Chromatography (404 C)

Fourth year Chemistry / Biochemistry students

by

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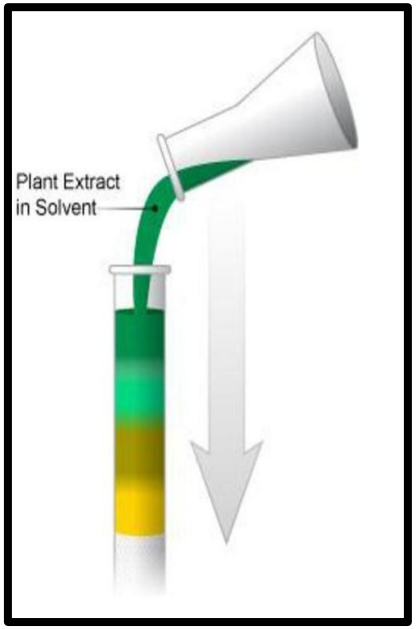
History

- Chromatography, literally "color writing", was first employed by Russian scientist Mikhail Tsvet in 1900.
- He continued to work with chromatography in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll, carotenes, and xanthophylls.
- Since these components have different colors (green, orange, and yellow, respectively) they gave the technique its name.

What is the meaning of chromatography?

Mikhail Tswett the Russian botanist who separated six pigments from a leaf pigment extraction.



He called the new technique chromatography because the result of analysis was (drawn in color) along the length of adsorbent column.

Chroma means: color and graphy means to draw or write

Chromatography: is a laboratory technique for the separation of compounds from a mixture.

Chromatography: is a method by which a mixture is separated by distributing its components between two phases.

- **The stationary phase remains fixed in place while the mobile phase carries the components of the mixture through the medium being used.**
- **The movement of the components in the mobile phase is controlled by the significance of their interactions with the mobile and/or stationary phases.**

Applications of chromatography

- The chromatographic technique is used for the separation of amino acids, proteins & carbohydrates.
- It is also used for the analysis of drugs, hormones and vitamins.
- Helpful for the qualitative & quantitative analysis of complex mixtures.
- The technique is also useful for the determination of molecular weight of proteins.

Types of chromatography

1) Analytical chromatography: produces small amount of purified sample and the objective is to separate compounds in order to identify them.

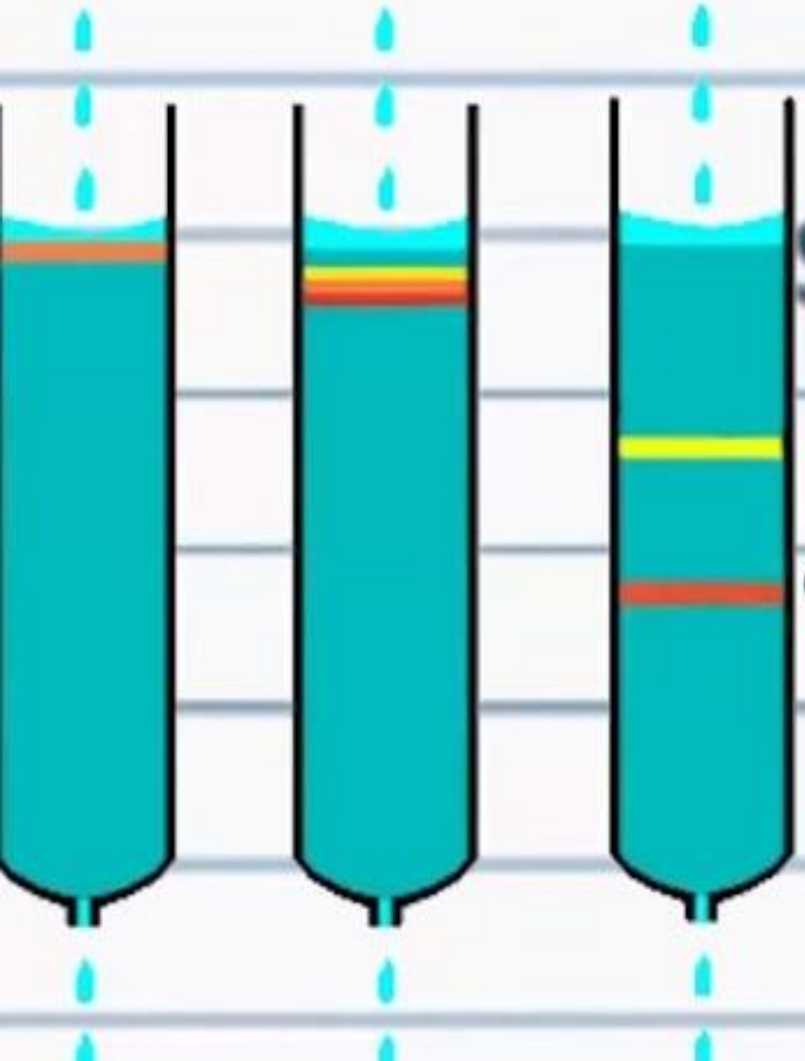
2) Preparative chromatography: produces large quantities of purified sample for further use including characterization and preparation of a commercial product. (This type is used in pharmaceutical industry).

Any chromatographic method contains:

1) **Solute or analyte (the substance to be separated)**
2) **The stationary phase**
3) **The mobile phase or solvent or eluent**

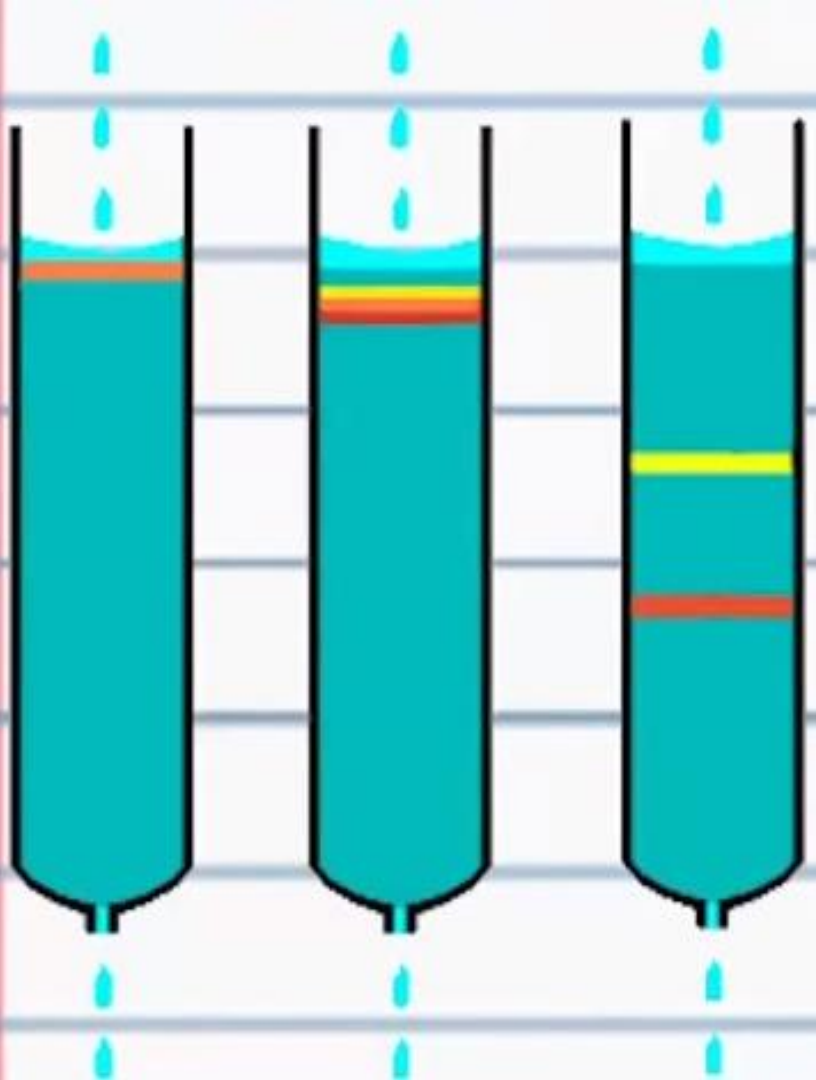
Normal-phase chromatography: the stationary phase is polar and the mobile phase is nonpolar.

Reversed phase: the stationary phase is nonpolar and the mobile phase is polar.



stationary phase

a solid, a thick liquid, or a bonded coating that stays fixed in one place



mobile phase
or
eluent

a liquid or gas that moves through it or across it

Classification of Chromatography

Planar chromatography: is one type of chromatography technique in which the stationary phase is on a flat plate and the mobile phase moves through stationary phase due to capillary action.

There are two types of planar chromatography:

A. Paper Chromatography

B. Thin layer chromatography TLC.

Column Chromatography: the stationary phase is held in narrow tube through which the mobile phase is forced under pressure or by gravity.

Types of Column Chromatography:

1) Adsorption column chromatography adsorbent.

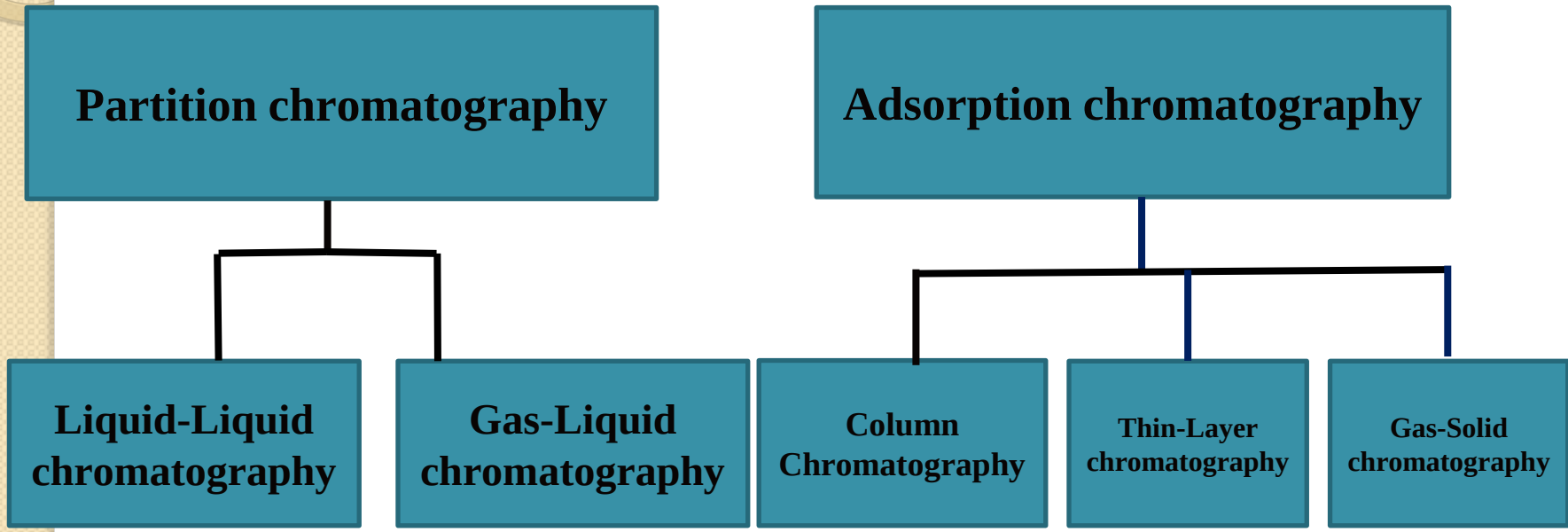
2) Partition column chromatography

3) Gel – filtration chromatography

4) Ion exchange column chromatography

5) Affinity chromatography

Classification of chromatography based on mechanism of separation



What are the Similarities Between Adsorption and Partition Chromatography?

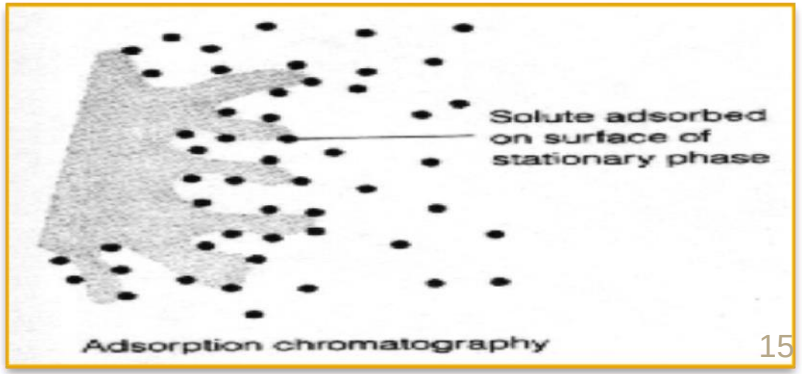
- Both adsorption and partition chromatography are varieties of chromatography
- Both types of chromatography function under the same principle of chromatography
- Both types are used to separate mixtures of compounds.
- Both adsorption and partition chromatography contain stationary and mobile phases.
- Adsorption and partition chromatography possess the ability to separate compounds in all three states; gas, liquid and solid.
- The mobile phase of both types is in liquid states.

What is the Difference Between Adsorption and Partition Chromatography?

Adsorption vs Partition Chromatography	
Adsorption chromatography is defined as a type of chromatography in which separation occurs based on adsorption.	Partition chromatography is a type of chromatography in which separation is based on partition.
Extraction	
Adsorption chromatography is a liquid-solid extraction.	Partition chromatography is a liquid-liquid extraction.
Stationary Phase	
The stationary phase is in the solid state of adsorption chromatography.	The stationary phase is a liquid state in partition chromatography.
Developments	
Adsorption chromatography was not further developed.	Partition chromatography leads to the development of other types of chromatography.

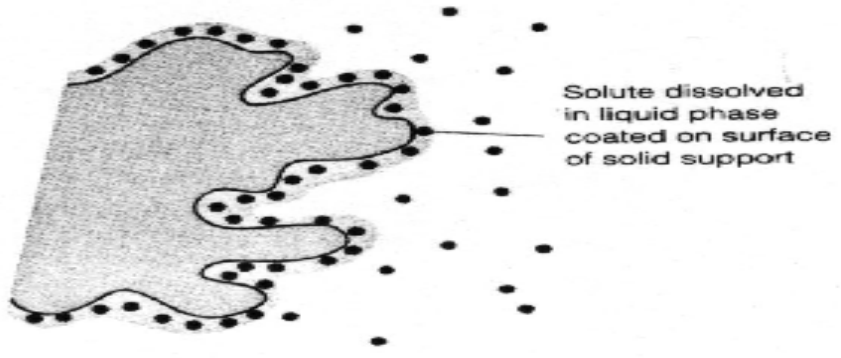
Adsorption chromatograaohy

- Adsorption chromatography is process of separation of components in a mixture introduced into chromatography system based on the relative difference in adsorption of components to stationary phase present in chromatography column
- Adsorption chromatography is one of the oldest types of chromatography.
- The equilibriation between the mobile and stationary phase accounts for the separation of different solutes.
- It utilizes a mobile liquid or gaseous phase that is adsorbed onto the surface of a stationary solid phase



Partition chromatography

- Chromatography in which separation is based mainly on differences between the solubility of the sample components in the stationary phase or on differences between the solubility of the components in the mobile and stationary phases.
- This form of chromatography is based on a thin film formed on the surface of a solid support by a liquid stationary phase.
- Solute equilibrates between the mobile phase and the stationary liquid.



Partition chromatography

Classification of chromatography based on the packing of stationary phase

Paper Chromatography (PC): stationary phase is thin film of liquid supported on an inert support.

Thin Layer Chromatography (TLC): stationary phase is thin layer of adsorbent material, usually silica gel, aluminum oxide (alumina), or cellulose which supported on glass, plastic, or aluminum foil.

Column Chromatography (CC): stationary phase is packed in glass column.

Classification of chromatography based on the force of separation

- Partition Chromatography
- Adsorption Chromatography
- Ion Exchange Chromatography
- Gel Filtration chromatography
- Affinity Chromatography

Table about Chromatography and its Applications

Chromatography	Application
Liquid chromatography	It is used in testing the water samples to know the pollution.
Gas chromatography	This type of chromatography is availed in the forensics lab to compare the fibers which are found on a victim body, detect bombs in airports and also used in identifying and quantifying the drugs like alcohol.
Thin-layer chromatography	It is availed in identifying the pesticides in the food and also used to work on the dye composition of fibers in the forensics laboratory.
Paper chromatography	It is availed in RNA fingerprinting and also in separating anions and amino acids.

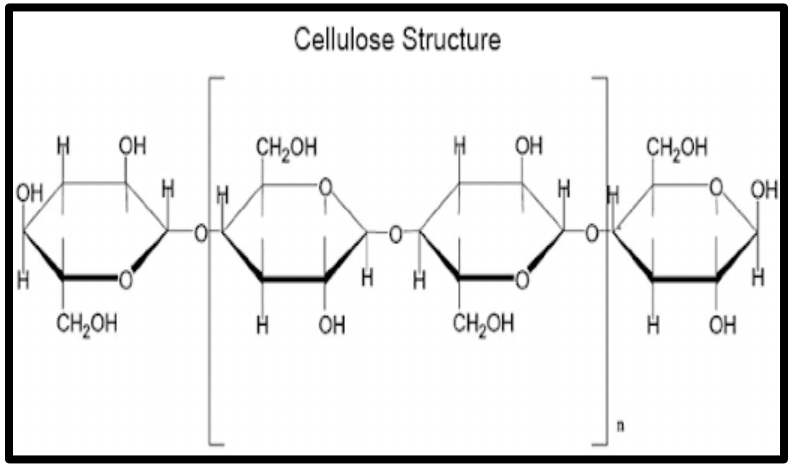
Paper Chromatography

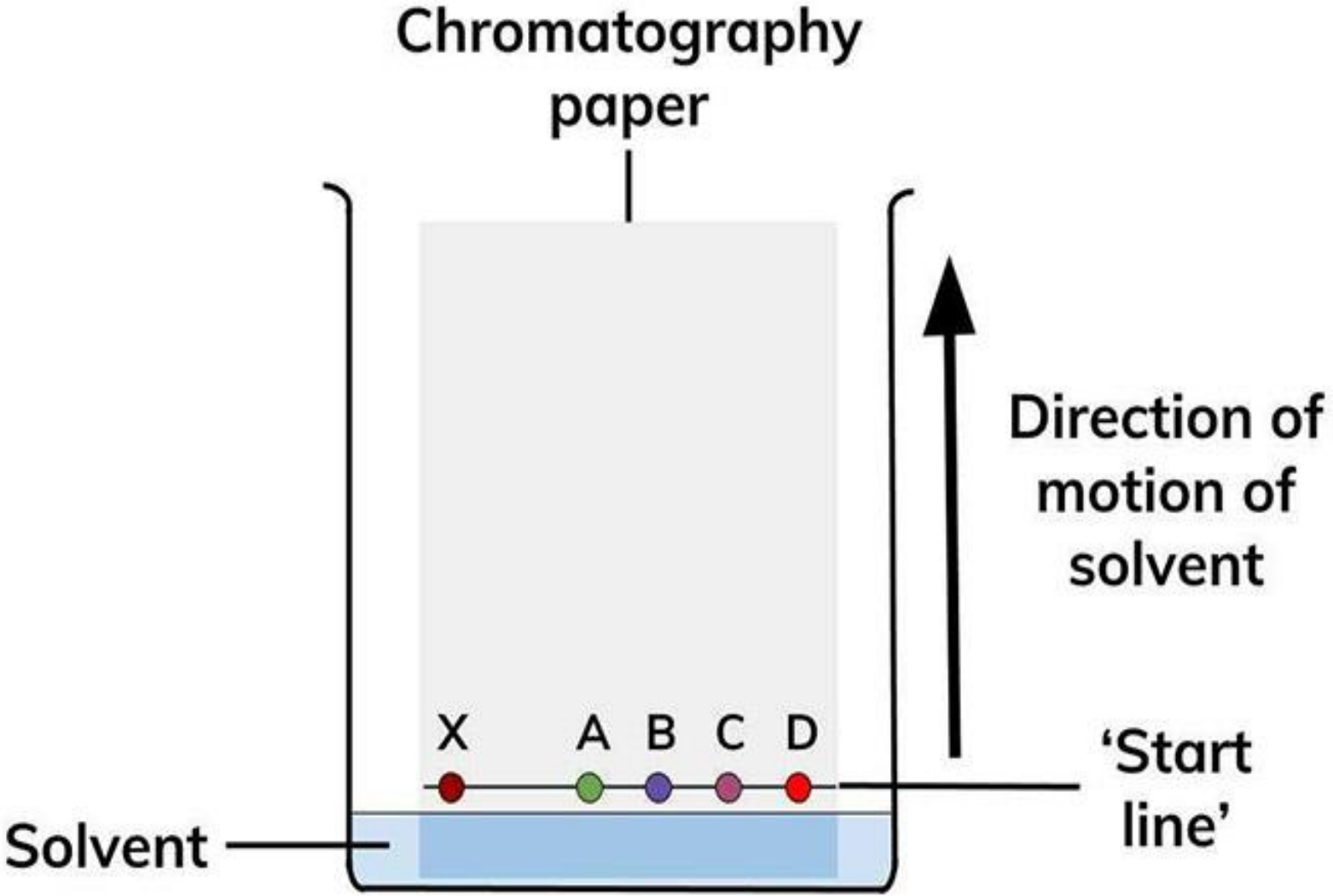
Paper Partition Chromatography

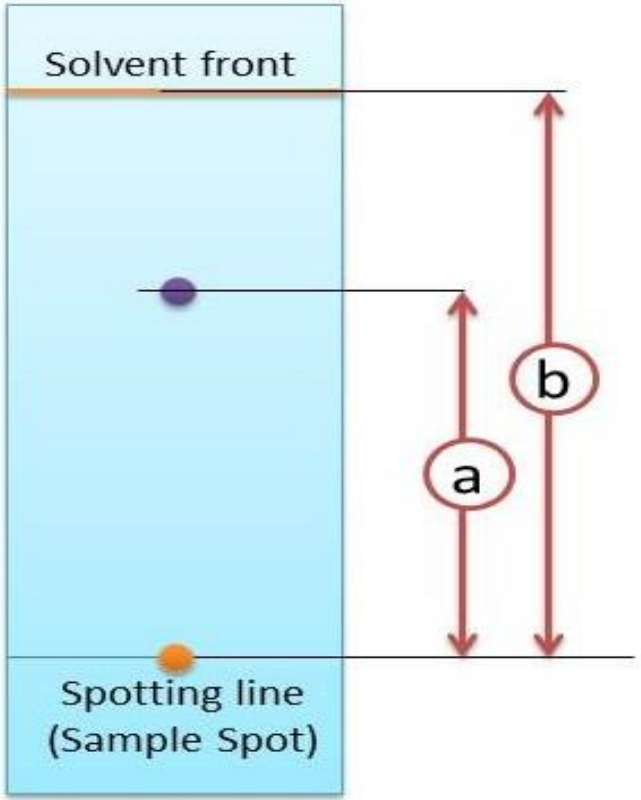
It is a method of **partition chromatography** using filter paper as a carrier or inert support.

In paper chromatography, the **stationary phase** is the water adsorbed between cellulose fibers of the paper (polar stationary phase).

The **mobile phase** is a suitable organic liquid solvent or mixture of solvents (non-polar mobile phase).




Chromatography paper
Direction of motion of solvent
X
A
B
C
D
'Start line'
Solvent



$$Rf = \frac{\text{distance travelled by the component}}{\text{distance travelled by the solvent}}$$

$$= \frac{a}{b}$$

Calculating the Retention Factor Value

The **retention factor or relative factor (R_f)**

the ratio of the distance moved by the solute and the distance moved by the solvent (known as the Solvent front) along the paper.

$$R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

$$0 < R_f < 1$$

Larger R_f value → more soluble
Smaller R_f value → less soluble
It is depend on:

1) Solvent
2) Concentration of Solute
3) Temperature
4) pH
5) Solid support

Types or Modes of Paper Chromatography

Based on the way the development of chromatogram on paper is done in procedures, we have, broadly, five types of chromatography.

1. Ascending chromatography: As the name indicates, the chromatogram ascends. Here, the development of paper occurs due to the solvent movement or upward travel on the paper.

The solvent reservoir is at the bottom of the beaker. The paper tip with sample spots just dips into the solvent at the bottom so that spots remain well above the solvent.

2. Descending chromatography: Here, the development of paper occurs due to solvent travel downwards on the paper.

The solvent reservoir is at the top. The movement of the solvent is assisted by gravity besides the capillary action.

3. Ascending- descending mode: Here solvent first travels upwards and then downwards on the paper.

Types or Modes of Paper Chromatography

4. Radial mode: Here, the solvent moves from the center (mid-point) towards the periphery of circular chromatography paper. The entire system is kept in a covered Petri dish for the development of the chromatogram.

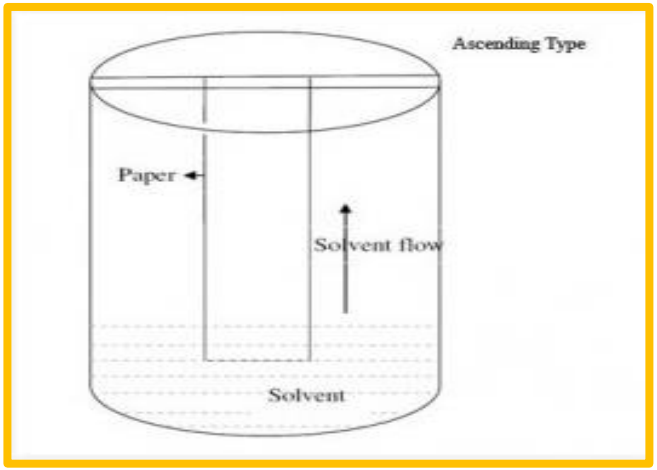
The wick at the center of paper dips into the mobile phase in a petri dish, by which the solvent drains on to the paper and moves the sample radially to form the sample spots of different compounds as concentric rings.

5. Two-dimensional chromatography: Here the chromatogram development occurs in two directions at right angles.

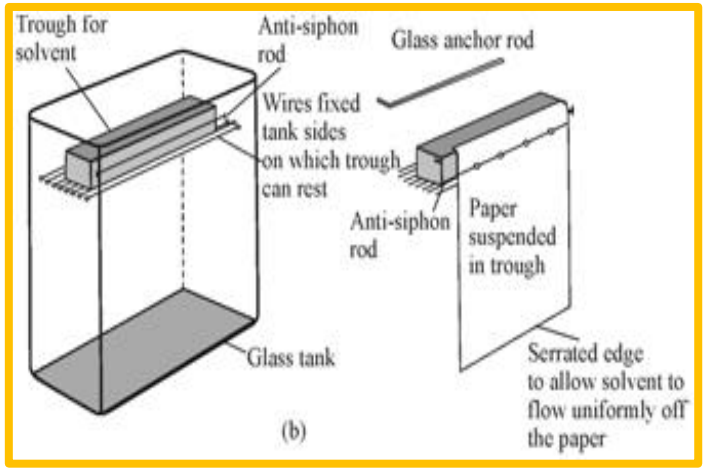
In this mode, the samples are spotted to one corner of rectangular paper and allowed for first development. Then the paper is again immersed in the mobile phase at a right angle to the previous development for the second chromatogram.

Types of paper chromatography

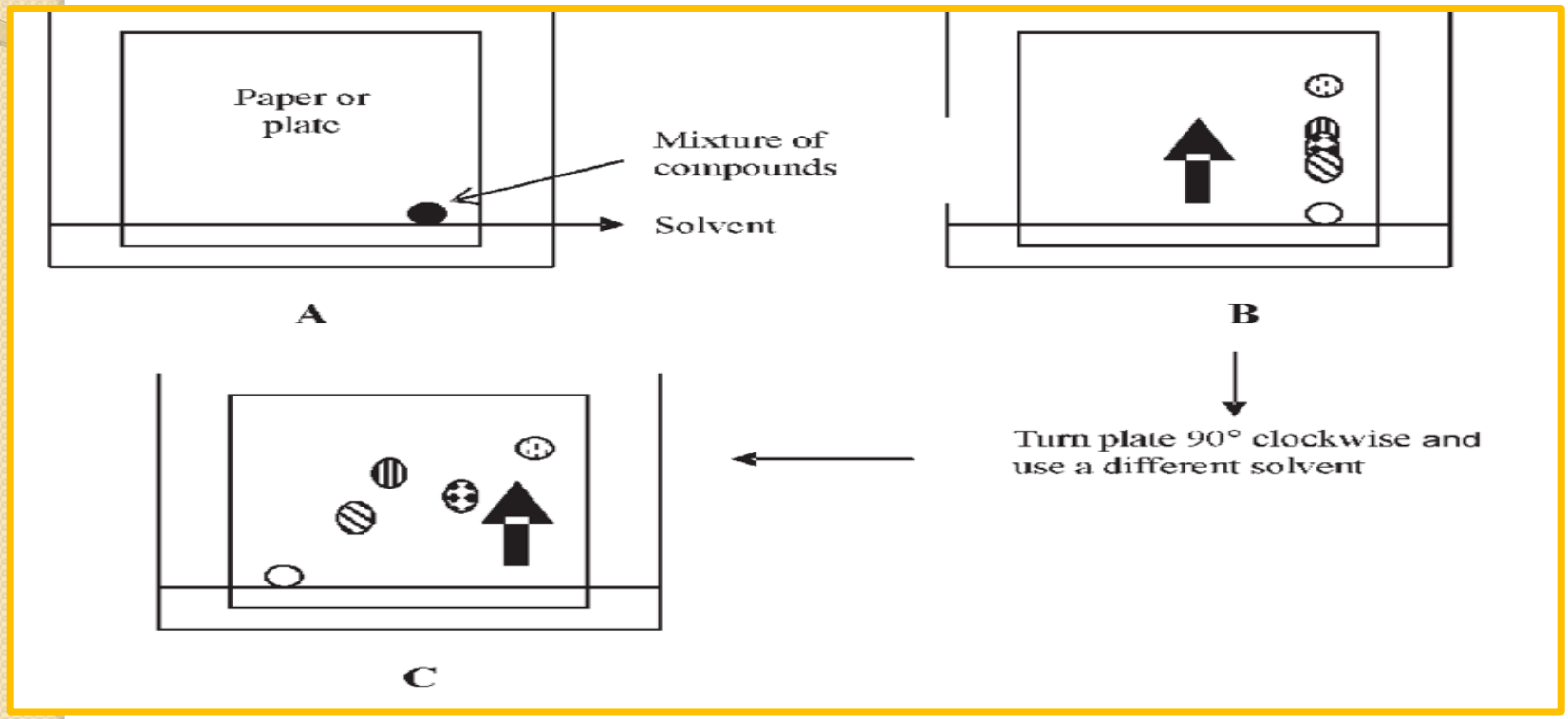
Ascending Paper Chromatography



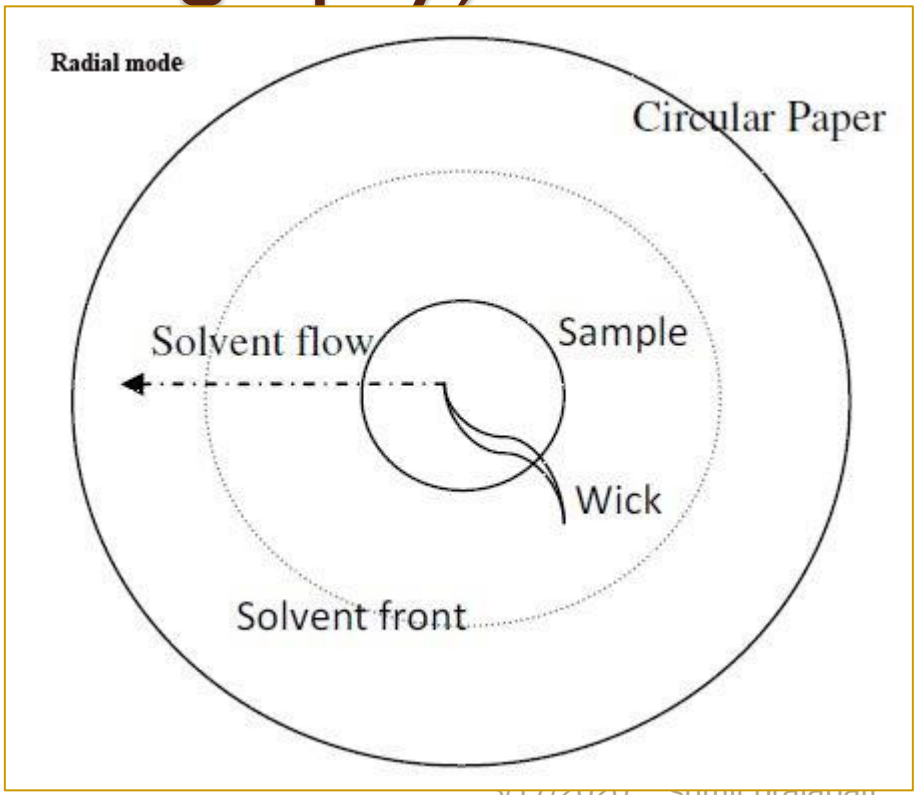
Descending Paper Chromatography



Two-Dimensional Chromatography



Radial mode (Circular chromatography)



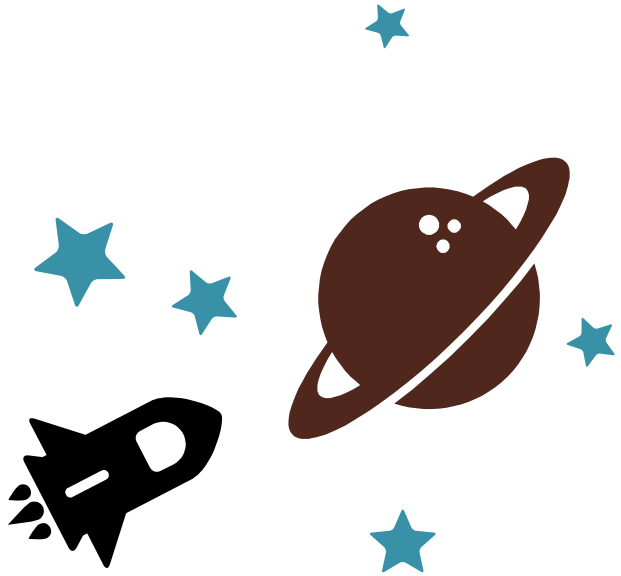
The advantages of paper chromatography are as follow:

- **Separation of compounds in a short time.**
- **In Comparison to other chromatographic methods, paper chromatography is a cheap technique.**
- **Organic and inorganic compounds can be identified by paper chromatography method.**
- **The setup of paper chromatography occupies less space than the other chromatographic method.**
- **Easy to handle and setup.**
- **The less sample quantity required for the analysis.**

The disadvantages of paper chromatography are as follow:

- **Volatile substances cannot be separated using paper chromatography techniques.**
- **Paper chromatography cannot be used with large amounts of sample.**
- **Paper chromatography cannot be separated complex mixture.**
- **As compared to the HPLC, or TLC paper chromatography has less accuracy.**
- **Data cannot be saved for long periods.**

Visualization technique



1) Colored spots are easily observed on developed chromatograms.

2) For colorless substances, physical and chemical methods are used to detect the spot:

a) Physical methods (Non-specific methods):

1) UV chamber for fluorescent compounds

2) (iodine chamber method).

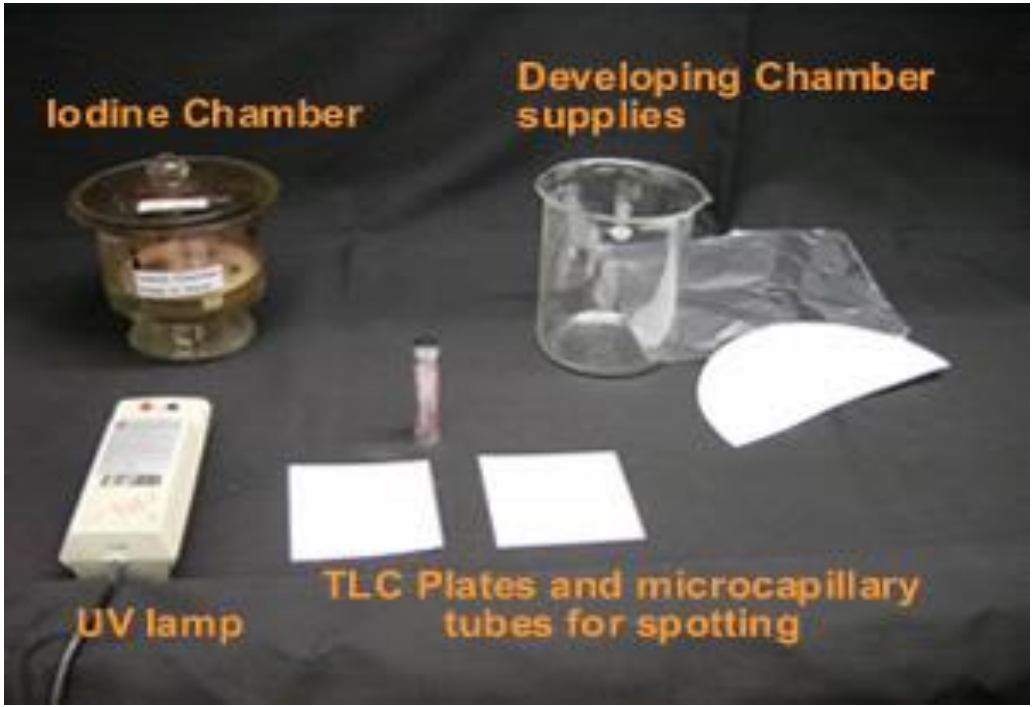
b) Chemical methods (Specific methods) or Spraying methods:

ex: 1) Ninhydrin in acetone for amino acids

2) Ferric chloride solution for phenolic compounds

3) Dragendroff's agent for alkaloids

4) Brady's reagent (2,4 – dinitrophenyl hydrazine in a mixture of methanol and sulphuric acid) for aldehydes and ketones


Iodine Chamber
Developing Chamber supplies
UV lamp
TLC Plates and microcapillary tubes for spotting

Visualization methods can be:

1) Non-destructive: compound is unchanged after the process.

2) Destructive: compound is converted into something new after the process.

Viewing a TLC plate under ultraviolet light is non-destructive, while using a chemical stain is destructive and iodine vapor method is considered (semi-destructive) because complexation is reversible, and the iodine will eventually evaporate from the TLC plate, leaving the original compound behind.