Separation Processes in Bioengineering

Experiment 3

Thin Layer Chromatography

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Chromatography

- Technique for separating the components, or solutes, of a mixture on the basis of the relative amounts of each solute distributed between a **moving fluid stream**, called the **mobile phase**, and a contiguous **stationary phase**.
- process for separating the components of a mixture by using a stationary phase and a mobile phase

Chromatography

- **Components of Chromatography**

- **Stationary phase:** composed of a “solid” phase or “a layer of a liquid adsorbed on the surface a solid support”.
- **Mobile Phase:** always composed of “liquid” or a “gas”
- **Sample to be separated**

Classification of Chromatograpic Methods

- ✓ On the basis of mechanism of separation
  1. Adsorption (Column, Thin layer Chromatography, Solid-gas Chromatography)
  2. Partition (Liquid- Liquid, Liquid –gas Chromatography)
  3. Ion Exchange (Cation Exchange, Anion Exchange Chromatography)
  4. Size Exclusion (Gel Filtration)
- ✓On the basis of chromatographic bed shape
  1. Two dimensional (Paper, Thin layer Chromatography)
  2. Three dimensional (Gel Filtration)
- ✓On the basis of phases
  1. Solid Phase (Solid-Liquid, Solid-gas chomatography)
  2. Liquid Phase (Liquid-Liquid, Liquid-gas Chromatography)

Thin Layer Chromatography

- *Thin Layer Chromatography* is an important technique for **identification** and **separation** of mixtures of organic compounds.
- is performed on a **supporting material** (a sheet of glass or metal foil)
- Supporting material usually coated with adsorbent such as *alumina (Al2O3) or silica (SiO2)*
- **mobile phase** consists of a *volatile* organic solvent or mixture of solvents.
- Several factors specify the efficiency of separation.

Thin Layer Chromatography

Adsorbent

- some adsorbents may be too **strongly** adsorbing or too **weakly** adsorbing.
- Approximately
- *Adsorbent* Alumina (Al2O3)
- Charcoal (C)
- Florisil (MgO/SiO2) (anhydrous)

Strongly adsorbent

- Silica gel (SiO2) → Least Strongly adsorbent

Thin Layer Chromatography

Eluting solvent

- solubility of compounds in eluting solvent affects **how fast they move up the TLC plate.**
- Ability of solvent to be adsorbed on the adsorbent.
- If solvent is too strongly adsorbed
  can fully displace all compounds.
  Results in non-effective separation because of moving up to solvent front
- If solvent is too weakly adsorbed
  may be insufficient to move any compounds fast enough for effective separation
- Generally mixtures of solvents are used to abtain optimum separation in TLC.

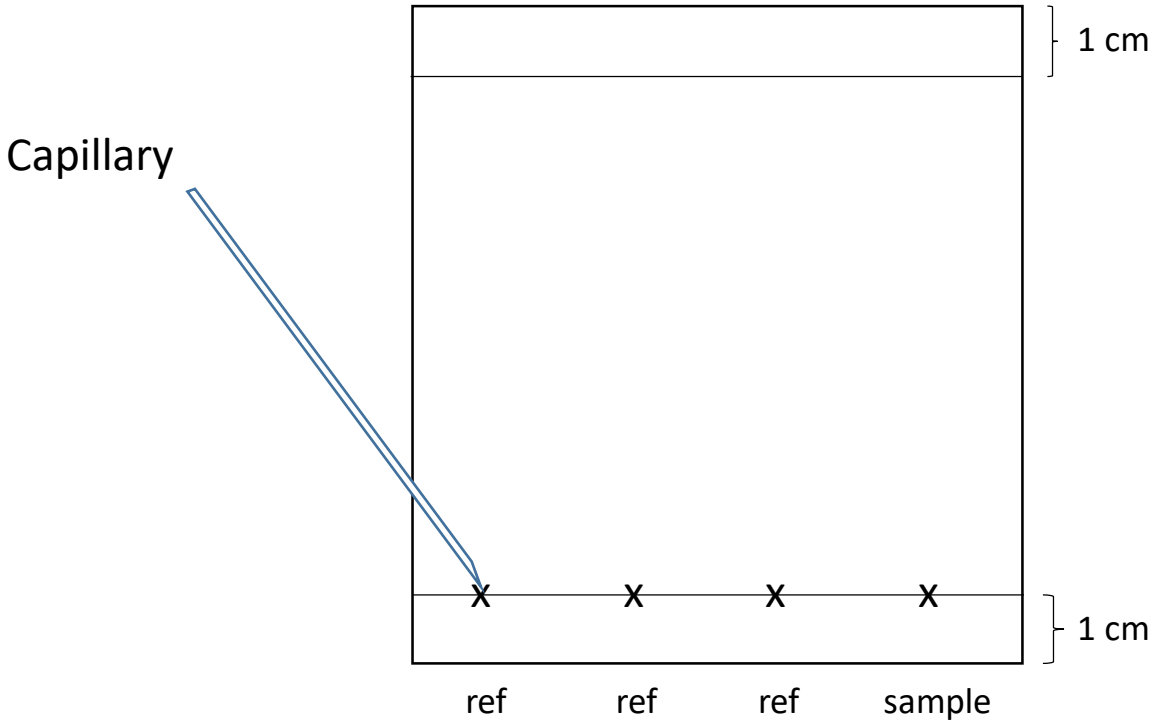
Thin Layer Chromatography

- **Selection of the particular combination of adsorbents and eluting solvents according to existing compound adjust success of separation in TLC**
- **Selection the eluting solvent basis on affinity to adsorbent.**
- **The affinity of the eluting solvent for the adsorbent should be comparable to the compounds being separated. So, this results in separation of compounds through difference in rates.**

Thin Layer Chromatography

- *BASIC CONCEPT* in three main step

1. Spotting

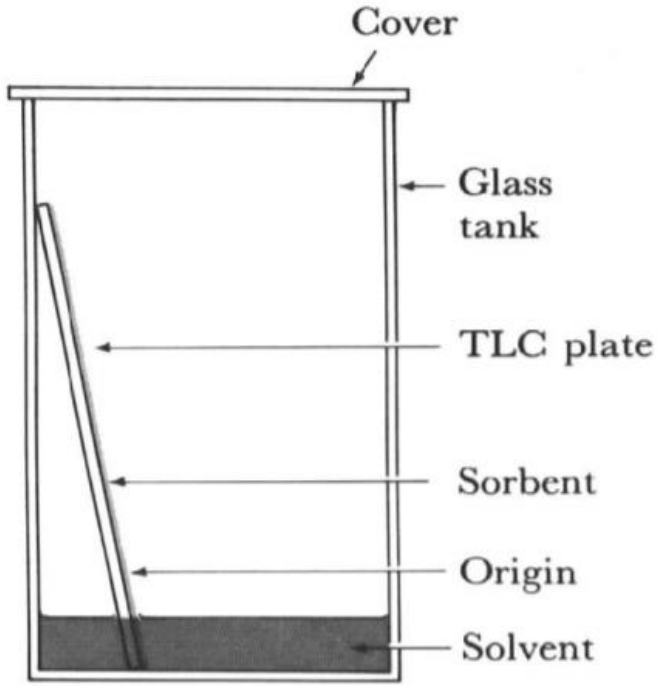


- Draw guide lines
- Prepare sample and references by using appropriate solvents.
- Transfer sample and references on plate, with help of capillary through repeated small touches.
- allowed to dry.

Thin Layer Chromatography

2. Development:

- The plate is placed in a closed vessel containing solvent (the mobile phase) so that the liquid level is below the spot.
- While the mobile phase are rising up the TLC plate by capillary action, the components dissolve in solvent and move through plate.

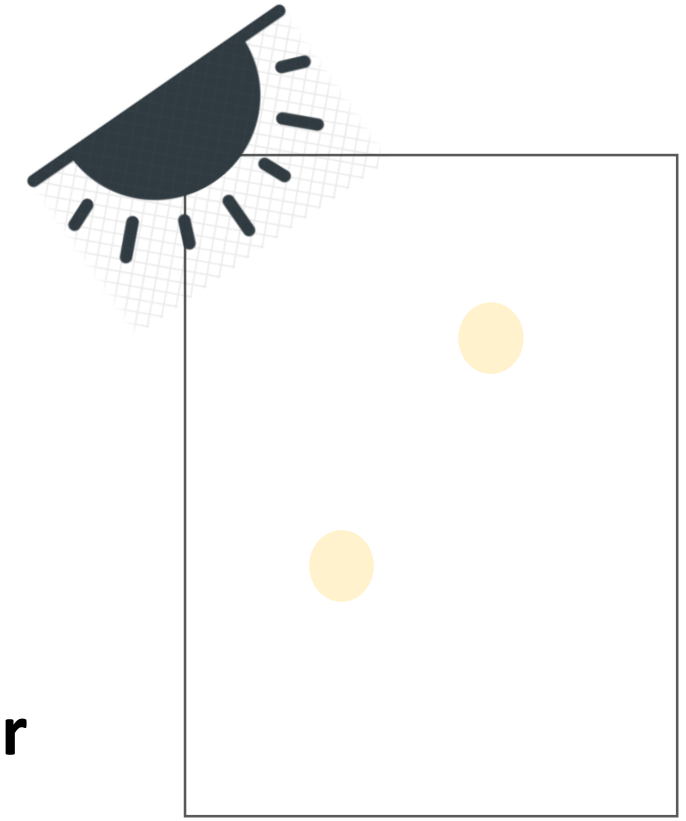


- **Less polar** substance binds weakly to adsorbent material -> elutes **FASTER**
- **More polar** substance binds strongly to the adsorbent material -> elutes **SLOWER**

Thin Layer Chromatography

3. Visualisation

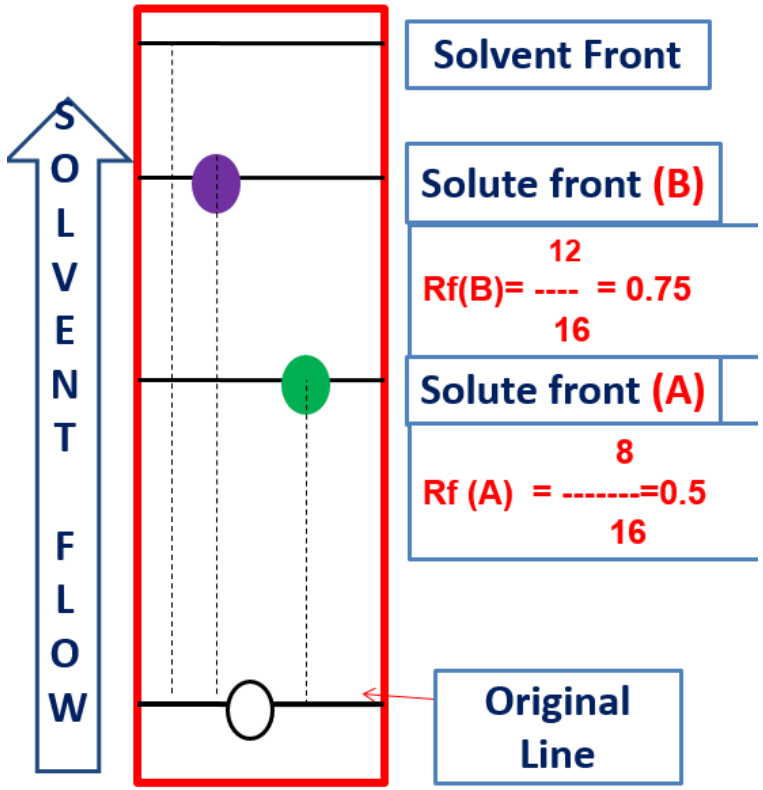
- *If compounds are visible :* the spots can be directly observed after development
- *Stains*



- **the most common visualization technique is to hold the plate under a UV lamp.**

Thin Layer Chromatography

- *Interpretting Outputs*
- Retention Factor (retardation factor)



$$R_f = \frac{Distance\ travelled\ by\ substance}{Distance\ travelled\ by\ solvent}$$

In general, **low polarity compounds have higher Rf values than higher polarity compounds.**

Thin Layer Chromatography

- Can be used to:

➢ Determine the purity of a substance

➢ Analysis fractions collected during purifications

➢ Monitor the progress of a reaction

➢ Identify compounds present in an existing substance

EXPERIMENT

AIM OF EXPERIMENT

- To learn

➢theory of Thin Layer Chromatography

➢Usage areas of TLC

➢ detection unknown compounds in a drug or mixture, by comparison to known reference standards.

➢ interpreting outputs.

EXPERIMETAL METHOD

- Preparing samples:

➢ 1 g of samples and references are dissolved in 20 ml of (1:1) mixture of dichloromethane and ethanol

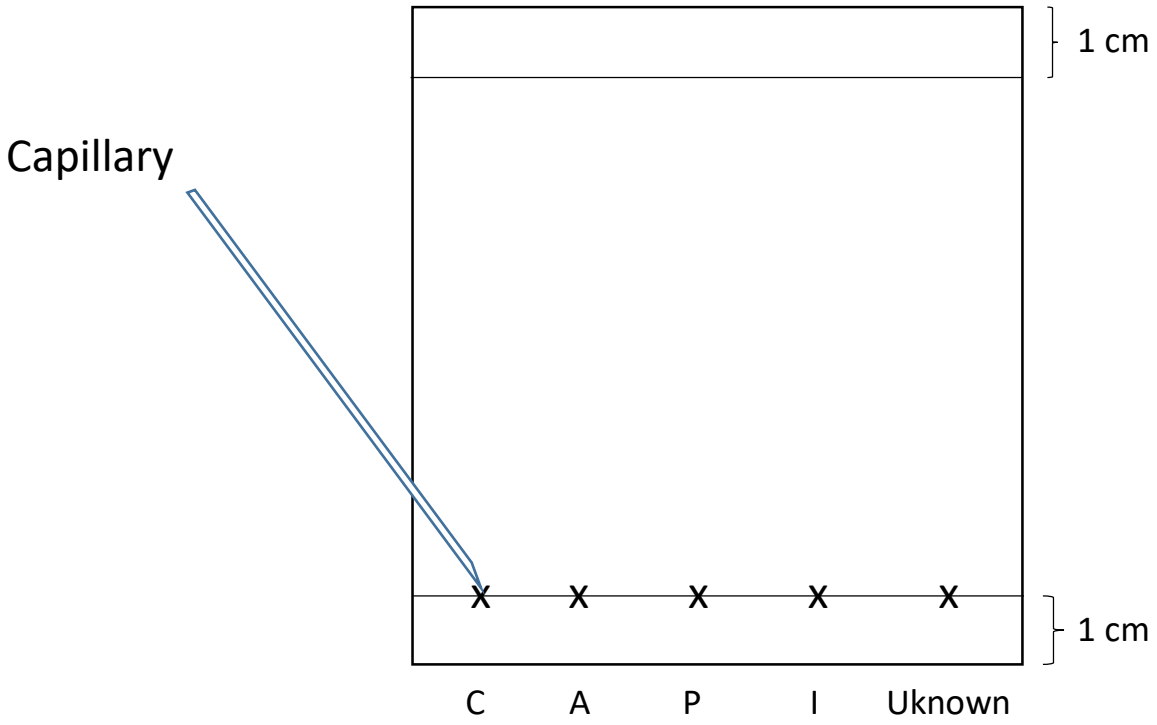
➢ Reference analgesics - Caffeine, Aspirine, Paracetemol, Ibuprofen

- Preparing Development (eluting) solvent:

➢ 0.5% acetic acid in ethyl acetate

EXPERIMETAL METHOD

- Spotting references and sample:



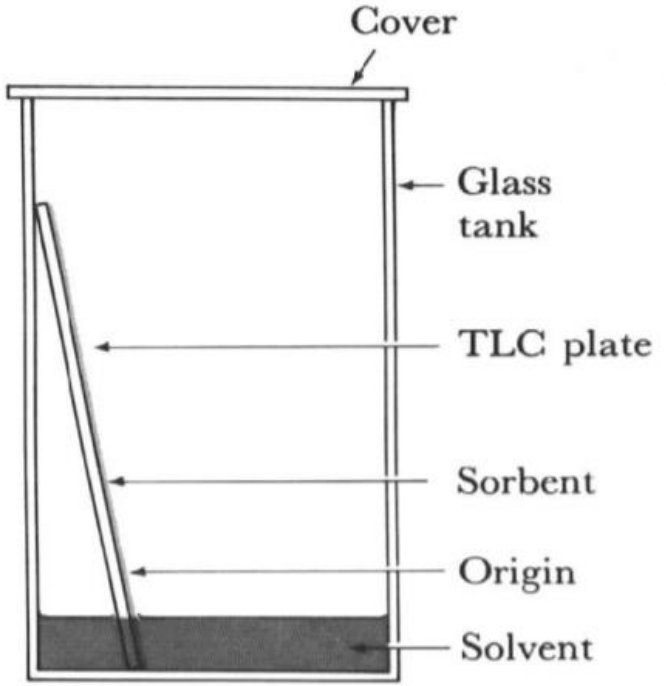
- After drawing guide lines on top and bottom of plate,
- spot the samples on the plate
- Examine whether enough sample applied or not, by using UV lamb

EXPERIMETAL METHOD

- <u>Developing TLC plate:</u>

➢ transfer solvent (eluting or developing solvent) to TLC chamber as a depth of 0.5 cm.

- When the solvent has risen to line at top (solvent front) of TLC plate, remove the plate from the developing chamber
- allow the solvent to evaporate.



EXPERIMETAL METHOD

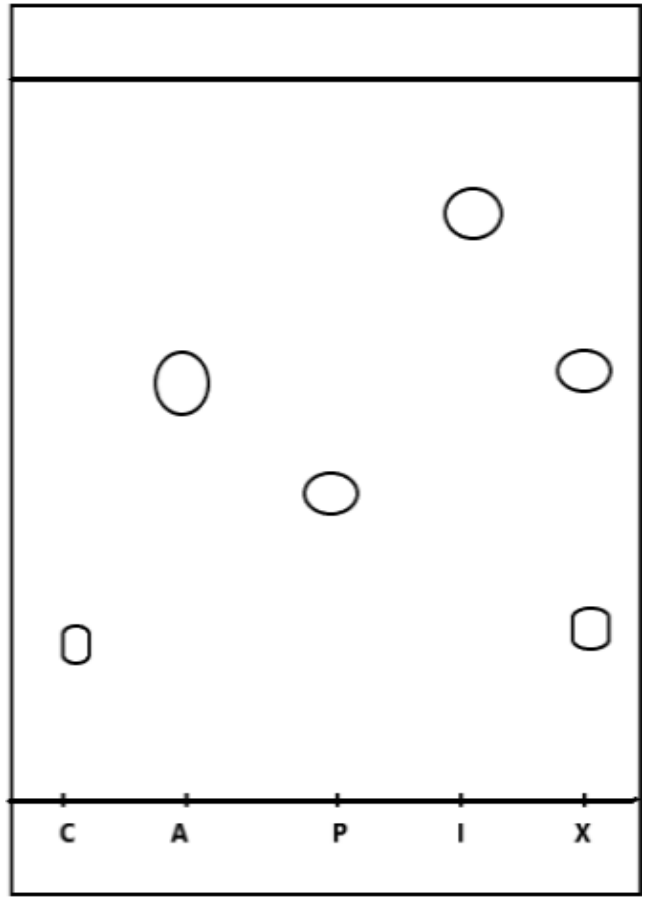
- <u>Visualisation:</u>

➢view plate under a UV lamp, after it is dry.

➢outline spots that you see on the plate.

- <u>Interpreting results:</u>

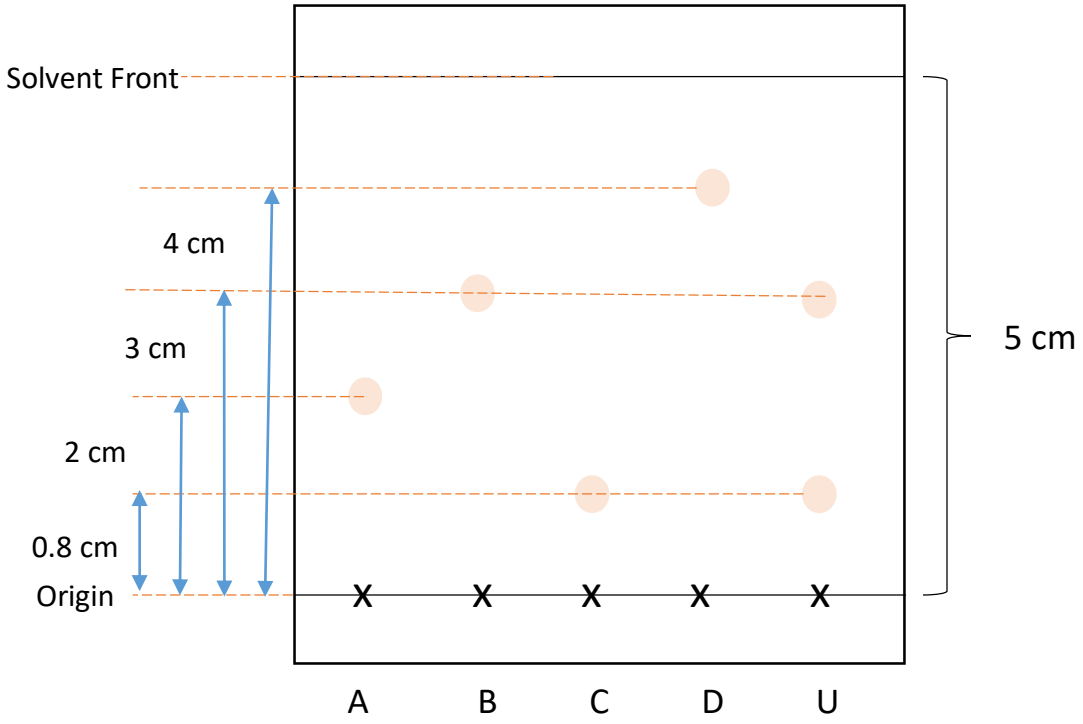
➢ Calculate Rf values of spots and interpret context of unknown sample.



Demonstration

- Demonstration videos can be available via:
- https://www.youtube.com/watch?v=qdmKGskCyh8
- https://www.youtube.com/watch?v=rMGQavOMAmc

Practice



- R_f(A) = 2cm/5cm =0.4
- R_f(B) = 3cm/5cm =0.6
- R_f(C) = 0.8cm/5cm =0.16
- R_f(D) = 4cm/5cm =0.8

➢ Unknown sample:

➢ Substance 1

- R_f(U) = 0.8cm/5cm = 0.16

➢ Substance 2

- R_f(U) = 3cm/5cm = 0.6

Unknown sample consists of compound C and B

REPORT

Should involve:

- Description of the experiment and discussion about the results.
- Factors affect Rf values of substance.
- Calculation of the Rf values.
- Interpretation context of substances

Contact

selcenarii@gmail.com