

Spectroscopy is one of the most powerful tool available for the study of atomic and molecular structure.

Absorption of Radiation:-

When electromagnetic radiations is passed through a matter.

The following changes occur.

1) As the photons of electromagnetic radiations are absorbed by the matter, electronic transition, vibrational changes (or) rotational changes may be occur.

After absorption, molecules get excited from the ground state excited state. Then they liberate energy quickly in the form of heat (or) reemit electromagnetic radiation.

2) Sometimes the portion of electromagnetic radiation which passes into the matter, instead of being absorbed may be scattered (or) reflected (or) rectified.

3) When the electromagnetic radiation is absorbed (or) scattered, it may undergo changes in polarization.

4) In some cases the molecules absorbs radiation and gets excited.

a) Fluorescence:-

If the excited molecules reemits the radiation almost within 10^{-8} seconds, it is called Fluorescence.

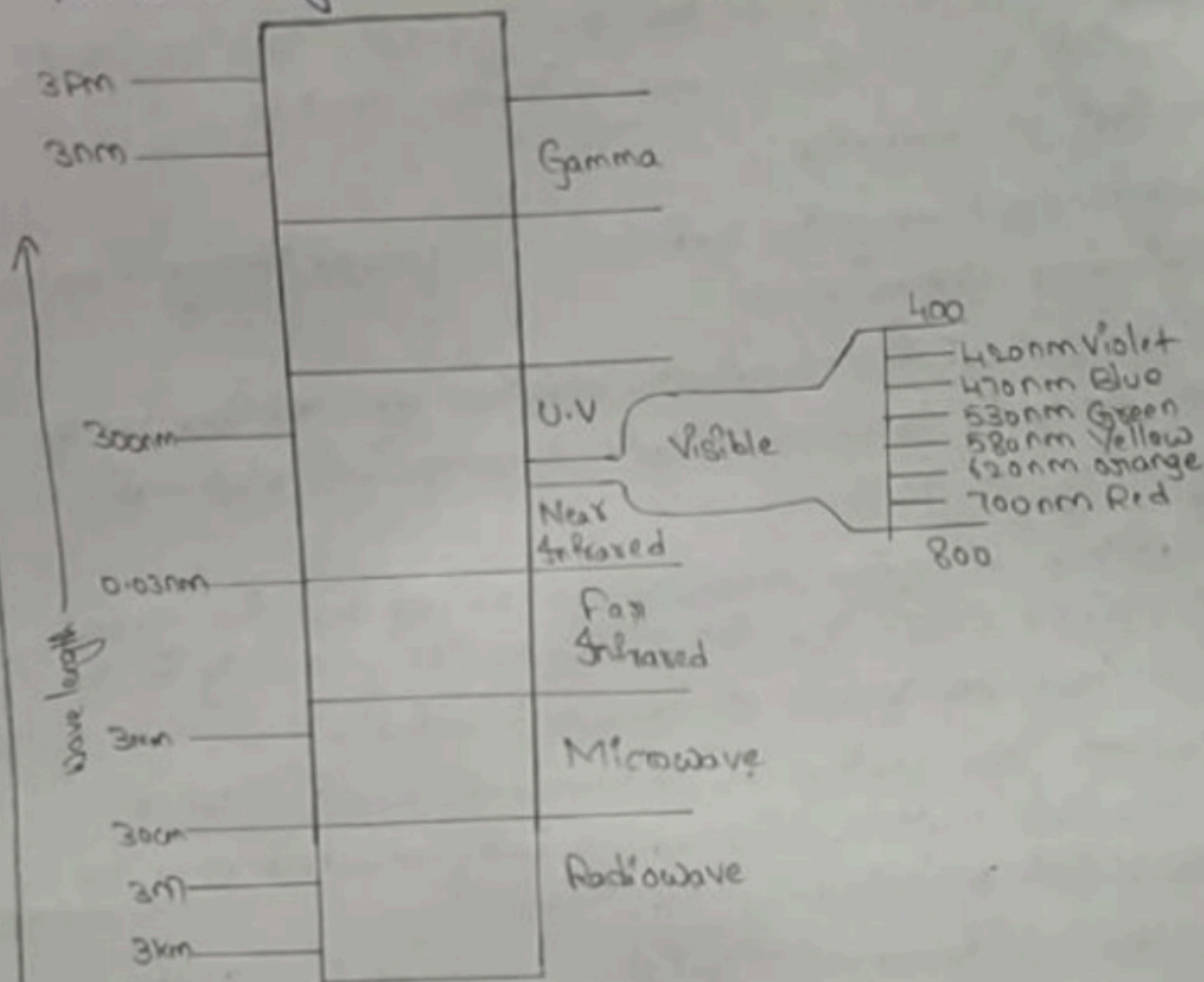
b) phosphorescence:-

If the excited molecules reemits the radiation after some time is called phosphorescence.

Electromagnetic spectrum:-

The entire range over which electromagnetic radiation exists is known as electromagnetic spectrum.

The electromagnetic spectrum covers larger range of wavelength



Electromagnetic spectrum.

Bee's law (or) Beer - Lambert's law:-

According to this law when a beam of monochromatic light is passed through a solution of an absorbing substance. The rate of decrease of intensity of radiation dI with thickness of the absorbing solution dx is proportional to the Intensity of Incident radiation I . As well as the Concentration of the solution c .

$$= \frac{dI}{dx} = -kIc \rightarrow \textcircled{1}$$

k = molar absorption Co-efficient.

on integrating the eq $\textcircled{1}$ b/w limits $I = I_0$ at $x=0$ and $I = I$ at $x=x$, we get.

$$\int_{I_0}^I \frac{dI}{I} = - \int_0^x k c dx$$

$$\ln \frac{I}{I_0} = -k c x \quad (\text{or}) \quad 2.303 \log \frac{I}{I_0} = -k c x \quad (\text{or})$$

$$\log \frac{I_0}{I} = \frac{k}{2.303} C x \rightarrow (2)$$

This is called Beer-Lambert's law.

The absorbance (A) is directly proportional to molar concentration (c) and thickness (or) path length (x)

Applications of Beer's Lambert's law:-

→ It is used in determination of concentration in unknown solution.

→ It is used in analysis of compounds

Limitations of Beer-Lambert's law:-

1) Beer-Lambert's law is not obeyed if the radiation used is not monochromatic

2) It is applicable only for dilute solutions.

3) The temperature of the system should not be allowed to vary to a large extent.

4) It is not applied to suspensions

5) Deviation may occur, if the solution contains impurities

(PH - Metro - Text book 4.15)

UV-Visible Spectroscopy:

→ UV visible spectra arise from the transition of valency electrons within a molecule (or) ion from lower energy level (Ground state E_0) to higher electronic energy level (E_1)

→ This transition occurs due to the absorption of UV wave length (100-400nm) (or) visible length (400-750nm) Types
→ The actual amount of energy required depends on the difference in energy b/w the Ground state and the excited State of the electrons.

$$\Delta E = E_L - E_0 = h\nu$$

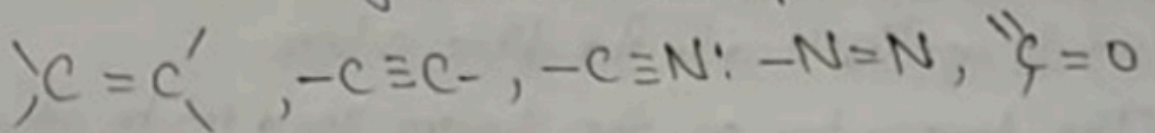
→ Electronic transition depends on the electronic structure of the absorption absorbing medium (Sample).

The absorption of UV-visible radiation in organic molecule is mainly due to presence of functional groups.

The two important groups responsible for absorption and position of absorption in UV-visible spectrum.

① Chromophores:

The presence of one (or) more unsaturated linkages (π -electrons) in a compound is responsible for the colour of the compound, these linkages referred chromophore.



Chromophores undergo $\pi \rightarrow \pi^*$ transitions in the short wave length regions of UV-radiation.

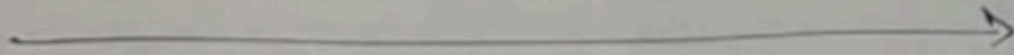
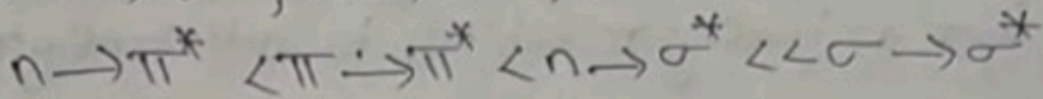
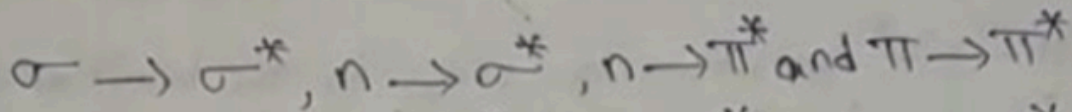
② Auxochrome:-

It can change the colour intensity

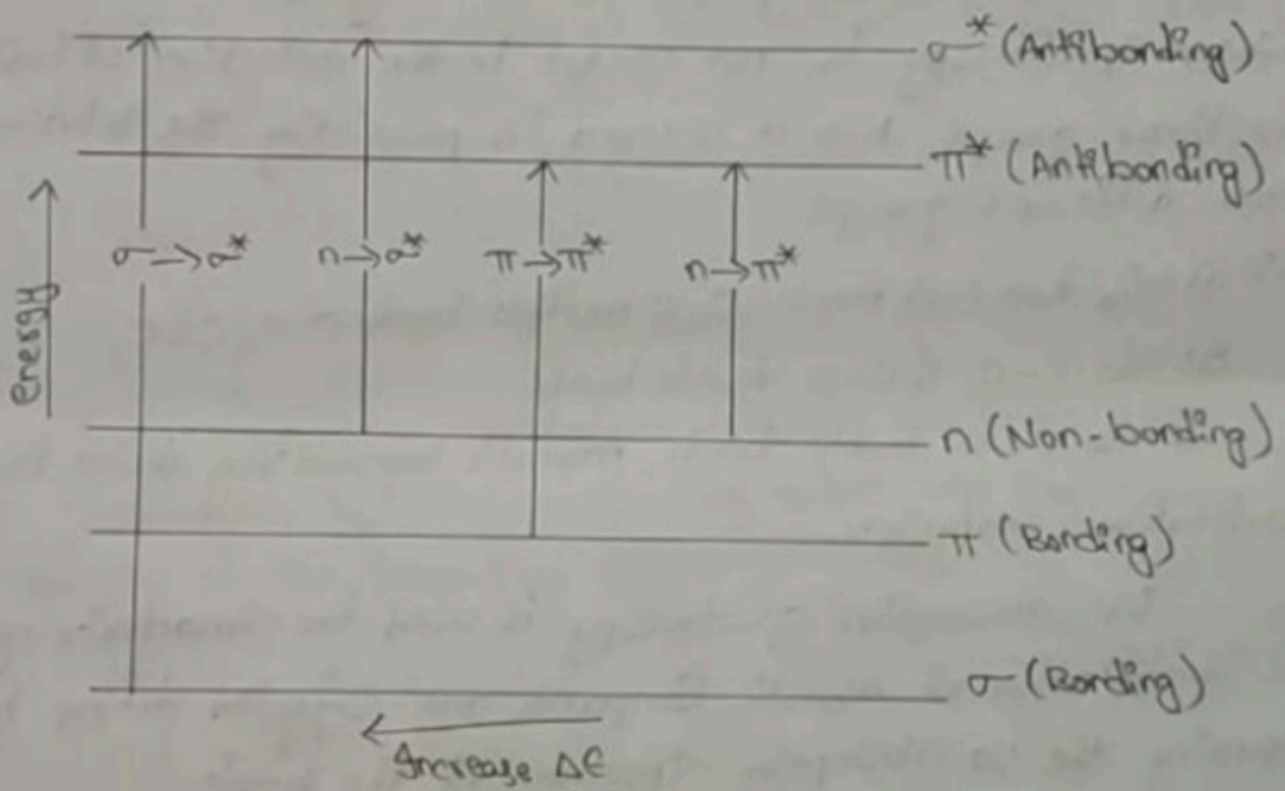
Ex:- $-\text{OH}$, $-\text{NH}_2$, $-\text{CO}$, $-\text{Br}$, $-\text{I}$ etc

Types of transitions involved in organic molecules.

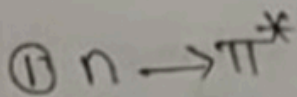
→ Energy absorbed in the visible and UV region by a molecule causes transitions of valence electrons in the molecule.



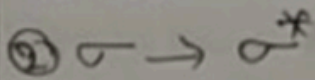
Increase energy



Types of transition.



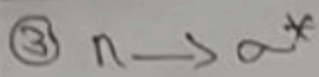
Shown by unsaturated molecules containing hetero atoms. It occurs due to the transition of non-bonding lone pairs of electron to the antibonding orbitals.



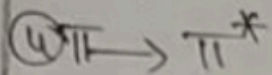
$\sigma - \sigma^*$ transitions occur in the compounds of single bonds.

These are no lone pairs of electrons.





$n \rightarrow \sigma^*$ are occur in the saturated Compounds lone pairs of electrons in addition to $\sigma \rightarrow \sigma^*$ transitions.



$\pi \rightarrow \pi^*$ transitions occur due to the transition of an electron from a bonding π -orbitals to anti-bonding π^* orbital.

Applications:-

① Predicting relationship b/w different groups:-

→ UV spectroscopy is not useful in the detection of individual functional groups, but it is used in predicting the relationship b/w different groups.

Ex:- ① b/w two (or) more C-C multiple bonds $C=C$, $C \equiv C$.

② b/w C-C & C-O double bonds

③ b/w C-C double bonds, Aromatic benzene ring double bonds.

Qualitative analysis:-

UV absorption spectroscopy is used for characterizing and identification of Aromatic Compounds and Conjugated defines by Comparing the UV absorption spectrum of the sample.

③ Detection of impurities:-

UV spectra is used detecting impurities in organic Compounds.

① The bands due to impurities are very intense.

② Saturated Compounds have little absorption band and in Saturated Compounds have strong absorption bands

Determination of molecular weight:-

Molecular weight of a compound can be determined if it can be converted into a suitable derivative, which gives an absorption band.

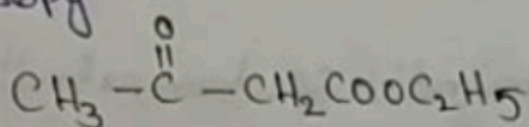
⑤ Dissociation Constants of Acids & Bases.

The dissociation constant (pK_a) of an acid (HA) can be determined by determining the ratio of $\frac{[HA]}{[A]}$ spectrophotometrically from the graph plotted b/w absorbance vs wave length of different pH values. This values are substituted in the equation.

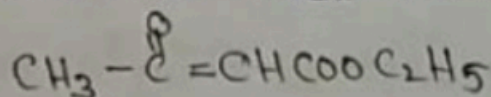
$$pK_a = pH + \log \frac{[HA]}{[A]}$$

⑥ Study of tautomeric equilibrium:-

The percentage of various keto and eno forms present in a tautomeric equilibrium can be determined by measuring the strength of the respective absorption bands using UV Spectroscopy



Keto form $\lambda_{max} = 275 \text{ nm}$



Enol form $\lambda_{max} = 244 \text{ nm}$

⑦ Studying kinetics of chemical reactions:-

Kinetics of chemical reactions can be studied using UV Spectroscopy by following the change in concentration of a product (or) a reactant with time during the reaction.

IR Spectroscopy

IR spectra is produced by the absorption of emf of a molecule in the IR region and the transitions occurs b/w vibrational levels.

→ The essential requirement for a molecule to show vibrational spectrum is that the dipole moment of the molecule must change during vibration.

→ This type of spectra is given by heteronuclear diatomic molecules and poly atomic molecules with and without dipole moment.

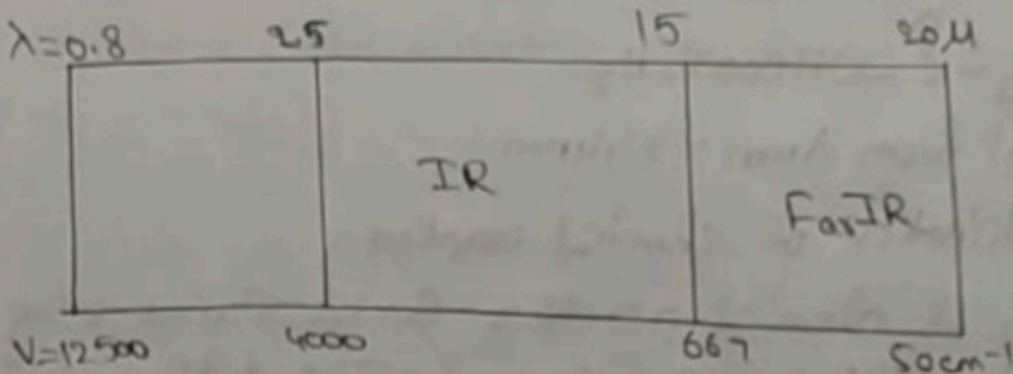
→ In polyatomic molecule, having dipole movement hence it becomes IR active.

→ Homonuclear diatomic molecules such as O_2 , N_2 , H_2 are IR inactive because they have no dipole movement.

→ The vibration of a diatomic molecule may be considered as the vibration of a simple harmonic oscillator.

Range of IR:

The Range in the electromagnetic spectrum extending from $12500 - 50 \text{ cm}^{-1}$



Range of IR radiation

Near IR :- This region is from $12500 - 4000 \text{ cm}^{-1}$

② IR :- This region from $4000 - 667 \text{ cm}^{-1}$.

③ Far IR :- This region is from $667 - 50 \text{ cm}^{-1}$

④ Fingerprint region :-

The vibrational spectral (IR) region of $1400 - 700 \text{ cm}^{-1}$ gives very rich and intense absorption bands. This region is termed as fingerprint region. The region $4000 - 1430 \text{ cm}^{-1}$ is known as group frequency region.

Uses :-

Identify the functional groups present.

Origin of IR spectrum :-

Since atoms in a molecule are continuously vibrating, molecules are also vibrating.

→ There are two kinds of fundamental vibrations.

① Stretching vibrations :-

During stretching the distance b/w two atoms decreases (or) increases, but bond angle remains changed.

② Bonding :-

During bonding bond angle increases and decreases but bond distance remains changed.

Applications of IR spectroscopy.

① Identify of the compound can be established.

→ The IR spectrum of the compound is compared with that of known compounds.

→ From the resemblance of the two spectra, the nature of the compound can be established.

→ This is because a particular group of atoms gives a characteristic absorption band in the IR spectrum.

→ IR spectra of both benzaldehyde (C_6H_5-CHO) and phenyl methyl ketone ($C_6H_5-CO-CH_3$) show a sharp absorption peak at 1700 cm^{-1} .

→ This indicates the presence of $C=O$ group in both the compounds.

② Detection of functional groups:-

→ In a given environment a certain functional group will absorb IR energy of very nearly the same wave length in all molecules.

Ex (i) Acetone and diethyl ketone ($C_2H_5-CO-C_2H_5$) give absorption peak at the same place.

② But Acetic Acid (CH_3COOH), and Cyclobutane

$\begin{array}{c} CH_2 - CH_2 \\ | \quad | \\ C=O \\ | \quad | \\ CH_2 - CH_2 \end{array}$ give absorption peak at different places.

③ Testing purity of a sample.

Pure sample will give a sharp and well-resolved absorption bands.

→ But impure sample will give a broad and poorly resolved absorption. Thus by comparison with IR spectra of pure compound, presence of impurity can be detected.

④ Determination of shape (or) symmetry of a molecule.

→ Whether the molecule is linear (or) non-linear can be found out by using IR spectra.

Ex:- IR spectra of NO_2 gives three peaks at 750 , 1323 and 1616 cm^{-1} .

① For non-linear molecule $(3n-6) = 3$ peaks

② For linear molecule $(3n-5)$

Since the spectra shows only 3 peaks, it is confirmed that NO_2 molecule is a non-linear molecule.

~~4) Determination of shape or symmetry~~

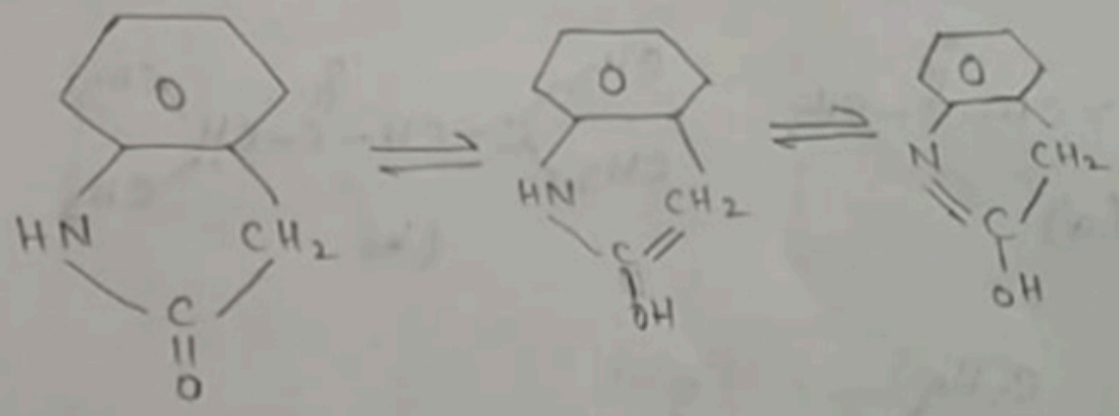
5) To study tautomerism:-

Tautomeric equilibrium can be studied with the help of IR spectroscopy.

Ex:-

The common systems such as keto-enol, lacto-lactum, and mercapto-thiamide, contain a group like $\text{C}=\text{O}$, $-\text{OH}$, $-\text{NH}$ (or) $\text{C}=\text{S}$. These groups show a characteristic absorption band in the IR spectrum, which enable us to find at which form predominates in the equilibrium.

Ex:-



6) Industrial applications:-

(a) Determination of structure of chemical products:-

During the polymerisation, the bulk polymer structure, can be determined using IR spectra.

(b) Determination of molecular weight

Molecular weight, of a compound can be determined by measuring end group concentrations, using IR spectroscopy.

(C) Crystallinity:

The physical structure like crystallinity can be studied through changes in IR spectra.

Ex:-

The absorption band at 934 cm^{-1} is for crystalline nylon 6:6

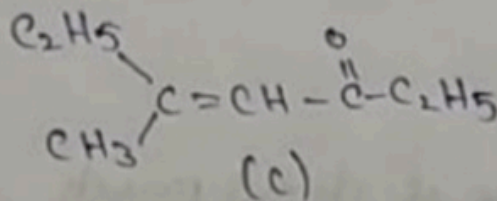
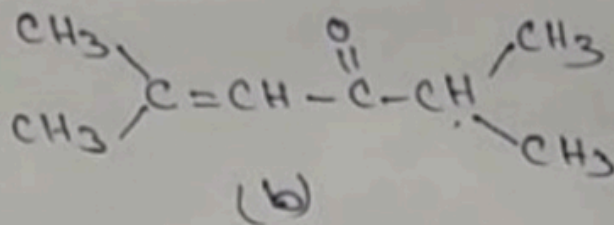
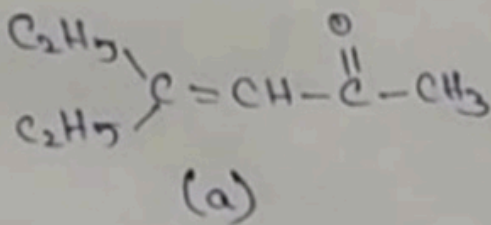
The absorption band at 1238 cm^{-1} is for amorphous nylon 6:6

⊕ Isomers can be identified in the fingerprint region

Similar molecules may show very similar spectra in the group frequency region ($4000-1430\text{ cm}^{-1}$). But they show some difference in the fingerprint region ($1400-700\text{ cm}^{-1}$)

Ex:-

Isomers of the compound $\text{C}_8\text{H}_{16}\text{O}$



High Performance liquid chromatography (HPLC)

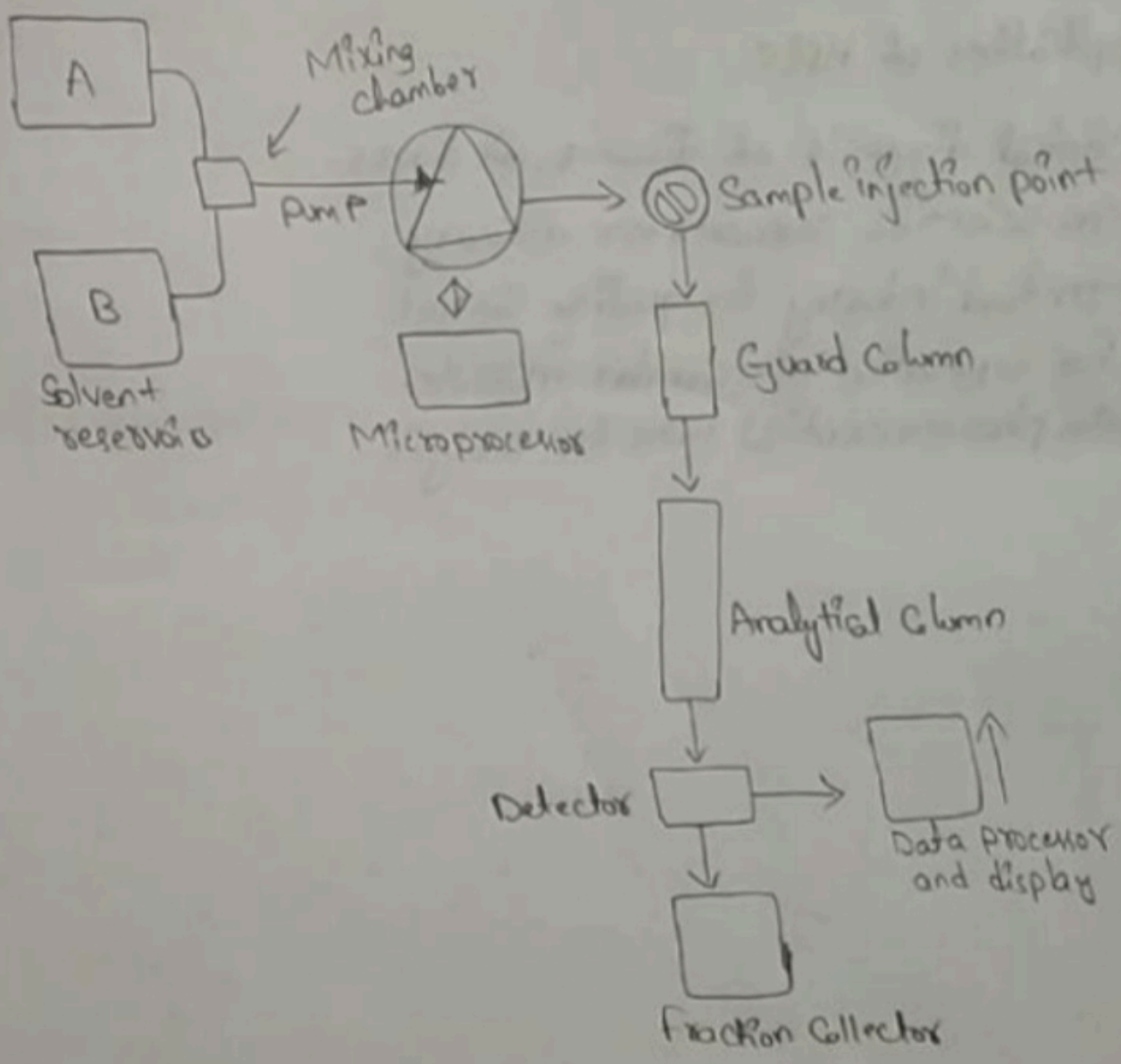
Principle:

HPLC is an advanced method of Column chromatography. The particle size of the adsorbent, used in the column is small and it helps to improve the resolution. The solvent is passed through the column under high pressure using pumps.

In general, the adsorbent with small particle size (3µm, 5µm, 10µm) with a very narrow size distribution will greatly improve the resolution. High pressure, in the Column, can be created using pump.

Instrumentation

The outline of the instrument is shown in figure



A schematic diagram of a HPLC system

HPLC consists of the following main components

- (i) Solvent reservoir
- (ii) A microprocessor-controlled high pressure pump to drive



the sample to the column

(iii) Sample injection port.

(iv) Guard Column and analytical Column containing the adsorbent
(stationary phase)

(v) Detector

(vi) Data processor with recorder

(vii) Fraction collector

Applications of HPLC.

1) Clinical diagnosis of diseases, disorders.

2) In scientific research for discovery.

3) In food industry for quality control.

4) For separation of similar molecules.

5) In pharmaceutical labs for analysis.