Introduction

- Chromatography means – Colour Writing
- It is new physical technique of separation, identification, identification and purification of components of a mixture.
- It is used in many areas of study particularly in chemistry, biology and medicine.
- Pigments, dyes, amino acids, vitamins, polymers, etc can be separated by using the chromatography technique.

- It is used for the purification and separation of organic as well as inorganic substances.

- Found useful for the fractionation of complex mixtures, separation of closely related compounds such as isomers and in the isolation of unstable substances.

- IUPAC (International Union for Pure and Applied Chemistry) defined Chromatography as -

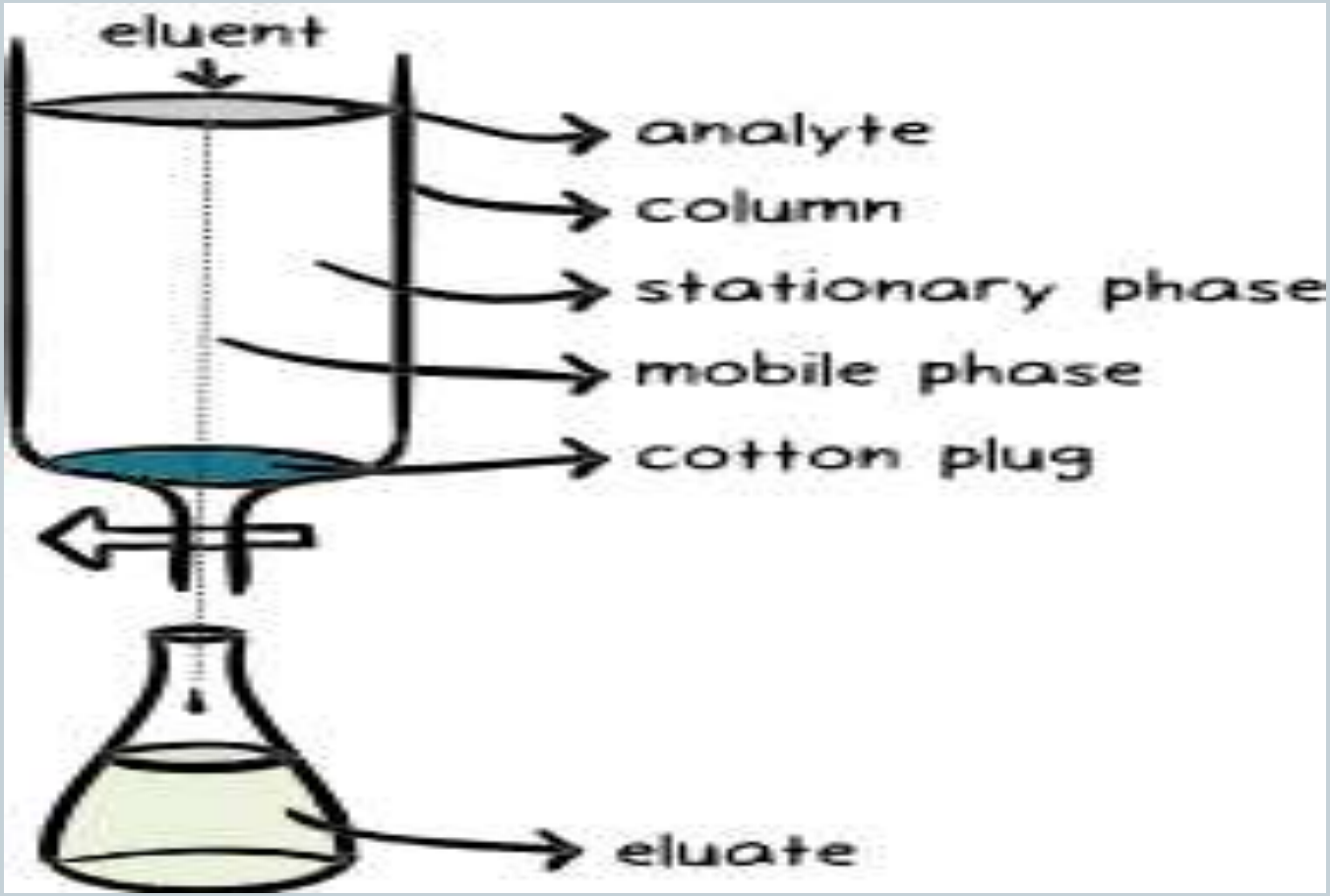
  a physical method of separation in which the components to be separated are, distributed between two phases, one of which is stationary phase while the other is mobile phase, moves in a definite direction.

- Chromatography is a separation technique that uses the size, shape, chemical properties or charge of molecules in a sample to separate the sample into its constituent components.
- It is often used to detect one, or a number of, components in a complex mixture.

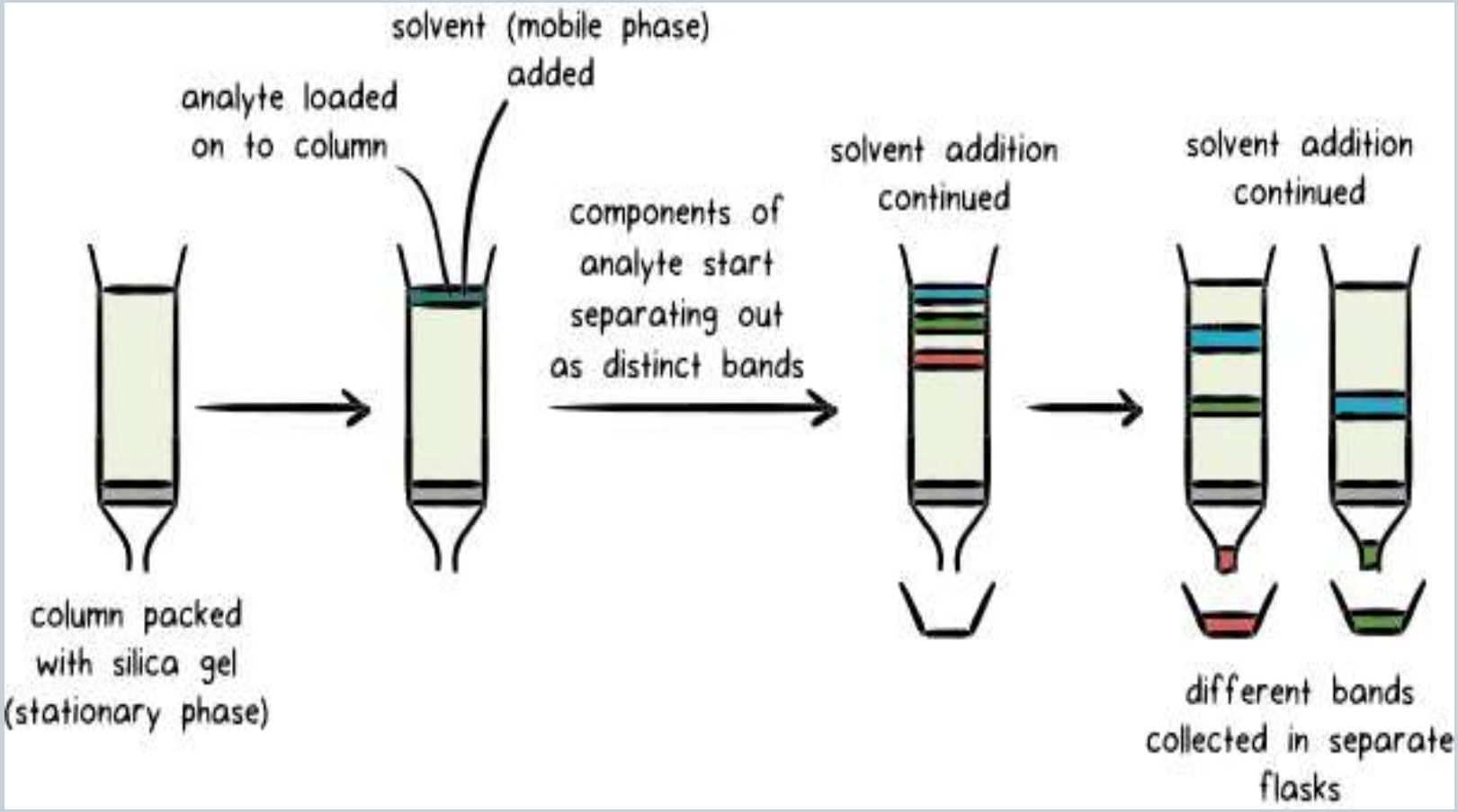
History of Chromatography

- The first true chromatography is usually attributed to the Russian-Italian botanist Mikhail Tsvet.
- Tsvet applied his observations with filter paper extraction to the new methods of column fractionation that had been developed in the 1890s for separating the components of petroleum.
- He used a liquid-adsorption column containing calcium carbonate to separate yellow, orange, and green plant pigments (what are known today as xanthophylls, carotenes, and chlorophylls, respectively).

Basics of Chromatography



TERM	DEFINITION
Mobile phase or carrier	solvent moving through the column
Stationary phase or adsorbent	substance that stays fixed inside the column
Eluent	fluid entering the column
Eluate	fluid exiting the column (that is collected in flasks)
Elution	the process of washing out a compound through a column using a suitable solvent
Analyte	mixture whose individual components have to be separated and analyzed


solvent (mobile phase) added
analyte loaded on to column
solvent addition continued
solvent addition continued
components of analyte start separating out as distinct bands
column packed with silica gel (stationary phase)
different bands collected in separate flasks

- The analyte is loaded over the silica bed (packed in the column) and allowed to adhere to the silica.
- Here, silica acts as the stationary phase.
- Solvent (mobile phase) is then made to flow through the silica bed (under gravity or pressure).
- The different components of the analyte exhibit varying degrees of adhesion to the silica and as a result they travel at different speeds through the stationary phase as the solvent flows through it, indicated by the separation of the different bands.
- *The components that adhere more strongly to the stationary phase travel more slowly compared to those with a weaker adhesion*.
- Analytical chromatography can be used to purify compounds ranging from milligram to gram scale.

Principle of separation of different components

- Differential affinities (strength of adhesion) of the various components of the analyte towards the stationary and mobile phase results in the differential separation of the components.

- Affinity, in turn, is dictated by two properties of the molecule: ‘Adsorption’ and ‘Solubility’.

- We can define adsorption as the property of how well a component of the mixture sticks to the stationary phase, while solubility is the property of how well a component of the mixture dissolves in the mobile phase.

- Higher the adsorption to the stationary phase, the slower the molecule will move through the column.

- Higher the solubility in the mobile phase, the faster the molecule will move through the column.

- So, the interplay between the above two factors determines the differential rates at which the different components of the analyte will move through the column.

- Adsorption and solubility of a molecule can be manipulated by choosing the appropriate stationary phase and mobile phase.

- Different chromatographic techniques –

(word file)

Thin layer chromatography

- Use of thin layer chromatography was first reported by two Russian scientists, N.A Izmailov and M.S Schreiber. Later this technique was developed further by other scientist.

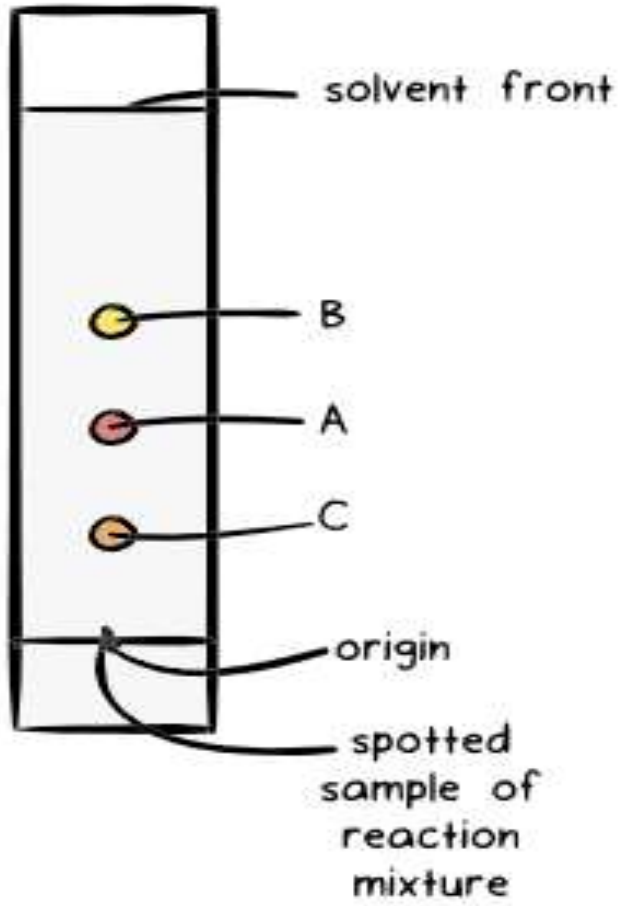
- Thin layer chromatography (TLC) depends on the separation principle.
- The separation relies on the relative affinity of compounds towards both the phases.
- The compounds in the mobile phase move over the surface of the stationary phase.

- The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast.
- Therefore, the separation of the mixture is attained.
- On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates.
- Their character and nature are identified by suitable detection techniques.

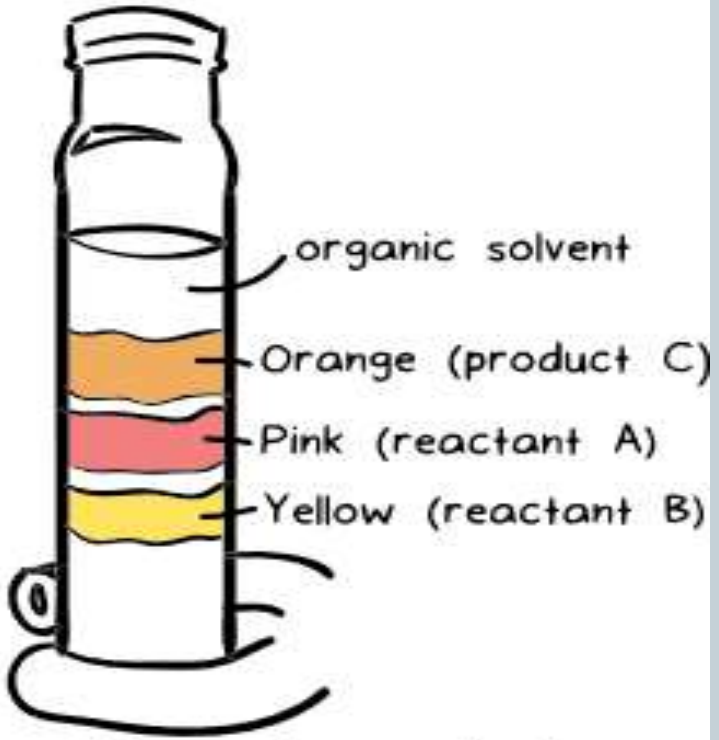
- The TLC plate is a thin piece of aluminium coated on one side with the stationary phase (this phase does not move), an inert material that does not react chemically with the sample components.
- A mobile phase, which consists of a solvent that moves through the stationary phase, is used to move the components of the sample up through the stationary phase.
- A drop of the sample is placed near the bottom end of the TLC plate. The bottom end of the TLC plate is then dipped into the solvent (mobile phase).
- The solvent creeps slowly up the TLC plate.

- The various components of the sample move along with the solvent at a rate that depends on how strongly they are bound to the stationary phase.
- Strongly bound substances hardly move at all, a very weakly bound substance may move at almost the same speed as the solvent.
- Thus the different components (or fractions) of the sample are separated into bands (or spots) along the length of the TLC plate.
- This separation process is the basis of the chromatography method

Thin Layer Chromatography (TLC)



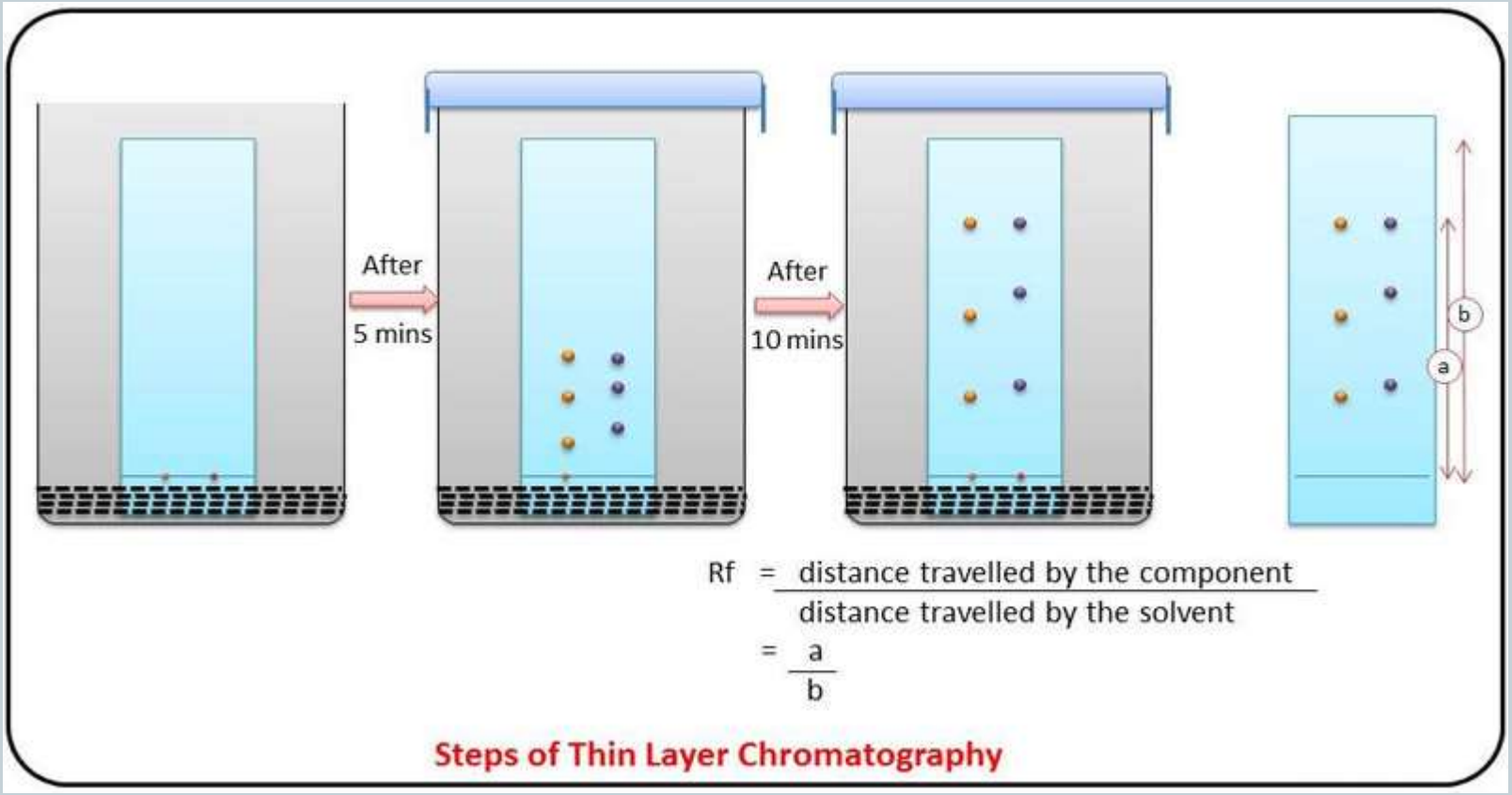
Column Chromatography



Glass column packed with silica in an organic solvent, reaction mixture loaded on the silica bed with help of a glass pipette

Components of Thin Layer Chromatography (TLC)

- **TLC plates,** preferably ready made with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in a fine particle size.
- **TLC chamber-** This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.
- **Mobile phase-** This comprises of a solvent or solvent mixture The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.
- **A filter paper-** This is moistened in the mobile phase, to be placed inside the chamber. This helps develop a uniform rise in a mobile phase over the length of the stationary phase.


After
5 mins
After
10 mins
b
a
Rf = distance travelled by the component / distance travelled by the solvent
= a / b
Steps of Thin Layer Chromatography

Applications of Thin Layer Chromatography (TLC)

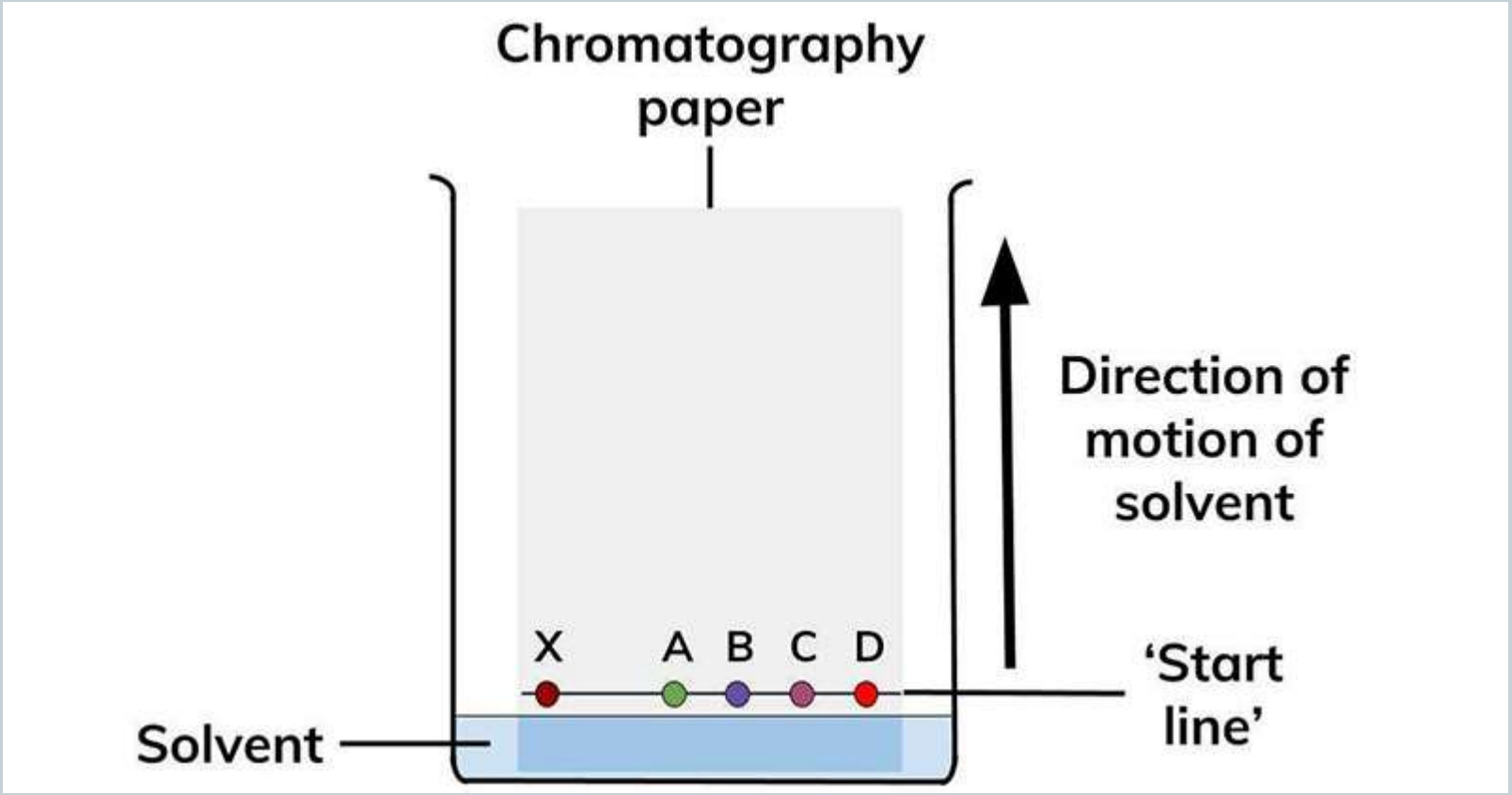
- In monitoring the progress of reactions
- Identify compounds present in a given mixture
- Determine the purity of a substance.
- Analyzing ceramides and fatty acids
- Detection of pesticides or insecticides in food and water
- Analyzing the dye composition of fibers in forensics
- Assaying the radiochemical purity of radiopharmaceuticals
- Identification of medicinal plants and their constituents

- https://www.khanacademy.org/test-prep/mcat/chemical-processes/separations-purifications/v/thin-layer-chromatography
- https://www.youtube.com/watch?v=lj5OWzhZSac
- https://www.youtube.com/watch?v=qdmKGskCyh8

Paper Chromatography

- Invented by Archer John Porter Martin and Richard Laurence Millington Synge.
- Its development successfully solved the problem of separating amino acids which are very similar to each other.
- https://www.youtube.com/watch?v=6o0yFvDYex4

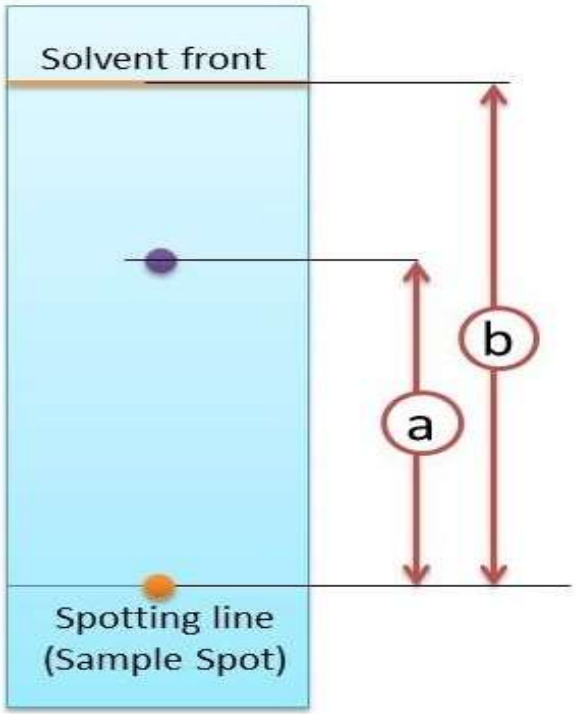
- Special papers are usually used for this technique.
- These papers should be highly purified.
- The papers used contain sufficient adsorbed water.
- Other liquids as silicone, paraffin oil are used
- Choice paper is dependent on the type of analysis under investigation
- Whatman chromatographic papers of different types are used.


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

- The principle of separation is mainly partition rather than adsorption.
- Substances are distributed between a stationary phase and mobile phase.
- Cellulose layers in filter paper contain moisture which acts as stationary phase.
- Organic solvents/buffers are used as mobile phase.
- The developing solution travels up the stationary phase carrying the sample with it.
- Components of the sample will separate readily according to how strongly they adsorb onto the stationary phase versus how readily they dissolve in the mobile phase.

Steps in Paper Chromatography

- Selection of Solid Support
- Selection of Mobile Phase
- Saturation of Tank
- Sample Preparation and Loading
- Development of the Chromatogram
- Drying of Chromatogram
- Detection



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

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

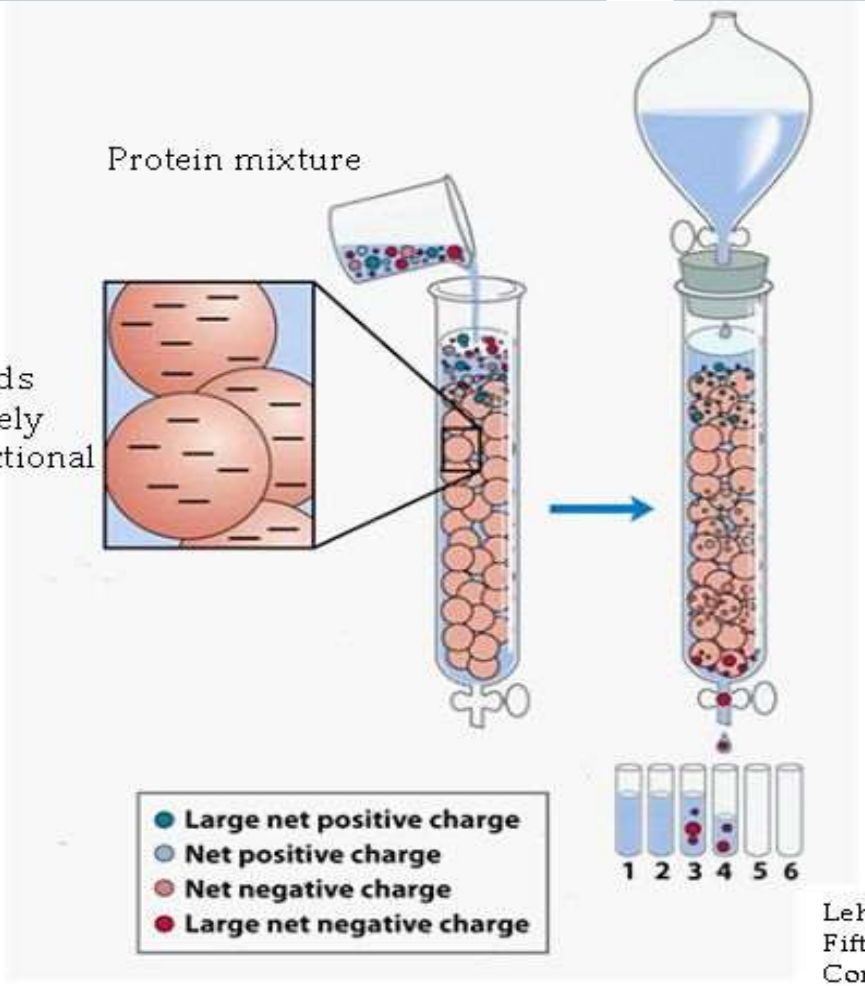
Calculating the Retention Factor Value

Applications of Paper Chromatography

- To check the control of purity of pharmaceuticals,
- For detection of adulterants,
- Detect the contaminants in foods and drinks,
- In the study of ripening and fermentation,
- For the detection of drugs and dopes in animals & humans
- In analysis of cosmetics
- Analysis of the reaction mixtures in biochemical labs

Ion-exchange Chromatography

- Originally introduced by Sir Thompson and JT Way.
- Method was used to treat clays with the salts, resulting in the extraction of ammonia in addition to release of calcium
- Compounds knows as “zeolites” were introduced to separate individual ions or electrically charged particles.
- Synthetic resins were developed from complex ion-exchange processes.
- https://www.youtube.com/watch?v=i4U4ndf2ayg&t=294s



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The Applications of Ion Exchange Chromatography

- Ion exchange is the most widely used chromatographic method for the separation and purification of charged biomolecules such as polypeptides, proteins, polynucleotides, and nucleic acids.
- Its widespread applicability, high capacity and simplicity, and its high resolution are the key reasons for its success as a separation method.

Ion exchange chromatography is widely used in several industrial applications some of which are as follows:

- Separation and Purification of blood components such as albumin,recombinant growth factors and enzymes.
- Biotechnology - Analytical applications such as quality control and process monitoring
- Food and clinical research - to study wheat varieties and the correlation of proteinuria with different renal diseases.
- Fermentation - Cation exchange resins are used to monitor the fermentation process during ß-galactosidase production.

Other methods

- Gel permeation chromatography
- Affinity chromatography
- Gas chromatography
- Supercritical fluid chromatography
- Capillary electrophoresis
- High performance chromatography