Thin Layer Chromatography

Based on differential Adsorption of Molecules

Thin Layer Chromatography

- Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis
- Thin Layer Chromatography can be defined as a method of separation or identification of a mixture of components into individual components by using finely divided adsorbent solid spread over a plate and liquid as a mobile phase
- Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material
- This layer of adsorbent is known as the stationary phase

Principle of Thin Layer Chromatography

- It is based on the principle of adsorption chromatography depending on adsorbent, its treatment and nature of solvents employed
- The components with more affinity towards stationary phase travels slower and Components with less affinity towards stationary phase travels faster
- Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate
- Their nature or character is identified by means of suitable detection techniques
- After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

Components of Thin Layer Chromatography

- **TLC plates -** 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.

- **Coating Material – Silica gel, Alumina Powder, Cellulose powder**
- **Added with binding agents to adhere on chromatography plate - Gypsum, starch, $CaSO_4$ or hydrated silicon oxide**

Preparation of Thin Layer: A continuous layer coated manually or with the use of applicator

The thickness of layer is 200um

Slurry of coating material is applied by:

- **Pouring**
- **Dipping**
- **Spraying**
- **Spreading**

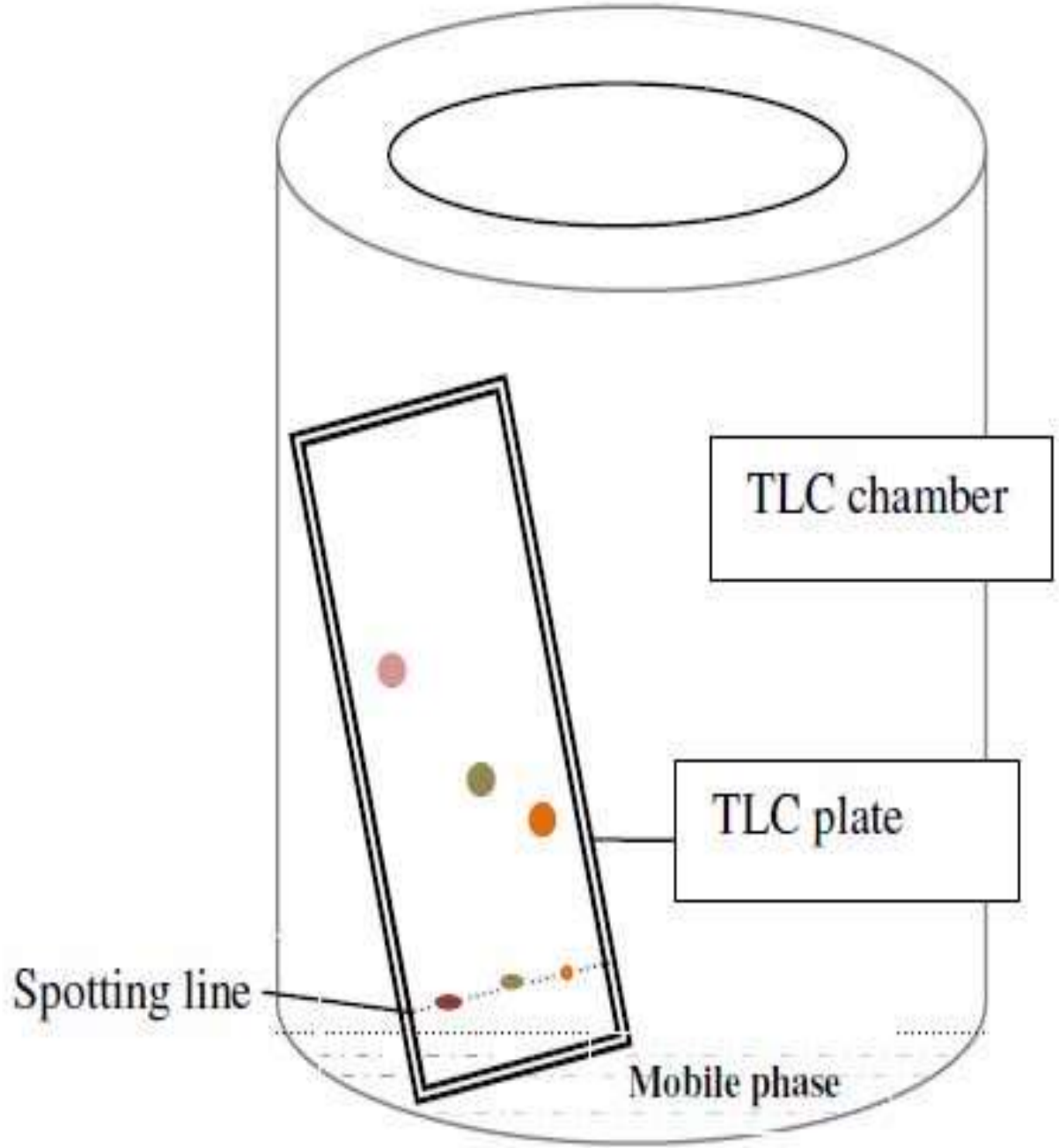
Activation of plates at 110^oC

Procedure of Thin Layer Chromatography

- The stationary phase is applied onto the plate uniformly and then allowed to dry and stabilize
- A thin mark is made at the bottom of the plate to apply the sample spots
- The mobile phase is poured into the TLC chamber to a leveled few centimeters
- The plate is then immersed, such that the sample spots are well above the level of mobile phase (but not immersed in the solvent) for development
- Sufficient time is given for the development of spots and the plates are then removed and allowed to dry
- The sample spots are then seen in a suitable methods as recommended for the given sample

Some common techniques for visualizing the results of a TLC plate include

- UV light
- Iodine Staining: is very useful in detecting carbohydrates since it turns black on contact with Iodine
- $KMnO_4$ stain (organic molecules)
- Ninhydrin Reagent: often used to detect amino acids and proteins

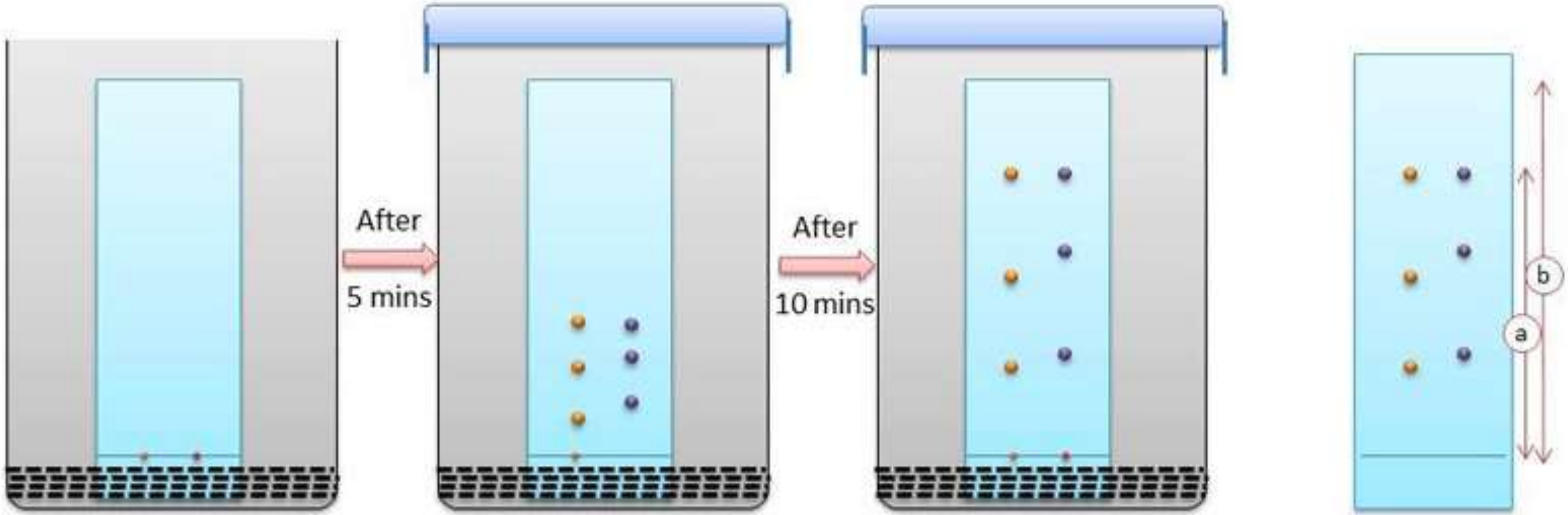

TLC chamber
TLC plate
Spotting line
Mobile phase

Retention Factor (Rf) Value

- The behaviour of a compound on a TLC is usually described in terms of its relative mobility
- Rf or Retention factor is a unique value for each compound under the same conditions

Factors affecting the separation:

- solvent system
- adsorbent
- thickness of the adsorbent
- amount of material spotted
- Temperature



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

Steps of Thin Layer Chromatography

Applications of Thin Layer Chromatography

- 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

Advantages of Thin Layer Chromatography

- It is a simple process with a short development time
- It helps with the visualization of separated compound spots easily
- It helps in isolating of most of the compounds
- The separation process is faster and the selectivity for compounds is higher (even small differences in chemistry is enough for clear separation)
- The purity standards of the given sample can be assessed easily
- It is a cheaper chromatographic technique

Limitations of Thin Layer Chromatography

- It cannot tell the difference between enantiomers and some isomers
- In order to identify specific compounds, the Rf values for the compounds of interest must be known beforehand
- TLC plates do not have long stationary phases, therefore, the length of separation is limited compared to other chromatographic techniques.