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Programme: M.Sc., Biomedical Science

Course Title : Biotechniques

Course Code : BMS35S1BT

Unit-IV

Thin Layer Chromatography

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KEY CONCEPTS

- Introduction
- General Principle
- TLC Technique
- Applications

Introduction

- Thin Layer Chromatography (TLC) is an important technique used for identification and separation of mixture of chemical compounds into its individual components.
- TLC is a form of liquid chromatography consisting of two phases: **A mobile phase (liquid)** and **A stationary phase (solid).**
- Differences in the interactions between the solutes and stationary and mobile phases enable separation.

Principle

- TLC is based on the principle of adsorption of partition chromatography or combination of both.
- It involves the distribution of components of a mixture to be separated between two phases.
- The components of the **mixture are partitioned between an adsorbent** (stationary phase), and a **solvent** (mobile phase).
- Different compounds will have different solubility and adsorption to the two phases between which they are to be partitioned.
- In TLC separation of the individual substances is based on their relative affinities towards stationary and mobile phases.

PRINCIPLE

- **The stationary phase:** is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.
- **The mobile phase:** is a developing liquid which flows through the stationary phase, carrying the samples with it.
- Components with more affinity towards stationary phase travels slower.
- Components with less affinity towards stationary phase travels faster.

METHOD

- **Adsorbents used as Stationary Phase:**
  - **Inorganic:** Silica Gel, Kieselguhr, Aluminium Silicate, Bentonite.
  - **Organic:** Cellulose & its acetylates, Charcoal & activated Charcoal, Dextran Gel, Polyamides.
- **Solvents used as Mobile Phase:**
  - Petroleum ether, Benzene, Carbon tetrachloride.
- **Selection of Adsorbents and Solvents:**
  - ✓ Adsorbent should not adhere to glass plate.
  - ✓ Solvents should be of high purity.
  - ✓ Selected based on the nature of the compound to be separated (polar or non polar.)

R_f Value

- R_f value indicates the position of migrated spots on chromatogram.
- In TLC the results are represented by R_f value which represents the migration of solute relative to the solvent front.
- The R_f value is calculated as:-

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

Factors affecting the R_f Value

- Nature of the adsorbent
- Mobile phase
- Thickness of the layer
- Temperature
- Activity
- Equilibrium
- Sample volume
- Loading process

TLC - Technique

STEP 1: Preparation of Slurry

- A plastic, glass or aluminum sheet is coated with a thin layer of silica gel (adsorbent).
- Plates must be dried, activated and stored in desiccator until used.

STEP 2: Preparation of Tank

- Solvent mixtures should be freshly prepared for analysis.
- Solvent is poured down side of the tank (1.5cm depth).
- Tank is covered with the glass lid and kept for saturation.

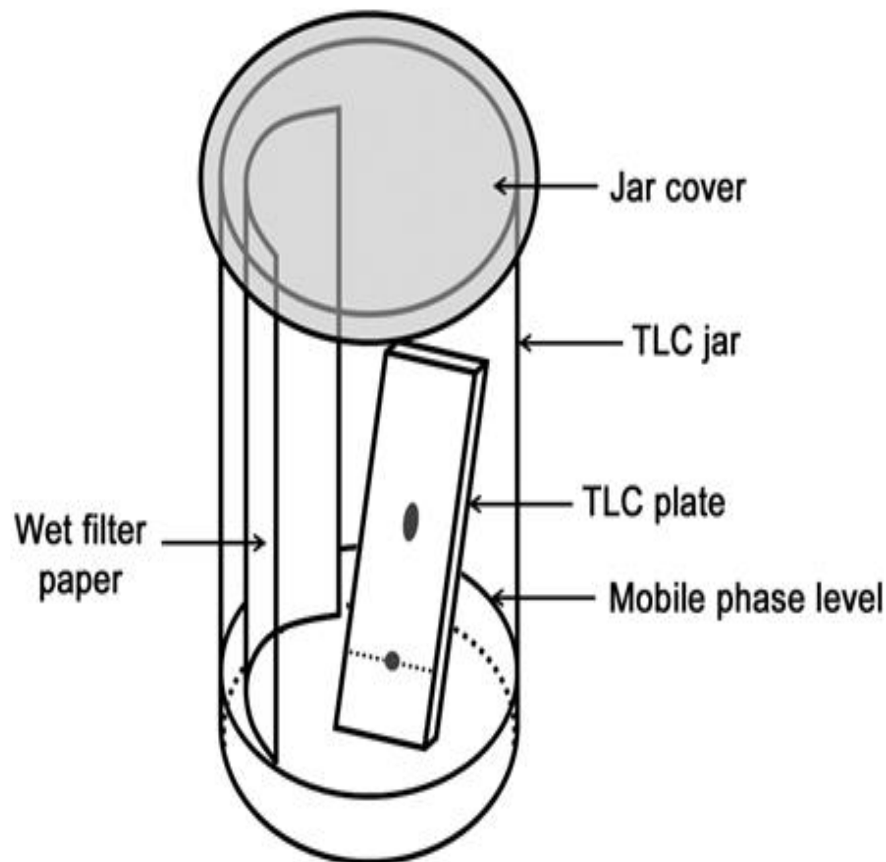
STEP 3: Application of Sample (Spot)

- A very small amount of sample (solution) to be analyzed is applied in a small spot with a capillary tube, ~1cm from the bottom of the TLC plate.

TLC - Technique

- The TLC is developed in a chamber which contains the mobile phase (solvent).
- When the mobile phase rises up the plate up by capillary action, the components dissolve in the solvent and move.

A TLC experimental set-up



http://nptel.ac.in/courses/102103047/module4/lec21/4.html

TLC - Technique

- Individual components in the sample move up at different rates.
- More **polar analytes interact** more **strongly** with the **stationary phase move** very s**lowly up.**
- More **nonpolar analytes interact less strongly** with the **polar silica gel** and **more strongly** with the less polar **mobile phase** mo**ve higher** up.
- Once the solvent reaches the top (below ~1-2 cm) of the TLC sheet the plate is removed from the developing chamber and position of solvent front is marked.

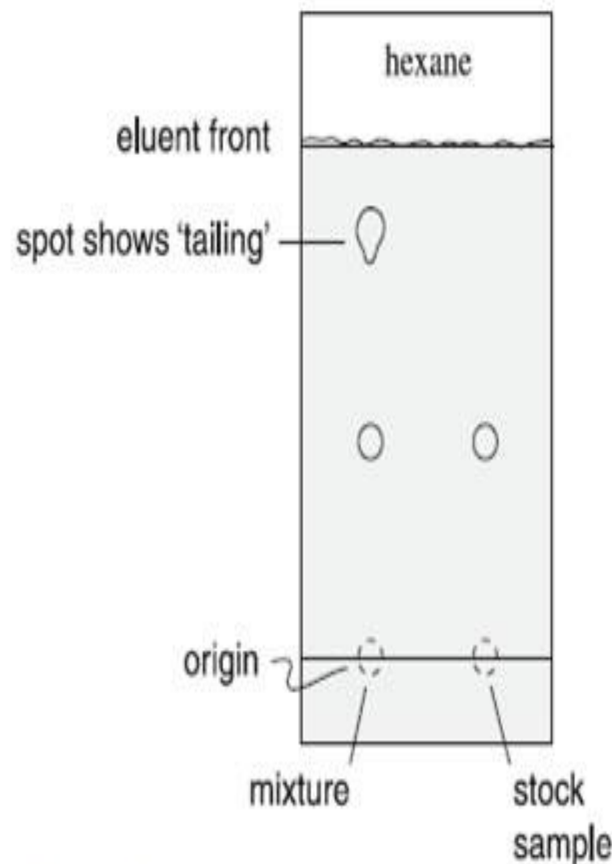
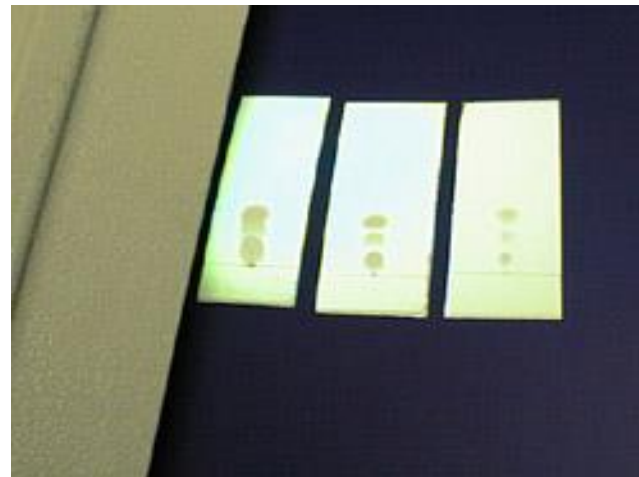


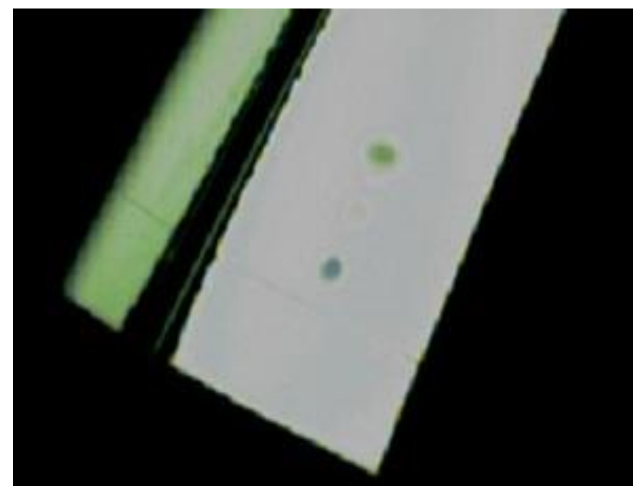
Figure 3 A completed chromatogram

http://classes.kvcc.edu/chm220/TLC%20Lab/lab/procedures1.htm

TLC - Technique

- The solvent is allowed to evaporate from the TLC sheet.
- As the compound is colorless, it can be visualized by suitable methods.
  - Lipids - Iodine vapors
  - Amino acids - Ninhydrin reagent.
- Also, manganese-activated zinc silicate (**fluorescent compound**), is added to the adsorbent that allows the visualization of spots under a black light (**UV_{254} lamp**).



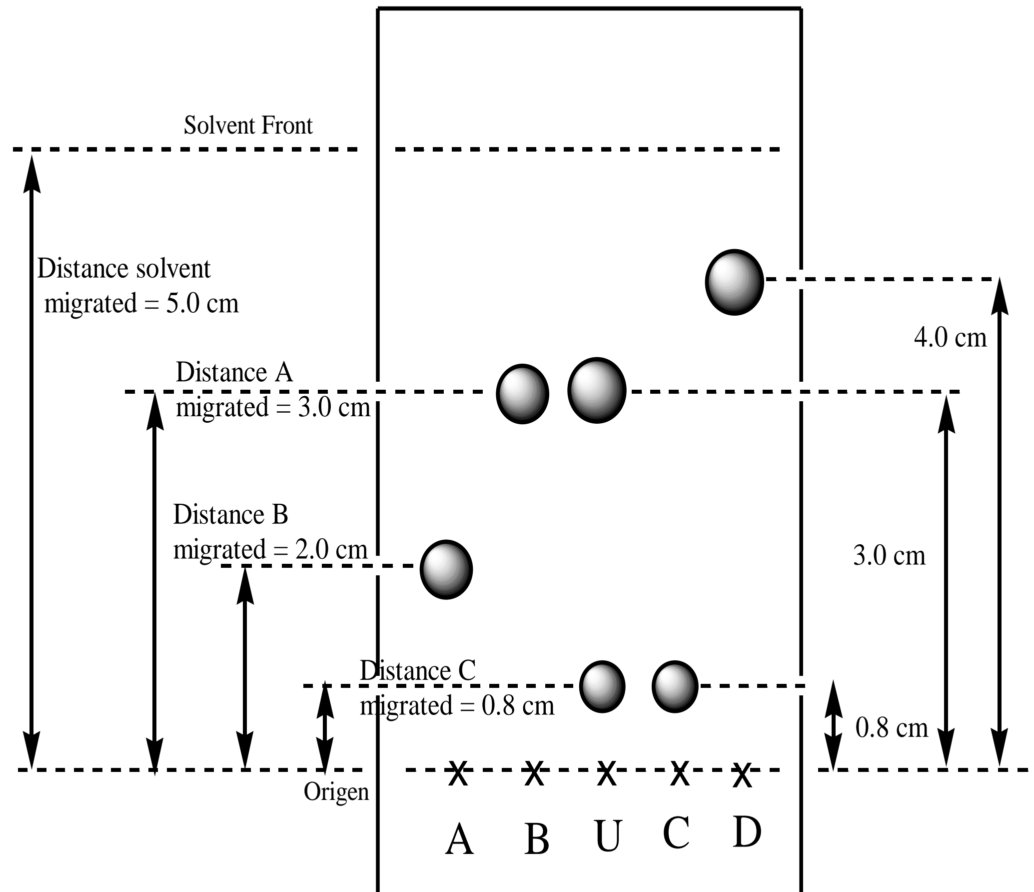


http://orgchemboulder.com/Technique/Procedures/TLC/TLC.shtml

- Once visible, the R_f value of each spot can be determined.

TLC – Calculation of R_f Value

$$R_f = \frac{\text{Distance from centre of solute spot (cm) to the baseline}}{\text{Distance from solvent front to baseline (cm)}}$$



$$R_f\,(A) = \frac{2.0\text{ cm}}{5.0\text{ cm}} = 0.40$$

$$R_f\,(B) = \frac{3.0\text{ cm}}{5.0\text{ cm}} = 0.60$$

$$R_f\,(C) = \frac{0.8\text{ cm}}{5.0\text{ cm}} = 0.16$$

$$R_f\,(D) = \frac{4.0\text{ cm}}{5.0\text{ cm}} = 0.80$$

$$R_f\,(U_1) = \frac{3.0\text{ cm}}{5.0\text{ cm}} = 0.60$$

$$R_f\,(U_2) = \frac{0.8\text{ cm}}{5.0\text{ cm}} = 0.16$$

https://slideplayer.com/slide/6184680/18/images/21/THIN+LAYER+CHROMATOGRAPHY.jpg

Results of TLC - R_f Value

- **Qualitative results of TLC**
  - expressed as fractions of 1.0
  - can be expressed from R_f values (Ex: R_f x 100)
  - no more than two decimal places

- **R_f values can be used to aid in the identification of a substance by comparison to <u>standards</u>.**

- **Comparison should be made <u>only</u> between spots on the same sheet, run at the same time.**

- Identical substances will have the same R_f value, whereas non- identical compounds will differ in their R_f values.

Applications

- TLC is used in qualitative and quantitative analysis to separate organic compounds and to test the purity of compounds.
- This technique is useful for separation of lipids, amino acids and sugars etc.
- It is useful in:
  - Identification of components of a mixture.
  - Following the course of a reaction,
  - Analyzing fractions collected during purification,
  - Analyzing the purity of a compound.

Advantages

- Low cost.
- Adoptable in most research and pharmaceutical laboratories.
- Short analysis time.
- Uses small quantities of solvent.
- Reliable and quick.
- Minimal training is required
- All spots can be visualized.