Sampling unit

- Sampling unit or injection port is attached to the column head.
- Since the sample should be in vapourized state, the injection port is provided with an oven that helps to maintain its temperature at about 20-50° C above the boiling point of the sample.
- Gaseous samples may be introduced by use of a gas tight hypodermic needle of 0.5-10 ml capacity.

- For Liquid samples, micro syringes of 0.1-iooμL capacity may be used.

Injections of samples into capillary columns :

(a) Split injections: it splits the volume of sample stream into two unequal flows by means of a needle valve , and allow the smaller flow to pass on to the columns and the bigger part is allowed to be vented to the atmosphere. This technique is not suitable when highest sensitivity is required.

(b) Splitless injectors: They allow all of the sample to pass through the column for loading. Sample should be very dilute to avoid overloading of the column and a high capacity column such as SCOT or heavily coated WCOT columns should be used.

(c) On column injectors: A syringe with a very fine quartz needle is used. Air cooled to -20°c below the b.p. of the sample. After then the warmer air is circulated to vaporize the sample.

(d) Automatic injectors: For improving the reproducibility and if a large number of samples are to be analyzed or operation is required without an attendant, automatic injectors are used.

- The solid samples are introduced as a solution or in a sealed glass ampoule, crushed in the gas stream with the help of a gas tight plunger, and the sample gets vapourized and flows into column under the influence of carrier gas.

Column unit

- Columns are of different shapes and sizes that includes: "U" tube type or coiled helix type.
- They are mainly made of copper, stainless steel, aluminium, Glass, nylon and other synthetic plastics.

Support material

- it's main function is to provide mechanical support to the liquid phase. An ideal support should have a large surface area, chemically inert, should get uniformly wet with liquid phase, should be thermostable.
- Commonly used solid phases are: diatomaceous earth or kieselguhr, glass beads, porous polymers, sand,etc.

Liquid phase

It should have the following requirements:

- It should be non-volatile
- Should have high decomposition temperature
- Should be chemically inert
- Should posses low vapour pressure at column temperature
- Should be chemically and structurally similar to that of the solute i.e., polar for polar solute.

Detectors

The eluted solute particles along with the carrier gas exit from the columns and enter the detector. The detectors then produce electrical signals proportional to the concentration of the components of solute. The signals are amplified and recorded as peaks at intervals on the chromatograph.

An ideal gas chromatographic detector has the following characteristics.

1. It should be highly sensitive towards wide range of compounds.
2. It should be stable during operation conditions.
3. It should produce uniform and linear responses towards wide range of vaporized solute particles.

4. It should have concentration reproducibility.

5. It should be easy to operate.

Generally, gas chromatography detectors are about 4-5 times more sensitive than the liquid chromatography detectors.

Some of the examples of detector are

(a) Thermal Conductivity Detector (TCD)/ Katharometer Detector

(b) Electron Capture Detector (ECD)

(c) Flame Ionization Detector (FID)

Q13. Discuss the applications of Gas chromatography.

Ans : **(Imp.)**

Qualitative Analysis

- Retention time data should be useful for identification of mixtures
- Comparing the retention time of the sample as well as the standard
- Checking the purity of a compound: compar the standard and sample
- Additional peaks are obtained.....impurities are present.....compound is not pure

Quantitative Analysis

- **Direct comparison method**
  - comparing the area of the peak, peak height, width of peak,
- **Calibration curves**
  - standards of varying concentration are used determine peak areas.
- **Internal standard method**
  - A known concentration of the internal standard is added separately to the standard solution
  - The peak area ratio of sample and internal standard....unknown concentration is easily determined.
- **Elemental Analysis**
  - Determination of C, H, O, S and N.
  - Determination of mixture of drugs
  - Isolation and identification of drugs
  - Isolation and identification of mixture of compounds
  - components(amino acids, plant extracts volatile oils)
- **Applications**
  - Qualitative Analysis - by comparing the retention time or volume of the sample to the standard / by collecting the individual components as they emerge from the chromatograph and identifying these compounds by other methods like UV, IR, NMR.
  - Quantitative Analysis-area under a single component elution peak is proportional to the quantity of the detected component/response factor of the detectors. It is done by:

I. Direct comparison method :

$A_{(sample)} / A_{(std)} = \alpha C_{(sample)} / C_{(std)}$

Where, a is the response factor determined for every pure compound under given conditions.

II. Calibration curve : a graph is plotted by taking peak areas on Y axis and concentration of standard compound on X axis. Concentration of unknown sample is then determined by plotting its peak area on same graph.

III. internal standard method: A known concentration of internal standard, which has similar retention characteristics as that of sample is added to both reference standard and test sample.

Pharmaceutical applications

- Quality control and analysis of drug products like antibiotics (penicillin), antivirals (amantidine), general anesthetics (chloroform, ether), sedatives/hypnotics (barbiturates), etc.
- Assay of drugs - purity of a compound can be determined for drugs like :
  - SAtropine sulphate
  - Clove oil
  - Stearic acid
  - In determining the levels of metabolites in body fluids like plasma, serum, urine, etc.

Miscellaneous

- Analysis of foods like carbohydrates, proteins, lipids, vitamins, steroids, drug and pesticides residues, trace elements.
- Pollutants like formaldehyde, carbon monoxide, benzene, DDT etc.
- Dairy product analysis like milk, butter-for detection of aldehydes, milk sugars, ketones and fatty acids.
- Separation and identification of volatile materials, plastics, natural and synthetic polymers, paints, and microbiological samples.

Q14. Discuss the principle and working of HPLC.

Ans :

Normal Phase HPLC : Principle

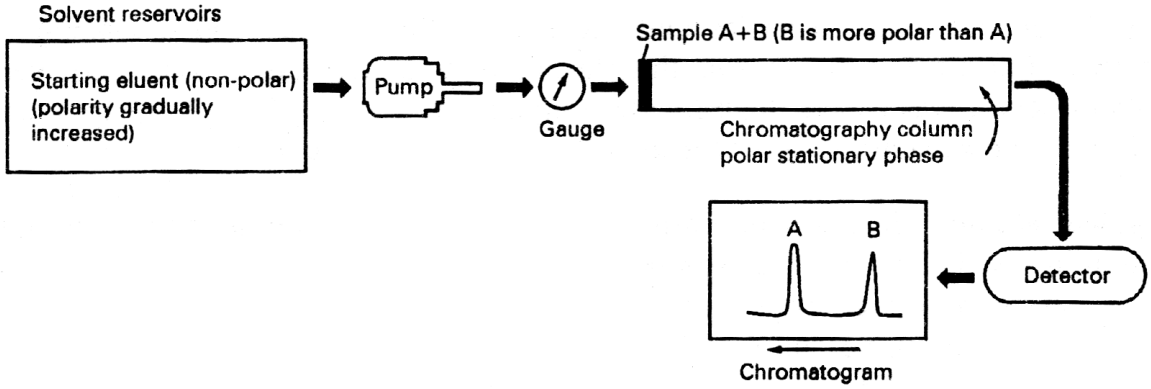


Fig:. Normal phase HPLC (diagrammatic representation)

Table. Stationary and Mobile Phases in Normal Phase HPLC

Stationary Phase (Commercial Name)	Mobile Phase Silica, Polar
1. Corasil	1. Hexane-Chloroform
	2 Durapak OPN
2. Isooctane-isopropanol	1. Hexane
	2. Hexane-isopropanol
3. BOP	1. Hexane-chloroform
	2. Isooctane-alcohol
4. Permaphase ETH	1. Hexane-alcohol
	2. $CHCl_3$

In normal phase HPLC, the chromatographic column is packed with polar (Si-OH) stationary phase materials. Assume that the sample to be analyzed is a mixture of A + B, where B is more polar than A and is injected into the column. On the highly polar stationary phase, B is relatively more strongly adsorbed than A to the Si-OH groups. The column elution starts with a less polar solvent such as hexane or isooctane, and the solvent polarity is gradually increased by adding $CHCl_3$ or alcohol to hexane. The less polar compounds elute first in the less polar solvents, the polar compounds are eluted later in the polar solvents. Thus, the less polar A moves faster than the polar B.

The solid stationary phase and the liquid mobile phase may be considered as a 2-phase system. Compound A is partitioned between the stationary phase and the mobile phase. Similarly, compound B is partitioned between the stationary phase and the mobile phase. B, being more polar, has more affinity (adsorption) to the stationary phase than A. The concentration of B is more in the stationary phase and less in the mobile phase; whereas for A, its concentration is less in the sationary phase and more in the mobile phase. The partition coefficients of A and 3 are different. The partition coefficient of B is more than that of A. A moves faster relative to B, and hence A is eluted first.

$$\text{Partition coefficient of A} = \frac{\text{Conc. of A is the sta. phase}}{\text{Conc. of A in the mob. phase}}$$

$$\text{Partition coefficient of B} = \frac{\text{Conc. of B is the sta. phase}}{\text{Conc. of B in the mob. phase}}$$

In normal phase HPLC, the polarity of the mobile phase is gradually increased during elution. Table gives some of the stationary phase and mobile phase solvent systems in normal phase HPLC.

Q15. What are the applications of HPLC.

Ans : **(Imp.)**

High-Performance Liquid Chromatography (HPLC)

Applications of HPLC

High-performance liquid chromatography (HPLC) is the most widely used instrumental technique. Its applications are:

1. Monitoring the progress of chemical reactions in organic research laboratories, drug R & D and drug manufacturing units.
2. Monitoring the progress of purifications (by column chromatography or crystallizations, etc.).
3. Determination of purity of samples.

4. Identification of unknown compounds by a comparison with standards using retention times.
5. Small-scale quantitative separations (preparative HPLC).
6. Detection and quantification of pesticides in the environment.
7. Analysis of drugs and their metabolites in biological fluids.
8. Forensic analysis of drugs, explosives, and poisons.
9. Detection and quantification of narcotics.

The HPLC is used for :

1) Separation of mixtures of compounds which are very similar in chemical structure.
2) Separation of polar and non-polar organic compounds
3) Separation of organic salts
4) Separation of non-volatile organic compounds such as amino acids and sugars, and
5) Separation of inorganic ions.

The HPLC is highly efficient technique, and requires extremely small amounts of the sample.

Q16. Draw a Neat diagram of HPLC instrument.

Ans :

HPLC Instrument

A schematic representation of a HPLC instrument is given in Fig. The basic units are the solvent reservoirs (mobile phase), pump, injection point (sample solution injection), a chromatography column packed with stationary phase material, detector and recorder.

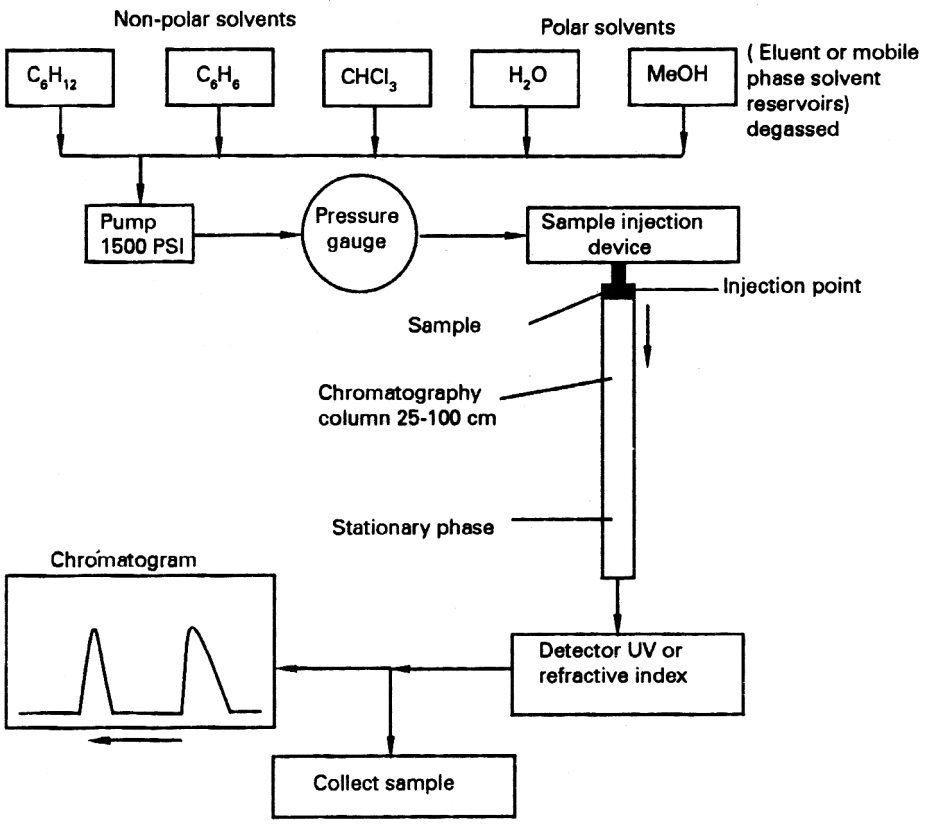


Fig:. HPLC instrument (diagrammatic representation)

Q17. Discuss and write the stationary phases and mobile phases in HPLC

Ans :

Stationary Phases in HPLC

The HPLC is broadly classified into two types: 1) Normal Phase HPLC, and 2) Reversed Phase HPLC. Highly polar stationary phase materials are used for normal phase HPLC. Non-polar or intermediate polar stationary phase materials are used for reversed phase HPLC. Both polar as well as non-polar stationary phase materials are extremely small, spherical, porous silica particles. The polar stationary phase silica particles have hydroxy functional groups on their surface while the non-polar and intermediate polar stationary phase silica particles have alkyl (C_8 or Ci_8) or nitrile functional groups on their surface. These alkyl or nitrile groups are bonded to silica. For this reason, they are known as bonded stationary phases.

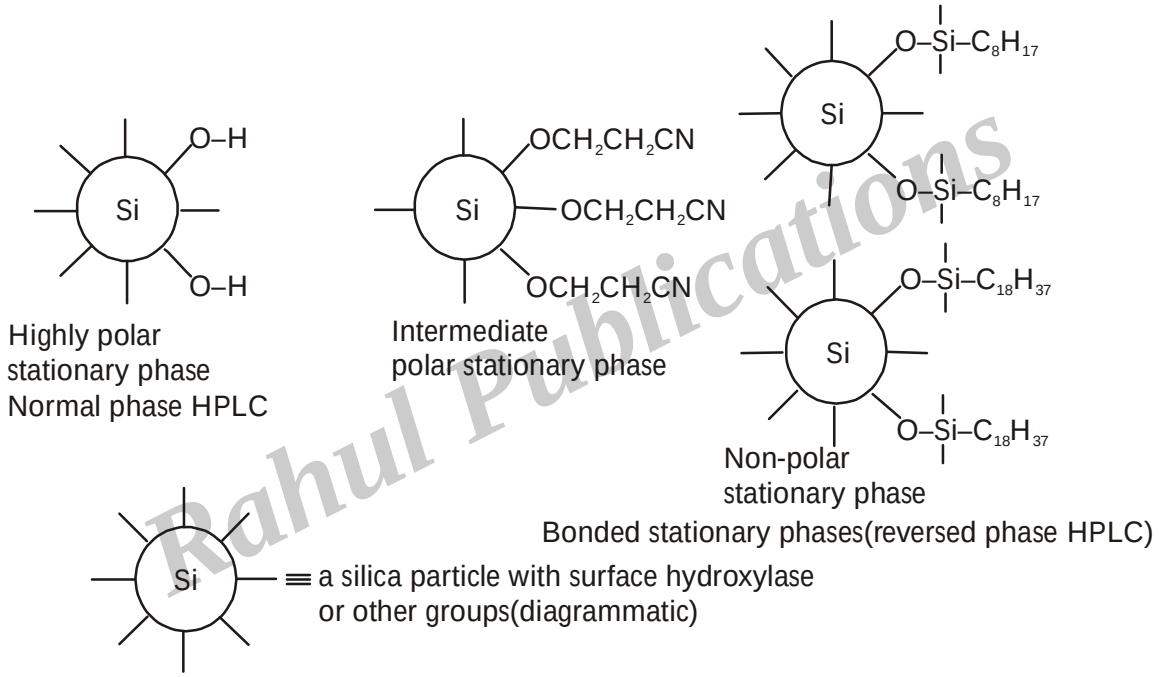


Fig:. Stationary phases in HPLC

Highly polar stationary phase SiO_2 particles, on heating with dil. HC1, give rise to a hydroxyl group bearing silica particles. These particles are extremely small, porous, spherical and therefore have a large surface area. This large surface area is responsible for their high performance in chromatographic separations (high performance means greater resolutions or separation power). The surface of these particles have OH functional groups (these silica particles are similar to the silica gel used in column chromatography and TLC, but are superior to them with respect to their uniform, spherical shape and porous nature, and hence are expensive). Because of the presence of OH groups, this material constitutes a highly polar stationary phase. It is a solid stationary phase and any separation of a mixture of compounds on this type of stationary phase is based on adsorption. Different compounds in the mixture have differing adsorption strengths and, in the presence of a suitable mobile phase solvent, get separated (exactly in the same way as discussed in column chromatography and TLC). A column packed with these silica particles is used for normal phase HPLC work.

Bonded stationary phases

These are of two types: They are: a) intermediate polar stationary phase and b) non-polar stationary phase.

(a) Intermediate polar stationary phase

The nitrile-bonded stationary phase is obtained by the reaction of silica with acrylonitrile. Nitrile groups are bonded to silica.

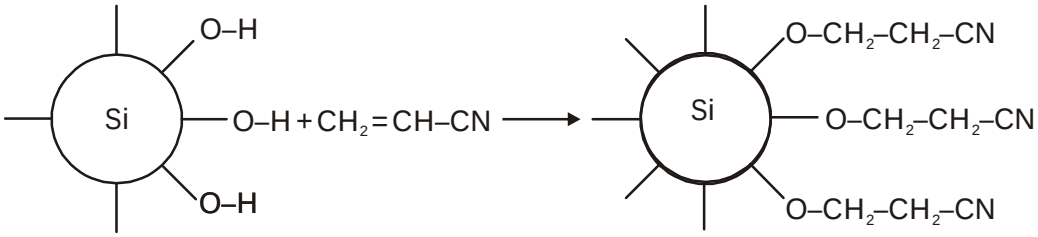


Fig:. Intermediate polar stationary phase

The bonded propanenitrile groups on the surface of the silica particle appear like brushes. These organic ligand (nitrile)-bearing silica particles are extremely small, spherical, and porous; therefore, the organic ligands have a large surface area. The $CH_2 - CH_2 - CN$ - type of stationary phase material is less polar than the Si-OH-type, but more polar than the C_8H_{17} or $C_{18}H_{37}$ n-alkyl type. Therefore, it is called an intermediate polar stationary phase. The $CH_2 - CH_2 - CN$ is equivalent to an organic liquid (CH_3CH_2CN is a liquid). Thus, this stationary phase is a liquid stationary phase. This liquid is supported by bonding to the silica particle. The silica particle is an inert support, bearing the organic liquid-type CH_2CH_2CN group. The separation of a mixture of compounds on liquid stationary phases is done by the differences in their partition coefficients in the presence of a suitable mobile phase. It should be noted that although these stationary phase particles are solid in nature, they should not be considered as a solid stationary phase.

(b) Mobile phase

The mobile phase in HPLC is a liquid. The liquids (column eluents) are pure organic solvents or solvent mixtures. Acetic acid, H_2O, and buffer solutions are also used as eluents. In normal phase HPLC, the starting eluent is generally a non-polar solvent such as n-hexane, n-octane, or $CHC1_3$ and the solvent polarity is gradually increased during elution. In reversed phase HPLC, the starting eluent is highly polar such as MeOH, or H_2O and the solvent polarity is gradually decreased during elution.

Highly pure and chromatography-grade solvents are used. They are degassed before use. The mobile phase liquid is pumped into the chromatography column at very high pressure.

Q18. Explain the analysis of paracetamol by using HPLC method.

Ans :

Experimental method

The HPLC analysis was performed on SHIMADZU LC 20 AT dual pump system with UV at ambient temperature. A Phenomenex C18 Column (250 mm x 4.6 mm 5 μ) was utilized. All the calculations concerning the quantitative analysis were performed with external standardization by the measurement of peak area that are integrated automatically by computer using spinchrom CFR software program.

Material and chemicals

Pharmaceutical grade paracetamol was procured from Emcure pharmaceutical Ltd. Pune HPLC grade acetonitrile procure from Fisher scientific pune and HPLC grade water from LOBA Chemicals pharmaceutical formulation and febrinil 650 was procure from commercial pharmacist shop standard working solution. Standard stock solution of paracetamol was prepared by dissolving accurately weighed 10 mg of drug in 10 mL of mobile phase (actonitrile and water 60 : 40 v/v) HPLC grade and filter through 0.25 μ membrane as external standard.

Sample solution

Twenty tablet of Febrinil each containing 650 mg of paracetamol weighted and finely powdered in mortar. A quantity equivalent to 50 mg of paracetamol weighed and transferred to a volumetric flask and dissolved in 50 mL of mobile phase i.e. mixture of acetonitrile and water (60 : 40 V/V.) This sample solution was stirred magnetically for five min. and centrifuged at 1000 rpm. It was diluted to get the solution of 0.5 mg/mL (500 μg) and 0.05 mg (50 μg) concentration filter through 0.25 μ membrane and degassed.

Results and Discussion

Method development HPLC analysis was performed by isocratic elution. The mobile phase was selected after several trials with acetonitrile and water maximum detection and sensitivity was observed in mobile phase ratio 60 : 40 v/v/ (ACN and Water). All the solvent were filtered through 0.45 μ Millipore filler before used and degassed in an ultrasonic bath. Standard sample solutions were also filtered through 0.25 μ membrane and degassed. 20 μL of standard solution was injected to Rhenodyne injector several time to optimized the condition. A steady baseline was recorded at the flow rate 1 mL/ min at the retention time 2.690 minute and UV detection at 210 nm. A typical chromatogram of paracetamol is given in Fig. 1 a and b. Throughout the study, the suitability of the chromatograph is system was monitored by calculating the resolution, selectivity and peak symmetry. Optimized chromatographic conditions are listed in Table 1. Table 1: Parameter Optimized condition Chromatograph Shimadzu HPLC Column Phenomenex C18 Column (250 mm x 4.6 mm 5 μ) Mobile phase Acetonitrile and water (60 : 40 V/V) Cont… 234 P. R. Solanki et al.: RP-HPLC Method for Estimation of…. Parameter Optimized condition Flow rate 1.0 mL/ min Detection UV at 210 nm Injection volume 20 μL Temperature Ambient Retention time of paracetamol 2.690 min Method was validated with respect to precision, accuracy stability, specificity, linearity, LOD, LOQ ruggedness and robustness according to ICH guidelines.

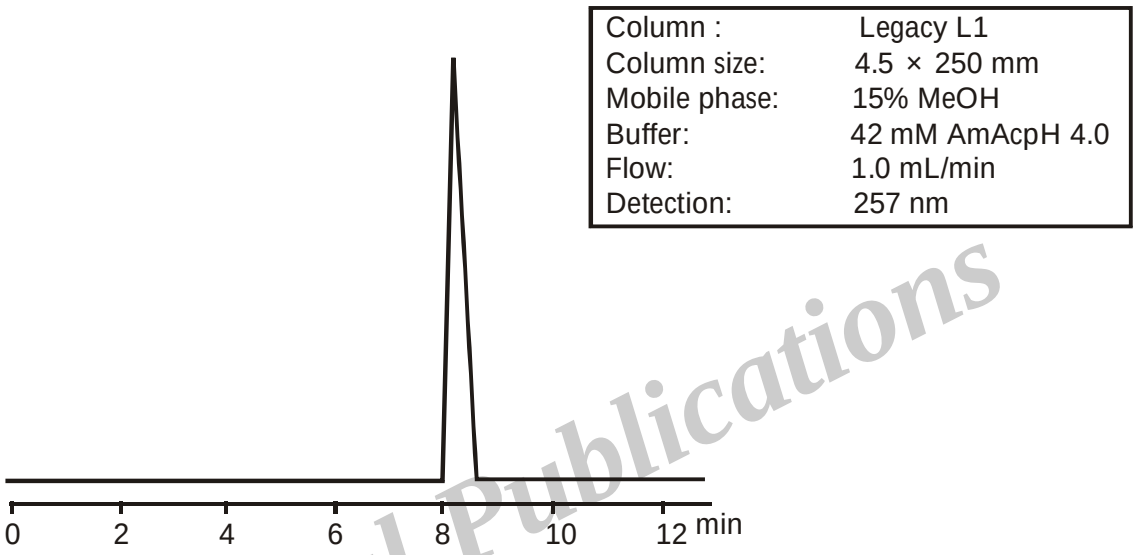
Accuracy

Accuracy of the method was determined by recovery experiments. The prepared standard solution was injected six times as a test sample from the respective area counts the concentration of the of paracetamol was calculated using the detector responses. The precision of the method was demonstrated by interday and intraday variation studies the response factor of the drug peak and % RSD were calculated. From the data developed HPLC method was found to be precise.

Linearity

The Linearity of the method was determined at seven concentration levels ranging from 20-100 mg/ mL. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was Y = 596.20 x + 12. 8458 (Table 3) shows the regression equation correlation coefficient (r2) RSD value of the slope, intercept LOD and LOQ values. The limit of detecting LOD and limit of quantification of (LOQ) the developed method by injecting progressively low concentration of std solution using the developed RP HPLC method. The LOD of drug was found to be 0.02 μg / mL and LOQ was 19.29 μg /mL. The ruggedness of the method was tested by injecting the standard working solution into different days. The high degree of reproductivity of detector

responses and retention times indicate that the method is fairly rugged. Fig. 1(a): Standard chromatograph 1(b): Sample chromatograph The stability of standard and sample solutions were evaluated under different storage condition for short term stability, solutions were kept of room temperature for 24 hrs. The long terms stability was assessed after storage of stock solution at 4o C for 15 days. The results were evaluated by comparing peak area ratio for standard solutions and sample solutions with those of freshly prepared solutions. The result found within 90-91 % of initial values indicates the stability of solution. The method developed was found to be specific for the quantitative determination of paracetamol bulk drugs like febrinil Sample solution was analyzed in triplicate after preparation of the drug solution as mentioned above is experimental section. The amount of paracetamol was found to be within the range of 90-91 % none of the excipients were found to interfere with the analyte peak and the results.



Short Question and Answers

1. Describe the technique used in column chromatography.

Ans :

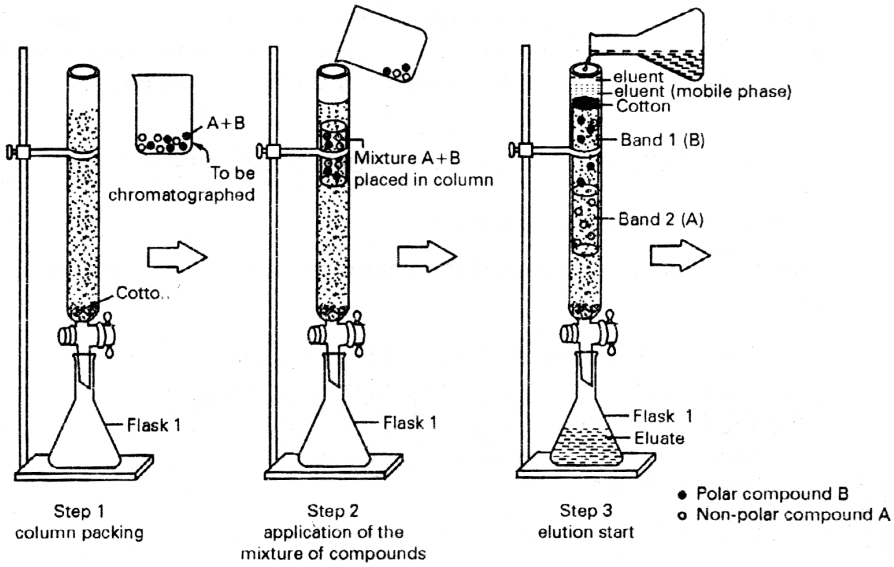
Column Chromatography

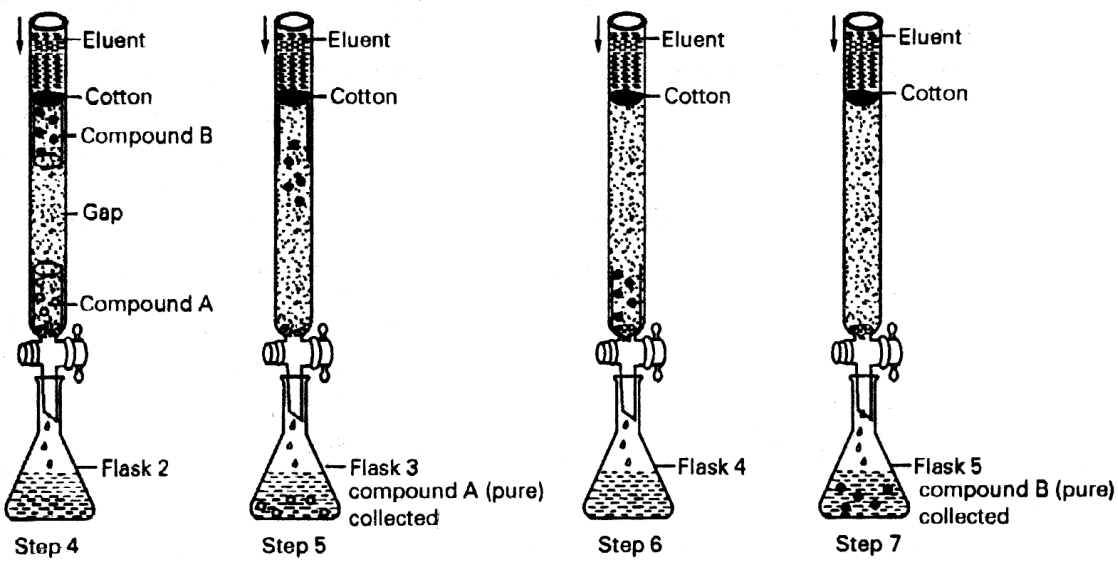
Column chromatography is a widely-used technique for the quantitative separation of complex mixtures of organic compounds in the gm-kg scale. It is mainly used in organic research laboratories for the separation and purification of reaction products. In the drug industry, two anti-cancer drugs - taxol and vincaleucoblastin are isolated in a pure form from their plant extracts by column chromatography.

In this type of chromatography, finely-powdered, porous solids such as silica gel or alumina, which constitute the stationary phase, are packed into a burette-like glass tube, commonly called the column. The column is clamped vertically. The mixture of compounds to be separated are dissolved in a very small volume of organic solvent and applied as a narrow band at the front end of the column. A liquid which is generally an organic solvent such as n-hexane, benzene, chloroform, constituting the mobile phase, is allowed to flow through the stationary phase by gravity. The compounds in the mixture have different adsorption strengths towards the material of the stationary phase. Therefore, the compounds in the mixture, when allowed to move down the column, move with different speeds. Weakly- adsorbed compounds move faster than the strongly adsorbed ones. The different speeds of movement of compounds in the column is known as the differential migration.

In chromatography techniques that use solid material as the stationary phase, e.g., column chromatography and thin-layer chromatography, the compounds in the mixture get separated due to their differences in adsorption strength towards the stationary phase. These techniques are therefore called adsorption chromatography and the stationary phase materials (silica gel, alumina) are called adsorbents.

The chromatography techniques that use. liquids as the mobile phase, e.g., column, thin-layer, ion-exchange, paper and high-performance liquid chromatography are all called liquid chromatography.





The solvent (mobile phase) introduced at the front end of the column stationary phase is called the eluent and that which leaves the column with or without the separated compounds, collected in conical flasks, is called eluate or column fraction. The process by which the compounds are carried through the stationary phase by the mobile phase is called the development or elution of the column.

2. Write about adsorbents used in column chromatography.

Ans :

Nature of Adsorbents

Stationary phase materials: Adsorbents

Table. Gives the list of adsorbents and the nature of their active sites used in column chromatography.

Sl.No.	Adsorbent	Nature of the surface active site
1.	Silica Gel	Acidic
2.	Alumina	Acidic and basic sites
3.	Magnesium Silicate	Acidic
4.	Kieselguhr	Neutral
5.	Charcoal	Neutral and Acidic
6.	Sucrose	Neutral
7.	Starch	Neutral

These adsorbents are finely-divided, porous particles with large surface areas ~ 50m^2/'g. The adsorbent may be directly taken into the column as the dry powder (dry-packing method), or it may be made into a slurry in an organic solvent and then poured into the column and the solvent drained off (wet-packing method). Silica gel and alumina are the two most common chromatographic adsorbents in use. They are cheap and readily available commercially.

Silica gel Adsorbents with the general formula $SiO_{2x}H_2O$ are called silica, silica gel, or silicic acid. The surface of the SiO_2 particle is covered by hydroxyl groups.

Free hydraxyl group Bound and reactive hydrocxl group

Fig:. The surface of a silica gel particle

It is the presence of these surface hydroxyl groups that is responsible for the selective adsorption properties of silica gel. Silica gel is therefore a highly polar, solid, stationary phase. The silica gel surface is weakly acidic (pH - 3 5).

Alumina, acidic ($A1_2O_3$) On heating hydrated alumina $Al_2O_3xH_2O$ to 300-400°C, most of the adsorbed water is drawn off, with the remainder of the water reacting with the surface $A1_2O_3$ to form hydroxyl groups. This type of alumina, used for column chromatography, is known as activated alumina. The surface of the particle of Al_2O_3 has hydroxy groups, which are acidic in nature (pH-4), and hence it is known as acidic alumina. Like silica gel, acidic alumina is also a highly polar, solid stationary phase.

Alumina, basic (Al_2O_3) Heating hydrated alumina to $Al_2O_3xH_2O$ to 800-1000°C removes the water molecules totally to give hydroxyl-free $A1_2O_3$, the oxide ions now give the basic properties to alumina. Basic alumina is also a polar, solid stationary phase.

3. Explain the Wet -packing technique.

Ans :

The chromatography column (thoroughly cleaned with chromic acid, washed with water, rinsed with methanol and dried) whose stopcock is without lubricating grease is clamped vertically, using two clamps. Using a glass rod the bottom end of the column is loosely plugged with a small piece of cotton and the column is filled with benzene to half level. 75 gm of silica gel is made into a slurry in about 400 ml of benzene in a 500 ml conical flask. It is shaken well to obtain a uniform slurry. This slurry is carefully transferred into the column with the stopcock slightly open. The slurry slowly settles down. Any excess benzene is drained out. When all the benzene is drained out and the slurry level in the column is unchanged, close the stopcock. The slurry height is about 2/3 of the length of the column. In a beaker, dissolve the mixture of o-nitroaniline and p-nitroaniline in a minimum volume of benzene (about 5 ml is needed for this). Using a droper, carefully transfer this solution onto the top of the silica gel slurry in the column and slightly open the stopcock. The' compound solution percolates and spreads as a small band at the top. Close the stopcock and insert a piece of cotton above the slurry, fill the column with benzene (mobile phase) and immediately open the stopcock. Adjust the eluate flow-rate to 10 ml/min. Feed the benzene continuously from the top. Collect the column eluate in 10 sequentially-labelled 250 ml conical flasks. In each conical flask, collect about 150 ml of eluate. Initially, an orange band is seen at the top of the column. After about 30 minutes one observes two bands, one a clear bright orange band moving faster than the pale yellow band, with a white gap in between them. The eluate in the flasks labelled 1, 2, 3 is colourless and is only pure benzene. The flasks 4, 5, 6 contain the orange compound (o-nitroaniline). The eluate in flask 7 and 8 is colourless and is pure benzene. The eluate in flasks 9 and 10 is pale yellow and contains the compound p-nitroaniline. The solutions in all the flasks are concentrated to a small volume (10-15 ml) and their TLC is examined. TLC gives information about the purity of the compounds in the 10 flasks. From the TLC information, the solutions in flasks 4, 5, 6 are combined, and the solvent is evaporated to give pure o-nitroaniline ~ 90 mg. Similarly, the solutions in flasks 9 and 10 are concentrated and the solvent is evaporated to give pure p-nitroaniline ~ 90 mg.

4. Describe the day - packing technique

Ans :

The above column chromatographic separation of o-and p-nitroanilines can also be performed using the dry-packing technique. In this method, fill the column with silica gel directly to about 3/4 level of the column (no solvent is used for this purpose). Now, disconnect the column, and withdraw about 1/5 of the silica gel from the column and transfer it into a beaker containing the mixture of o- and p- nitroanilines and a few millilitres of $CHC1_3$. With continuous stirring on a hot-water bath, evaporate the $CHC1_3$. The mixture of compounds, o- and p-nitroanilines are now adsorbed to the silica gel. It appears as a yellow powder. Now, clamp the column vertically and carefully pour this yellow powder at the top, place a piece of cotton, open the stopper and fill the column with benzene. Adjust the eluate flow rate to 10 ml/min. Feed benzene continuously from the top. Collect the column eluate in 10 serially-labelled 250 ml conical flasks. Concentrate the eluate fractions on a water bath and check the TLC. As in the wet-packing technique, the 1, 2, 3 fractions do not contain any compound, 4, 5, 6 fractions contain orange-yellow o-nitroaniline (~ 90 mg), and fractions 9 and 10 contain pale yellow p-nitroaniline (~ 90 mg).

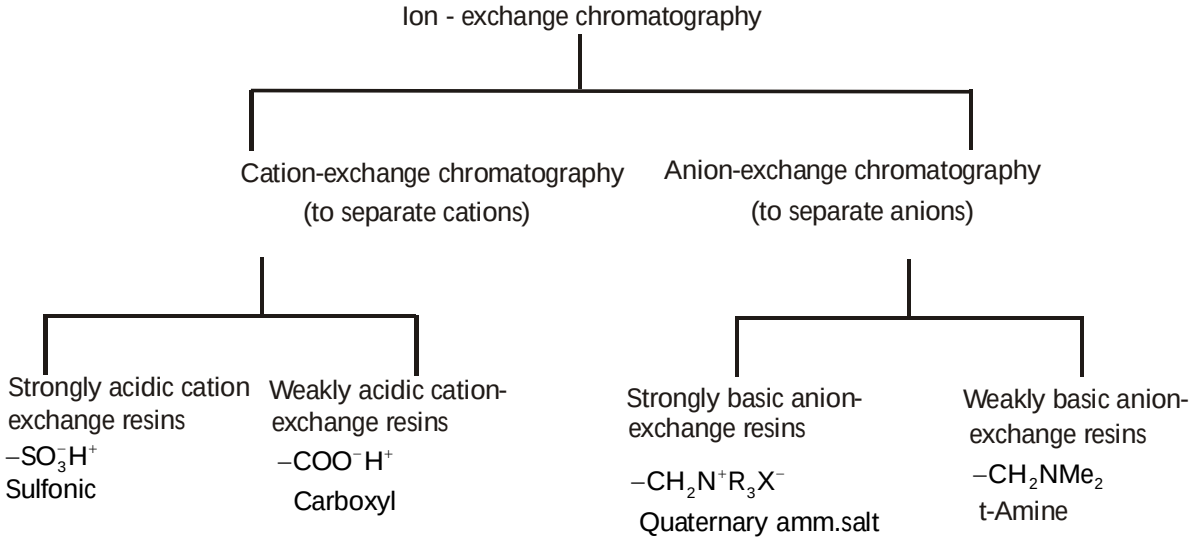
5. Write the priciple invvolved in Ion exchange chromatography.

Ans :

Ion-exchange chromatography is a technique for separating mixtures of charged compounds, such as cations (K^+, Na^+, Ca^{++}, Cu^{++}, Lanthanides), anions (Cl^-, Br^-, I^-), amino acids (ala, asp, orn), proteins or neutral molecules that can develop a charge in acidic or basic media such as carboxylic acids and amines.

Ion-exchange chromatographic separations are done by using porous resin beads (granules) to which are. bonded acidic groups such as $-SO_3H^+$ or $-COO^-H^+$, or basic groups such as $-CH_2N^+$, R_3X^- or $-CH_2NR_2$.

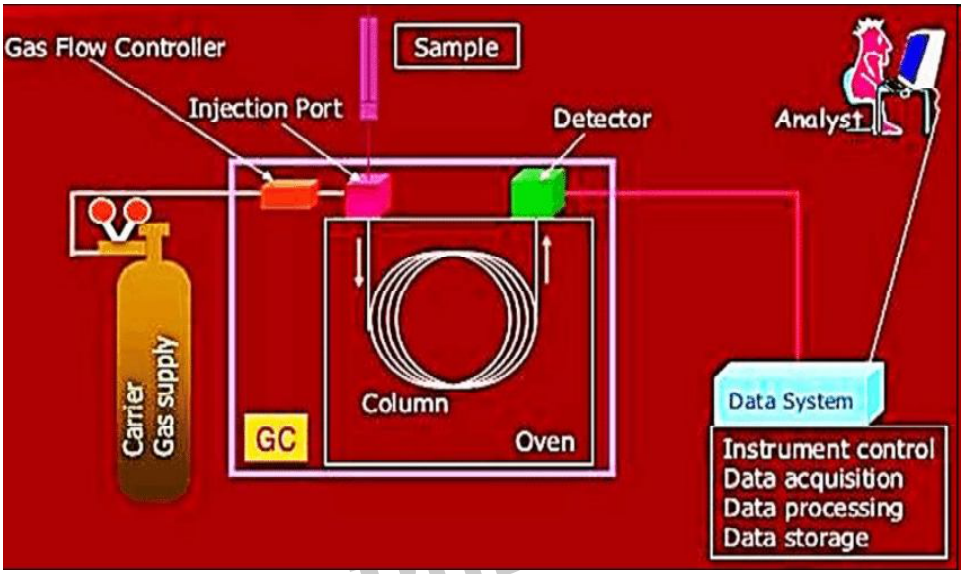
Depending on the type of bonded group used for ion-exchange chromatography, this technique is classified into two classes: (1) cation - exchange chromatography, and (2) anion - exchange chromatography.



Cation-exchange resins are of two types: (1) strongly acidic cation-exchange resins, and (2) weakly acidic cation-exchange resins. Anion-exchange resins are of two types: (1) strongly basic anion-exchange resins, and (2) weakly basic anion-exchange resins. These resins are used as stationary phases in ion-exchange chromatography.

6. Draw the block diagram of Gas chromatography.

Ans :



7. Discuss the applications of Gas chromatography.

Ans :

Qualitative Analysis

- Retention time data should be useful for identification of mixtures
- Comparing the retention time of the sample as well as the standard
- Checking the purity of a compound: compar the standard and sample
- Additional peaks are obtained.....impurities are present.....compound is not pure

Quantitative Analysis

- **Direct comparison method**
  - comparing the area of the peak, peak height, width of peak,
- **Calibration curves**
  - standards of varying concentration are used determine peak areas.
- **Internal standard method**
  - A known concentration of the internal standard is added separately to the standard solution
  - The peak area ratio of sample and internal standard....unknown concentration is easily determined.

- **Elemental Analysis**
  - Determination of C, H, O, S and N.
  - Determination of mixture of drugs
  - Isolation and identification of drugs
  - Isolation and identification of mixture of compounds
  - components(amino acids, plant extracts volatile oils)
- **Applications**
  - Qualitative Analysis - by comparing the retention time or volume of the sample to the standard / by collecting the individual components as they emerge from the chromatograph and identifying these compounds by other methods like UV, IR, NMR.
  - Quantitative Analysis-area under a single component elution peak is proportional to the quantity of the detected component/response factor of the detectors. It is done by:

I. Direct comparison method :

$A_{(sample)} / A_{(std)} = \alpha\, C_{(sample)} / C_{(std)}$

Where, a is the response factor determined for every pure compound under given conditions.

II. Calibration curve : a graph is plotted by taking peak areas on Y axis and concentration of standard compound on X axis. Concentration of unknown sample is then determined by plotting its peak area on same graph.

III. internal standard method: A known concentration of internal standard, which has similar retention characteristics as that of sample is added to both reference standard and test sample.

Pharmaceutical applications

- Quality control and analysis of drug products like antibiotics (penicillin), antivirals (amantidine), general anesthetics (chloroform, ether), sedatives/hypnotics (barbiturates), etc.
- Assay of drugs - purity of a compound can be determined for drugs like :
  - SAtropine sulphate
  - Clove oil
  - Stearic acid
  - In determining the levels of metabolites in body fluids like plasma, serum, urine, etc.

Miscellaneous

- Analysis of foods like carbohydrates, proteins, lipids, vitamins, steroids, drug and pesticides residues, trace elements.
- Pollutants like formaldehyde, carbon monoxide, benzene, DDT etc.
- Dairy product analysis like milk, butter-for detection of aldehydes, milk sugars, ketones and fatty acids.
- Separation and identification of volatile materials, plastics, natural and synthetic polymers, paints, and microbiological samples.

8. What are the applications of HPLC.

Ans :

High-Performance Liquid Chromatography (HPLC)

Applications of HPLC

High-performance liquid chromatography (HPLC) is the most widely used instrumental technique. Its applications are:

1. Monitoring the progress of chemical reactions in organic research laboratories, drug R & D and drug manufacturing units.
2. Monitoring the progress of purifications (by column chromatography or crystallizations, etc.).
3. Determination of purity of samples.
4. Identification of unknown compounds by a comparison with standards using retention times.
5. Small-scale quantitative separations (preparative HPLC).
6. Detection and quantification of pesticides in the environment.
7. Analysis of drugs and their metabolites in biological fluids.
8. Forensic analysis of drugs, explosives, and poisons.
9. Detection and quantification of narcotics.

The HPLC is used for :

1) Separation of mixtures of compounds which are very similar in chemical structure.
2) Separation of polar and non-polar organic compounds
3) Separation of organic salts
4) Separation of non-volatile organic compounds such as amino acids and sugars, and
5) Separation of inorganic ions.

The HPLC is highly efficient technique, and requires extremely small amounts of the sample.

Choose the Correct Answers

1. The stationary phase material in column chromatography is also called _____. [a]
   (a) Adsorbent (b) Absorbate
   (c) Eluent (d) None of these

2. The stationary phase in TLC generally is _____. [a]
   (a) Silica gel G (b) Silica gel
   (c) Sand (d) None of these

3. In column chromatography, with a polar stationary phase such as silica gel and a non- polar mobile phase, _____ compounds are eluted first. [b]
   (a) Polar (b) Non-polar
   (c) Intermediate polar (d) None of these

4. Adsorption of organic compounds to the stationary phase, such as silica gel, is due to _____. [d]
   (a) Van der Waals' forces (b) Dipole forces
   (c) H-bonding forces (d) None of these

5. In column chromatography the compound which is _____ adsorbed, elutes first. [b]
   (a) Strongly (b) Weakly
   (c) Medium strength (d) None of these

6. In column chromatography the compounds with _____ value of partition coef-ficient elute first. [b]
   (a) Highest (b) Lowest
   (c) Medium (d) None of these

7. The differential migration of compounds in the column is due to _____ differences. [c]
   (a) Adsorption strengths (b) Partition coefficients
   (c) Both (a) & (b) (d) None of these

8. A commonly used adsorbent in column chromatography is _____. [d]
   (a) Silica gel G (b) Alumina
   (c) Cellulose (d) Silica gel 200 mesh

9. The solvent used for the preparation of TLC microslides is _____. [a]
   (a) $CHC1_3$ + MeOH (b) MeOH only
   (c) $CHC1_3$ only (d) None of these

10. Preparative TLC is _____ a technique. [a]
   (a) Separation (b) Identification
   (c) both a & b (d) None of these

11. Colourless compounds on a TLC plate are detected by the use of _____. [d]

(a) Iodine vapour (b) UV light

(c) Colouration reagents (d) None of these

12. R_f means _____. [a]

(a) Retardation factor (b) Reference factor

(c) Retention factor (d) None of these

13. A TLC experiment of a sample requires _____. [a]

(a) 5 to 10 min (b) 2 hrs

(c) 1 day (d) None of these

14. In a strongly acidic cation-exchange resin, the active or mobile ion is _____. [a]

(a) a cation (b) An anion

(c) H_3O^+ (d) All of the above

15. The ion-exchange phenomenon is _____. [a]

(a) Stoichiometric (b) Non-stoichiometric

(a) Metric (b) All of the above

16. Ion-exchange resins swell in water because resin is a _____. [a]

(a) Cross-linked polymer (b) Linear polymer

(c) Water-soluble polymer (d) All of the above

17. Resin $-CH_2N^+Me_3Cl + SO_4^{-2}$ _____. [b]

(a) Resin $-CH_2 - N^+Me_3SO_4^{-2} + Cl^-$ (b) $(\text{Resin} - CH_2 - N^+Me_3)_2SO_4^{-2} + 2\ Cl^-)$

(c) $-CH_2 - N^+Me_3SO_4^{-2} + Cl^-$ (d) All of the above

18. Ninhydrin is _____. [b]

(a) Indan-l,2,3-trione (b) Indan-l,2,3-trione hydrate

(c) Indole (d) All of the above

19. The strength of adsorption of a cation M^{+1} to the cation-exchange resin is dependent on the _____. [b]

(a) Naked radius of the cation (b) Hydrated radius of the cation

(c) Indole (d) All of the above

20. To separate a mixture of anions we use _____. [b]

(a) a cation-exchange resin (b) an anion-exchange resin

(c) an amino acid analyzer (d) All of the above

21. The stationary phase in paper chromatography is _____. [a]

(a) Water (b) Paper or cellulose

(c) Silica gel (d) All of the above

22. Paper chromatography is an example of _____ chromatography. [b]

(a) Solid-liquid (b) Liquid-liquid

(c) Gas-liquid (d) All of the above

23. In a two-dimensional paper chromatography, _____ solvent/(s) is/are used two times. [b]

(a) Same (b) Different

(c) All (d) All of the above

24. Identification of unknown compounds by TLC and paper chromatography is done by comparison of the _____. [b]

(a) Retention times (b) Rf values

(c) Colour matching (d) None of the above

25. In descending paper chromatography of a mixture of compounds, the compound with the _____. value of partition coefficient moves faster. [b]

(a) Highest (b) Lowest

(c) Low (d) All of the above

26. Reversed phase HPLC is a _____ chromatography. [a]

(a) Liquid-liquid (b) Solid-liquid

(c) Gas-liquid (d) All of the above

27. The octadecyl bonded stationary phase is _____. [a]

(a) Non-polar (b) Highly polar

(c) Intermediately polar (d) None of the above

28. Octyl or octadecyl groups, used as stationary phases in reversed phase HPLC, are bonded to _____. [a]

(a) Silica (b) Carbon

(c) Water (d) All of the above

29. In reversed phase HPLC the _____ compound in the mixture is eluted first. [a]

(a) Most polar (b) Least polar

(c) Intermediately polar (d) None of these

30. In normal phase HPLC, the _____ compound in the mixture is eluted first. [b]

(a) Most polar (b) Least polar

(c) Intermediately polar (d) None of these

Fill in the Blanks

1. Separating cations k^{T}, Na^{-1}, ca^{+1} in a mixture by _____ chromotography.
2. The stationary phase used in strongly acidic cation - exchange resin. _____
3. The time spent by the compound in the column is called _____.
4. The principle involved in _____ is partition or distibution of compounds b/w st phases mobile phase.
5. The technique used for separation of cations & anions is _____
6. An Instrumental method. of chromatrography used for purity check & identification of unknown compounds by _____.
7. The techniques use a liquid as the stationary phase are called _____
8. The techniques use a solid as the stationary phase are called _____

ANSWERS

1. Ion - exchange
2. Zero carb - 225
3. Retention time
4. Chromatograph
5. Ion - Exchange chromatrography
6. HPLC chromatography technique
7. Partition chromotography
8. Adsortion chromatography

FACULTY OF SCIENCE

B.Sc. III-Year, V-Semester Examination

Model Paper - I

SPECTROSCOPY AND CHROMATOGRAPHY

CHEMISTRY - V

Time : 3 Hours] [Max. Marks : 80

PART - A (5 × 4 = 20 Marks)

Answer any Five Questions

ANSWERS

1. Explain the types of electronic transitions. **(Unit-I, SQA-2)**
2. Define Terms Employed In Absorption Spectroscopy. **(Unit-I, SQA-9)**
3. Define chemical shift. **(Unit-II, SQA-1)**
4. What are equivalent and nonequivalent proton give examples. **(Unit-II, SQA-2)**
5. Explain the Batch Extraction. **(Unit-III, SQA-3)**
6. How do you determine iron(III) by solvent extraction technique. **(Unit-III, SQA-5)**
7. Write the priciple invvolved in Ion exchange chromatography. **(Unit-IV, SQA-5)**
8. Discuss the applications of Gas chromatography. **(Unit-IV, SQA-7)**

PART - B (15 × 4 = 60 Marks)

Answer all the questions

9. a) Determine the rotational energy of rigid diatomic molecules. **(Unit-I, Q.No. 11)**

(OR)

b) Classification of Molecules based on moment of inertia. **(Unit-I, Q.No. 20)**

10. a) Explain spin-spin splitting of the signals. **(Unit-II, Q.No. 4)**

(OR)

b) Determine the molecular formula by Mass Spectrometry. **(Unit-II, Q.No. 22)**

11. a) Explain the technique of counter current extraction. **(Unit-III, Q.No. 5)**

(OR)

b) Describe the main features of (i) Descending (ii) Ascending (iii) Horizontal paper chromatography. **(Unit-III, Q.No. 15)**

12. a) Explain the principle involved in cation exchange chromatography. **(Unit-IV, Q.No. 6)**

(OR)

b) Explain the instrumentation in gas chromatography. **(Unit-IV, Q.No. 12)**

FACULTY OF SCIENCE

B.Sc. III-Year, V-Semester Examination

Model Paper - II

SPECTROSCOPY AND CHROMATOGRAPHY

CHEMISTRY - V

Time : 3 Hours] [Max. Marks : 80

PART - A (5 × 4 = 20 Marks)

Answer any Five Questions

ANSWERS

1. Explain bathochromic and hypsochromic shifts. **(Unit-I, SQA-5)**
2. Give the fingerprint region of Infrared spectrum. **(Unit-I, SQA-8)**
3. What do you mean by the base peak. **(Unit-II, SQA-11)**
4. Define Nitrogen rule. **(Unit-II, SQA-10)**
5. Explain the Technique of thin layer chromatography (TLC). **(Unit-III, SQA-7)**
6. Explain the technique & applications of two dimensional paper chromatography. **(Unit-III, SQA-12)**
7. Draw the block diagram of Gas chromatography. **(Unit-IV, SQA-6)**
8. What are the applications of HPLC. **(Unit-IV, SQA-8)**

PART - B (15 × 4 = 60 Marks)

Answer all the questions

9. a) Determine the bond length and moment of Inertia from rotational spectra. **(Unit-I, Q.No. 12)**

(OR)

b) Determination of Bond length of rigid diatomic molecules HCl. **(Unit-I, Q.No. 21)**

10. a) Give the factors effecting the chemical shift. **(Unit-II, Q.No. 5)**

(OR)

b) Write about Spin-spin Coupling. **(Unit-II, Q.No. 7)**

11. a) Classify the chromatography methods based on the **(Unit-III, Q.No. 8)**

(i) Nature of the mobile phase

(ii) The Nature of the mobile phase

(OR)

b) Describe the main features of, **(Unit-III, Q.No. 15)**

(i) Descending

(ii) Ascending

(iii) Horizontal paper chromatography.

12. a) Explain the chromatography separation of a mixture of compounds based on the differences in there adsorption strengths and partition co-efficients **(Unit-IV, Q.No. 4)**

(OR)

b) Write about cation exchange Resins with chemical structures. **(Unit-IV, Q.No. 8)**

FACULTY OF SCIENCE

B.Sc. III-Year, V-Semester Examination

Model Paper - III

SPECTROSCOPY AND CHROMATOGRAPHY

CHEMISTRY - V

Time : 3 Hours] [Max. Marks : 80

PART - A (5 × 4 = 20 Marks)

Answer any Five Questions

ANSWERS

1. Write types of molecular spectra. **(Unit-I, SQA-1)**
2. Write the selection rules of IR spectroscopy. **(Unit-I, SQA-7)**
3. What is meant by (n + 1) rule in spin-spin coupling? **(Unit-II, SQA-6)**
4. Give the fragmentation pattern of Ethylbromide. **(Unit-II, SQA-13)**
5. Explain the Technique of thin layer chromatography (TLC). **(Unit-III, SQA-7)**
6. What are R_f values. How do you calculate and write there applications. **(Unit-III, SQA-11)**
7. Describe the technique used in column chromatography. **(Unit-IV, SQA-1)**
8. Describe the day - packing technique **(Unit-IV, SQA-4)**

PART - B (15 × 4 = 60 Marks)

Answer all the questions

9. a) Explain the energy levels of simple harmonic oscillator. **(Unit-I, Q.No. 13)**

(OR)

b) Define Beer-Lambert's Law. **(Unit-I, Q.No. 23)**

10. a) Discuss the principles of NMR. **(Unit-II, Q.No. 1)**

(OR)

b) Represent the types of ions in mass spectrum. **(Unit-II, Q.No. 16)**

11. a) Discuss the technique of continuous extraction of liquids. **(Unit-III, Q.No. 4)**

(OR)

b) Describe the technique of paper chromatography justify that it is a liquid - liquid partition chromatography. **(Unit-III, Q.No. 14)**

12. a) Write the priciple invvolved in Ion exchange chromatography. **(Unit-IV, Q.No. 7)**

(OR)

b) Write the principle involved in Gas chromotography. **(Unit-IV, Q.No. 10)**