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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

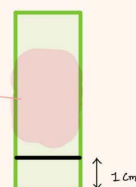
Electrophoresis– Introduction, factors affecting electrophoretic mobility, Techniques of paper, gel, capillary electrophoresis, applications

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What Is Thin Layer Chromatography?

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel.



On completion of the separation, each component appears as spots separated vertically. **Each spot has a retention factor (Rf) expressed as:**

$$Rf = \frac{\text{dist. travelled by sample}}{\text{dist. travelled by solvent}}$$

[The factors affecting retardation factor are the solvent system, amount of material spotted, adsorbent and temperature.] TLC is one of the fastest, least expensive, simplest and easiest chromatography technique.

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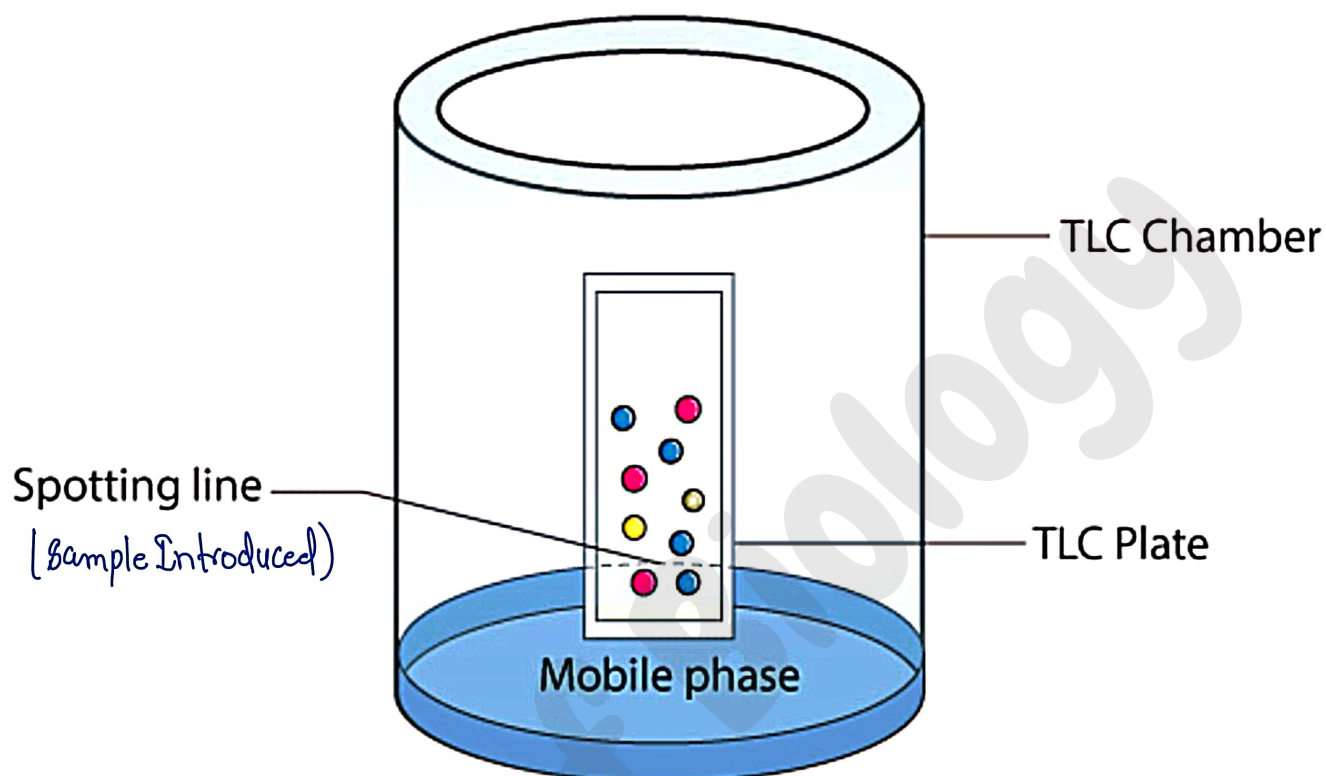


Diagram of Thin Layer Chromatography

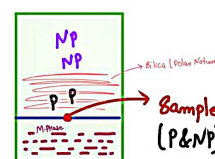
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Thin Layer Chromatography Principle

Like other chromatographic techniques, 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.

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Thin Layer Chromatography Procedure

Before starting with the Thin Layer Chromatography Experiment, let us understand the different components required to conduct the procedure along with the phases involved.

1. Thin Layer Chromatography **Plates** – ready-made plates are used which are chemically inert and stable. The stationary phase is applied on its surface in the form of a thin layer. The stationary phase on the plate has a fine particle size and also has a uniform thickness.
2. Thin Layer Chromatography **Chamber** – **Chamber** is used to develop plates. It is responsible to keep a steady environment inside which will help in developing spots. Also, it prevents the solvent evaporation and keeps the entire process dust-free.
3. Thin Layer Chromatography Mobile phase – Mobile phase is the one that moves and consists of a solvent mixture or a solvent. This phase should be particulate-free. The higher the quality of purity the development of spots is better.
4. Thin Layer Chromatography Filter Paper – It has to be placed inside the chamber. It is moistened in the mobile phase.

→ R₀F Value (Retardation/Retention factor) →

$$R_{0}F = \frac{\text{Distance travelled by solute/sample}}{\text{Distance travelled by solvent.}}$$

→ R₀F Value ranges from 0-1

$$\text{Ideal Value} = 0.3 - 0.8$$

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Thin Layer Chromatography Experiment

The stationary phase that is applied to the plate is made to dry and stabilize.

- To apply sample spots, thin marks are made at the bottom of the plate with the help of a pencil.
- Apply sample solutions to the marked spots.
- Pour the mobile phase into the TLC chamber and to maintain equal humidity, place a moistened filter paper in the mobile phase.
- Place the plate in the TLC chamber and close it with a lid. It is kept in such a way that the sample faces the mobile phase.
- Immerse the plate for development. Remember to keep the sample spots well above the level of the mobile phase. Do not immerse it in the solvent.
- Wait till the development of spots. Once the spots are developed, take out the plates and dry them. The sample spots can be observed under a UV light chamber.

Disadvantages Of Thin Layer Chromatography:

1. Thin Layer Chromatography plates do not have longer stationary phase.
2. When compared to other chromatographic techniques the length of separation is limited.
3. The results generated from TLC are difficult to reproduce.
4. Since TLC operates as an open system, some factors such as humidity and temperature can be can affect the final outcome of the chromatogram.
5. The detection limit is high and therefore if you want a lower detection limit, you cannot use TLC.
6. It is only a qualitative analysis technique and not quantitative.

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Thin Layer Chromatography Applications

- The qualitative testing of Various medicines such as sedatives, local anaesthetics, anticonvulsant tranquilisers, analgesics, antihistamines, steroids, hypnotics is done by TLC.
- TLC is extremely useful in Biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, urine, body fluids, serum, etc.
- Thin layer chromatography can be used to identify natural products like essential oils or volatile oil, fixed oil, glycosides, waxes, alkaloids, etc.
- It is widely used in separating multicomponent pharmaceutical formulations.
- It is used for the purification of samples and direct comparison is done between the sample and the authentic sample.
- It is used in the food industry, to separate and identify colours, sweetenin agent, and preservatives
- It is used in the cosmetic industry.
- It is used to study if a reaction is complete.

Advantages Of Thin Layer Chromatography –

- An easy method of separation of the components.
- In this technique, fewer types of equipment are used. ...
- All components of UV light is achievable to visualize.
- The non-volatile compounds can be separated by this method.
- Microlitre quantity of sample can also be separated in TLC.