UNIT –III

Introduction to chromatography →

Adsorption and partition column chromatography-Methodology, advantages, disadvantages and applications.

Thin layer chromatography- Introduction, Principle, Methodology, Rf values, advantages, disadvantages and applications.

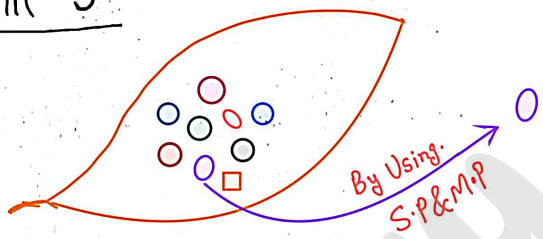
Paper chromatography-Introduction, methodology, development techniques, advantages, disadvantages and applications

Electrophoresis– Introduction, factors affecting electrophoretic mobility, Techniques of paper, gel, capillary electrophoresis, applications

Unit-3

1.

Chromatography →



It is the Separation of Mixture into Indivisual Component by Using Stationary Phase & Mobile Phase.

On the basis of Stationary Phase & Mobile Phase Nature.

⇓

Chromatography are divided into four types

(a) Gas-Solid Chromatography.
(b) Gas-Liquid Chromatography.
(c) Solid-Liquid Chromatog.
(d) Liq.-Liq. Chromatography

On the Basis of Principle Chromatography are Classified into →
or
Mechanism of Separation.

(a) Adsorption Chrom. → Oldest type of Chromatography.

- → Mobile Phase ⇒ Liquid or Gaseous form.
- → Stationary Phase ⇒ Solid Phase.

(b) Partition Chromatography ⇒ (eg → Paper Chromat.)$^{2.}$

* Here, Both Phases are in Liquid form either it is Stationary or Mobile Phase.

If Gas is Mobile Phase then it is called Gas-Liquid Chromatography

(c) Ion-Exchange Chromatography →

(d) Size-Exclusion Chromatography → Also Known as Gel Permeation Chromatography

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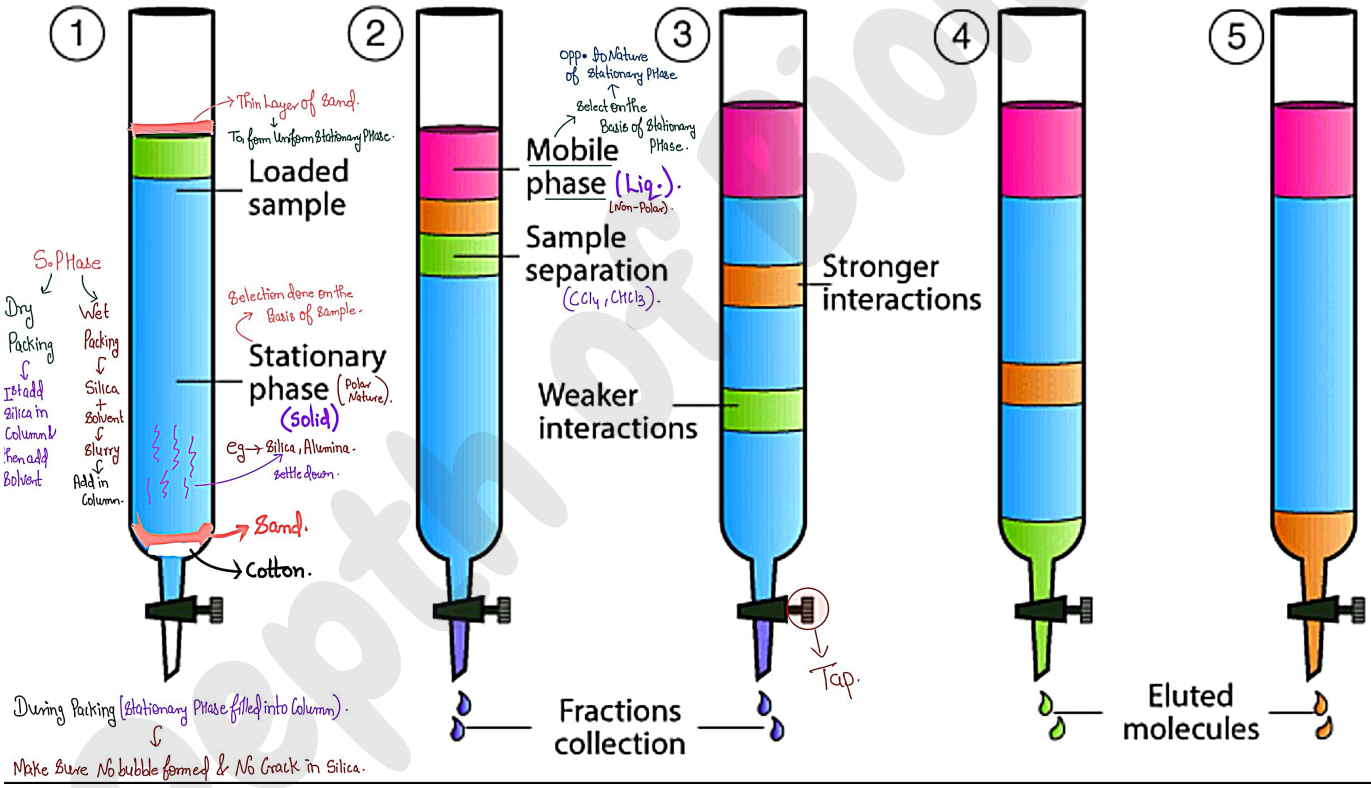
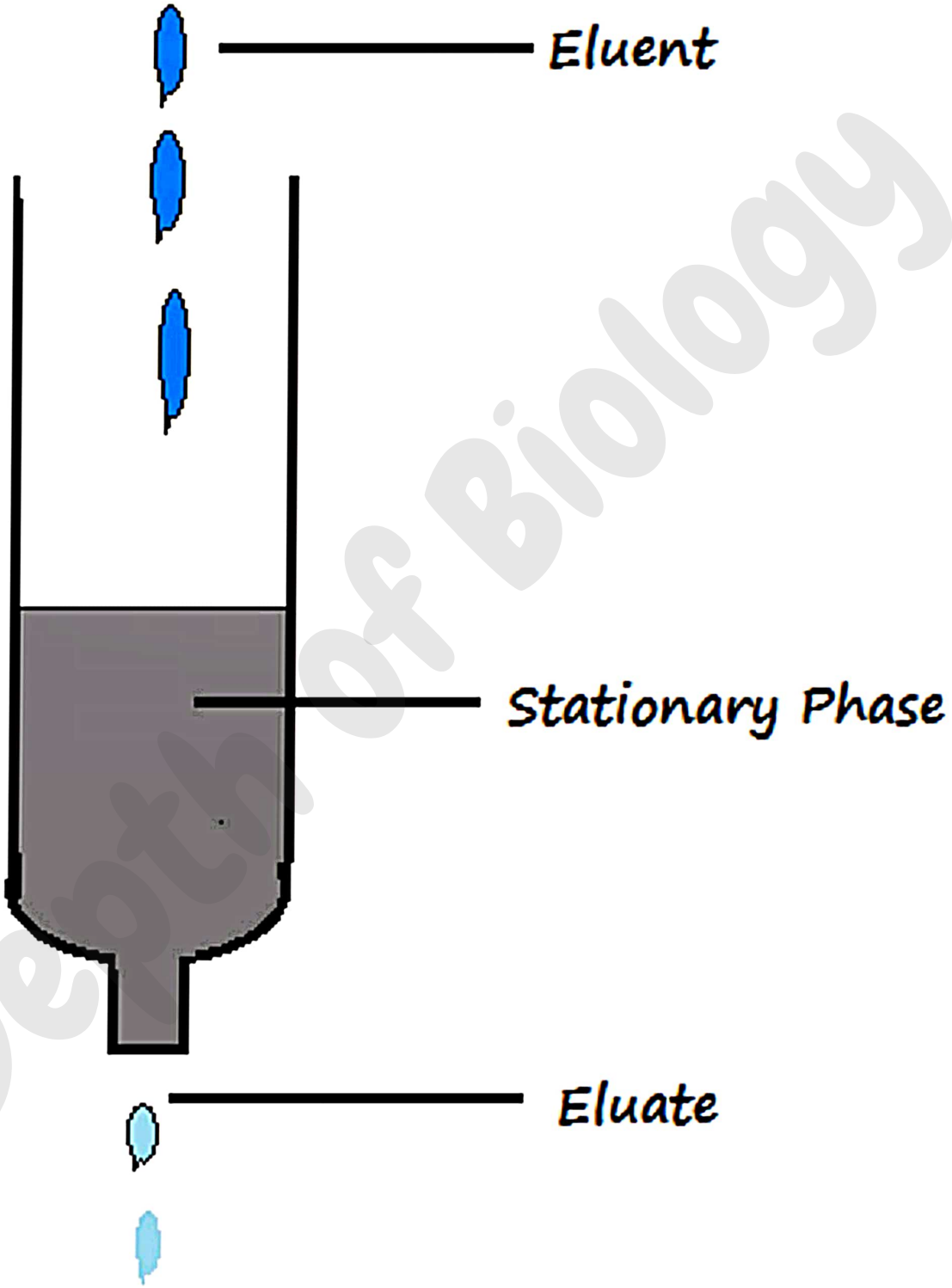
1. Adsorption Column Chromatography

- Here Column is Used for Purification Process So, it is Called Column Chromatography.
- Column is tube like structure** made up of Glass or Plastic.
- In Case of Column Chromatography Stationary Phase Ont in solid form.

& Mobile Phase is Ont in Liquid form.





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3.

⇒ Column Chromatography performed either Manually or Automatic.

⇒ For Automatic Column Chromatography AKTA Purifier is Used.

⇒ Steps Involved in Column Chromatography →

(i) Pick a Stationary Phase / Selection of Stationary Phase →

✱ Stationary Phase Selection is done on the basis of Sample

✱ Silica, Alumina most Commonly Used Stationary Phase in Column Chromatography.

(ii) Column Packing → Stationary Phase packed into Column.

• First of all we have to ready the Column. Because Column is open from both side. So we can't add directly Silica or Stationary Phase directly into the Column. So ready the Column means

• Use Cotton plug at the bottom of Column side. Cotton must be Used in proper amount, so we can easily perform Column Chromatography

4.

- Because if Cotton Quantity is More then Solvent Solution is not passed from it.

& if Cotton is present in Less amount then More Solvent is passed from Column. (So, we Use Cotton in appropiate amount)

☆ Then after Cotton we apply / Use Sand (Acid Washed sand) at the Curvy part of Column.

⇒ Then we apply Stationary Phase into Column.

⇝ Stationary Phase are apply by 2 Methods ⇒

(a) Dry Packing Method.

⇓

Here, firstly we filled / apply Powdered form Silica into Column. Then, we add Solvent Solution into Column. This type of Packing Called Dry Packing & Method is Dry Packing Method.

(b) Wet Packing Method

⇓

Here Silica & Solvent Solution Mixed for some time & a Slurry is formed then this Slurry further applied to Column this is called Wet Packing (Commonly Used).

Then after sometime Silica is settle down & a thin layer of Sand is applied on the top.

Sand is Inert material (Non-reactive).

✱ Column top & bottom Sand is applied so, a Uniform Stationary Phase is formed.

✱ During Packing of Column we must ensure that No bubbles formed during Packing. & Silica not be dry or dried Because If Silica is dry then Cracking occur & purification process affect.

(iii) Mobile Phase Selection → Mobile Phase Select on the basis of Stationary Phase

- If Stationary Phase is polar then Mobile Phase must be Non-Polar or Less polar.
- Because if both Stationary Phase & Mobile Phase have same polarity then they form bond & Mobile phase not easily pass from the Sample

6.

Now for the purification →

- Sample is passed/run on packed Column.
- Then after Sample loading, Solvent Solution is loading

Example of Solvent → ① CCl_4 ② $CHCl_3$ ③ Methanol ④ Acetic Acid.

✡ Since Our Stationary Phase is polar in Nature (Silica).

→ So, Solvent Solution must be less polar.

✡ Now Sample Contain both Polar & Non-polar Molecule.

& the polar molecule of Sample form bond with Stationary (Polar) Phase & they move Very Slowly in Column.

& the Non-polar molecule of Sample move faster with Solvent (Non-Polar) or Less polar.

⇒ Then we remove the Tap of the Column & then Collect it.

⇒ & after Collection Analyse it

Advantages of Column Chromatograph

- Column chromatography is advantageous over most other chromatographic techniques because it can be used to separate and purify substantial quantities of components from a mixture.

- Column chromatography can also be used for analytical purposes.

Disadvantages of Column Chromatograph

Packing of the column requires some technical skill.

The technique is time-consuming and tedious, especially for larger samples.

It requires constant attention while the experiment is being performed: collection vessels must be frequently switched and solvent should be added continuously to the top of the column at a rate sufficient to cover the adsorbent.

COLUMN CHROMATOGRAPHY

APPLICATIONS

- Separation of mixture of compounds
- Purification process
- Isolation of active constituents
- Estimation of drugs in formulation
- Isolation of active constituents
- Determination of primary and secondary glycosides in digitalis leaf.
- separation of diastereomers

7.

Partition Chromatography.

Paper Chromatography →

- Here We Used paper for the Separation of Compound, So It is Called Paper Chromatography.

Principle of Paper Chromatography →

☆ On the basis of Nature of sample we Can Select the paper

There are different types of paper Used in Paper Chromatography.

1. Simple Paper → Whatman Paper (available in different grades) (grade means pore size different).

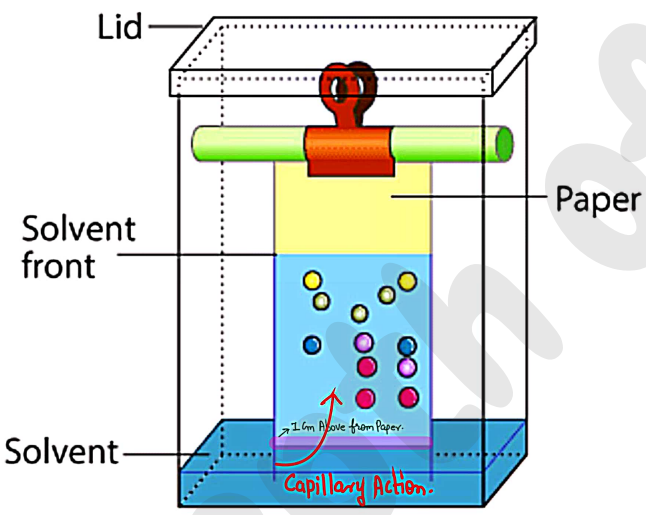
Or Some Modified paper →

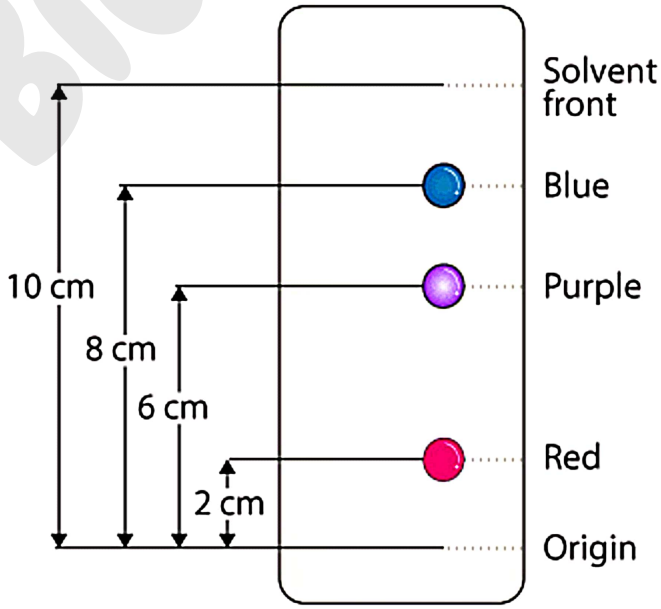
(a) Hydrophilic papers → Paper modified with Methanol, Glycol, Glycerol.

(b) Hydrophobic Papers → Used in Reversed Chromatography

(c) Thin Layer apply (Like Silica, Alumina) on paper.

PAPER CHROMATOGRAPHY





8.

Solvent Solution → Pure Solvent, Buffer Solution or Mixture of Solvents.

- Hydrophilic Solution →

  Methanol : H_2O (4:1)

  Isopropanol : NH_3 : H_2O (9:1:2)

- Hydrophobic Solution →

  Dimethylether : Cyclohexane

✱ Then after Selection of Paper draw a line 1Cm above from the base of paper. (Here Sample is applied which we want to Analyze) Means paper is loaded.

⇒ Then paper is placed in Chamber (Chamber are made up of Glass/plastic)

✱ Before placing this paper into Chamber. The Chamber Must be Saturated. Saturated means we placed Solvent Solution in Chamber 20-30minutes before paper is applied.

- Then paper is Hang after 20-30 minutes with the Help of Clip & the line which we draw in paper not dipped in Solvent solution because if it is dipped then Sample is dissolved & goes into Solvent solution.

- Then after dipping the paper in solvent, Solvent moves on paper Via Capillary action in upward direction.

Due to this Upward movement of Solvent Sample is Seperated.

- Suppose our Solvent is less polar in Nature the Movement (Mobile Phase) of Solvent on paper / Sample leads to Seperation of less polar Molecule from Sample.

☆ At the end colour spot Can Visually detected on paper

There are types of test ⟶ Specific test → (Here we detect easily Nature (A, G, F, T, R))

↓

Non-Specific Test → for Colourless Compound (Here we detect the Number Not the Nature)

→ R.F Value (Retardation/Retention factor) → 10.

$$R.F = \frac{\text{Distance travelled by Solute/Sample}}{\text{Distance travelled by Solvent.}}$$

→ R.F Value ranges from 0-1

Ideal Value = 0.3-0.8

Advantages of Paper Chromatography

- Fewer quantitative material is required.
- Compound separation happens quickly.
- For analysis, a small sample size is needed.
- When compared to other chromatography techniques, paper chromatography is a cheap procedure.
- Paper chromatography can distinguish between organic and inorganic substances.
- Compared to other chromatographic techniques, paper chromatography requires less room.
- Easy to put up and utilize.
- Only a Few samples are needed for the analysis, and the technique is less expensive.

Disadvantages of Paper Chromatography

- Techniques for paper chromatography cannot distinguish between volatile compounds.
- Paper chromatography is not able to handle large sample volumes.
- Quantitative analysis could be more helpful in paper chromatography.
- Paper chromatography cannot separate complex mixtures.
- The accuracy of paper chromatography is inferior to that of HPLC, TLC, or HPTLC.
- Data can only be kept for a short time.

APPLICATIONS OF PAPER CHROMATOGRAPHY

- **Separation of Ink Dyes**
  - To compare ink dyes use in any company
- **Food Colouring**
  - To differentiate colouring agent used in food products
- **To isolate plant pigment from root and leaves**
- **Separation of mixture of drugs**
- **Separation of carbohydrates, vitamins, amino acids etc.**