Experiment 11+12

chromatography

In general:

- The mixture is dissolved in fluid (gas, solvent,....) called mobile phase (eluent), which carries it through a system (column, capillary tube, plate or sheet) called stationary phase (adsorbent) on which the material is fixed.
- The different constituents of the mixture, have different affinities for stationary phase

((What is affinity?? It's the tendency of a chemical species to interact with stationary phase))

In general:

- The separation is based on different partitioning between mobile and stationary phases.

- Chromatography can be used to purify a mixture, to analyze mixture to know concentration of such analyte, to identify the constituents of an unknown and to separate the mixture.

Chromatograpy is a separation technique used in many fields such as medicine, biochemistry, pharmacy,.....

Chromatography is classified into:

- 1-adsorption chromatography
- 2-partition chromatography
- 3- ion exchange chromatography
- 4- size exclusion chromatography

Adsorption Chromatography

- Separation depend on the interaction between the constituents of the analyte and stationary phase (adsorbent)

- • Stationary phase: solid ⇒ $CaCO_3$
- ⇒ alumina $Al_2O_3.xH_2O$

- • Mobile phase (eluent): gas or liquid
- (The choice of eluent depends on polarity and solubility)

- Example: column chromatography

**The strongly adsorbed component moves slowly through the column and vice versa

partition chromatography

- Separation depend on difference of partition coefficient of two solvent.
- Stationary phase: water molecules bound on cellulose paper or silica gel
- Mobile phase: organic solvent

Example: paper chromatography

Size exclusion chromatography

- Separation depends on size of analyte
- Large size molecules emerge first where small size one retained in stationary phase.
- Effective for separation and analysis of a mixture of polymers.

Ion exchange chromatography

- Exchange of ions between analyte and stationary phase such as resin or zeolite.

- Resin is an unreactive polymer with certain functional group can exchange ions

1- Cation exchange resin is polymer with –SO3H,
-OH , - CH2 SO3H

2- Anion exchange resin is polymer with –NR2 ,
- NRH , -NH2

Ion Exchange Chromatography

- Certain natural minerals such as zeolites have ion exchange capacity ???

(They have power for substituting ions in solutions filtered through them by ions found in their structure).

from this point → Synthetic ion exchange resins → made of polystyrene OR cellulose

Ion Exchange Chromatography

- illustrative example for the exchange process:

Ion exchange capacity of the resin =

$$\frac{\text{mmoles of counter ions}}{\text{mass in grams of dry resin}}$$

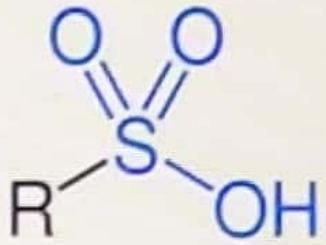
Ion Exchange Chromatography

1- cationic ion exchange:

$Resin\text{-}H^+ + M^+ \rightarrow Resin\text{-}M^+ + H^+$

In the solution — in the eluting solution

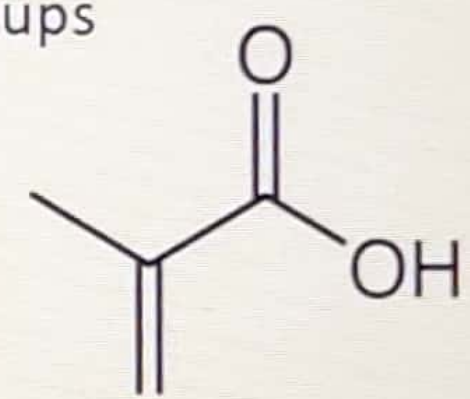
(Acidic functional group)



A- strong cationic exchanger

high rate of exchange

ex.: resins substituted with sulfonic acid groups



B- weak cationic exchanger

low rate of exchange

ex: resins cross linked with methacrylic acid groups

Ion Exchange Chromatography

1- anionic ion exchange:

$Resin\text{-}OH^- + A^- \rightarrow Resin\text{-}A^- + OH^-$

In the solution — in the eluting solution

(Basic functional group)

A- strong anionic exchanger

high rate of exchange

ex.: resins prepared with R_3-N groups

B- weak anionic exchanger

low rate of exchange

ex: resins prepared with secondary or primary amine groups

- In today experiment, stationary phase is cation exchange resin and mobile phase is distilled water

$$R\text{-}SO_3H + M^{n+} \underset{\text{regeneration}}{\overset{\text{exchange}}{\rightleftharpoons}} (R\text{-}SO_3)_nM + nH^+$$

$$R\text{-}SO_3H + Ca^{2+} \underset{\text{regeneration}}{\overset{\text{exchange}}{\rightleftharpoons}} (R\text{-}SO_3)_2Ca + 2H^+$$

Steps:

- Cover the resin with 6M HCl to activate resin thus enlarge surface area and efficiency of exchanger
- put the resin in a column (buret) as a slurry rather than dry packing to increase surface area and to increase efficiency of exchange

Steps

- wash by distilled water to remove excess H+ of the regeneration
- make test by methyl orange. It should be neutral color of indicator (yellow)
- add 10.0 ml unknown gradually and alternatively with distilled water
- collect effluents in flask by washing with distilled water to 200 ml to collect H+ of the exchange
- make test by methyl orange. It should be neutral color of indicator (yellow)
- titrate effluent against NaOH as titrant using Phenolphthalein as indicator.

Calculations

- (M x V) NaOH = number of moles OH^- = number of moles H^+
- Number of moles Ca^{2+} = number of moles H^+/ 2
- M =number of moles Ca^{2+} / volume ;

(volume = 10 x 10-3)

- ppm Ca2+ = M x Mw x 1000
- Capacity resin= number of moles Ca^{2+} / mass of resin

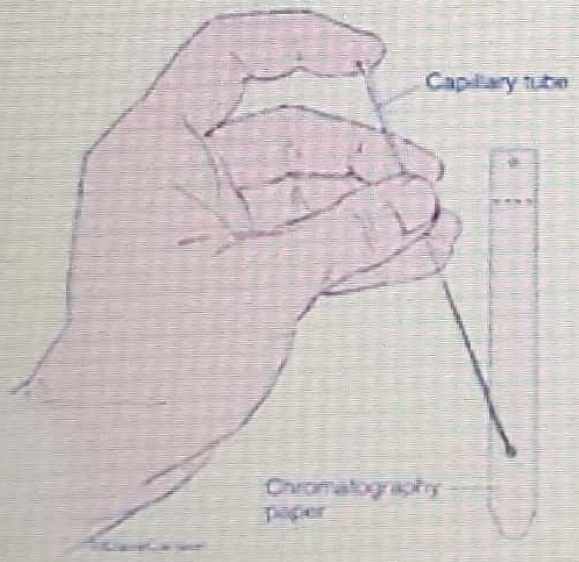
Paper Chromatography

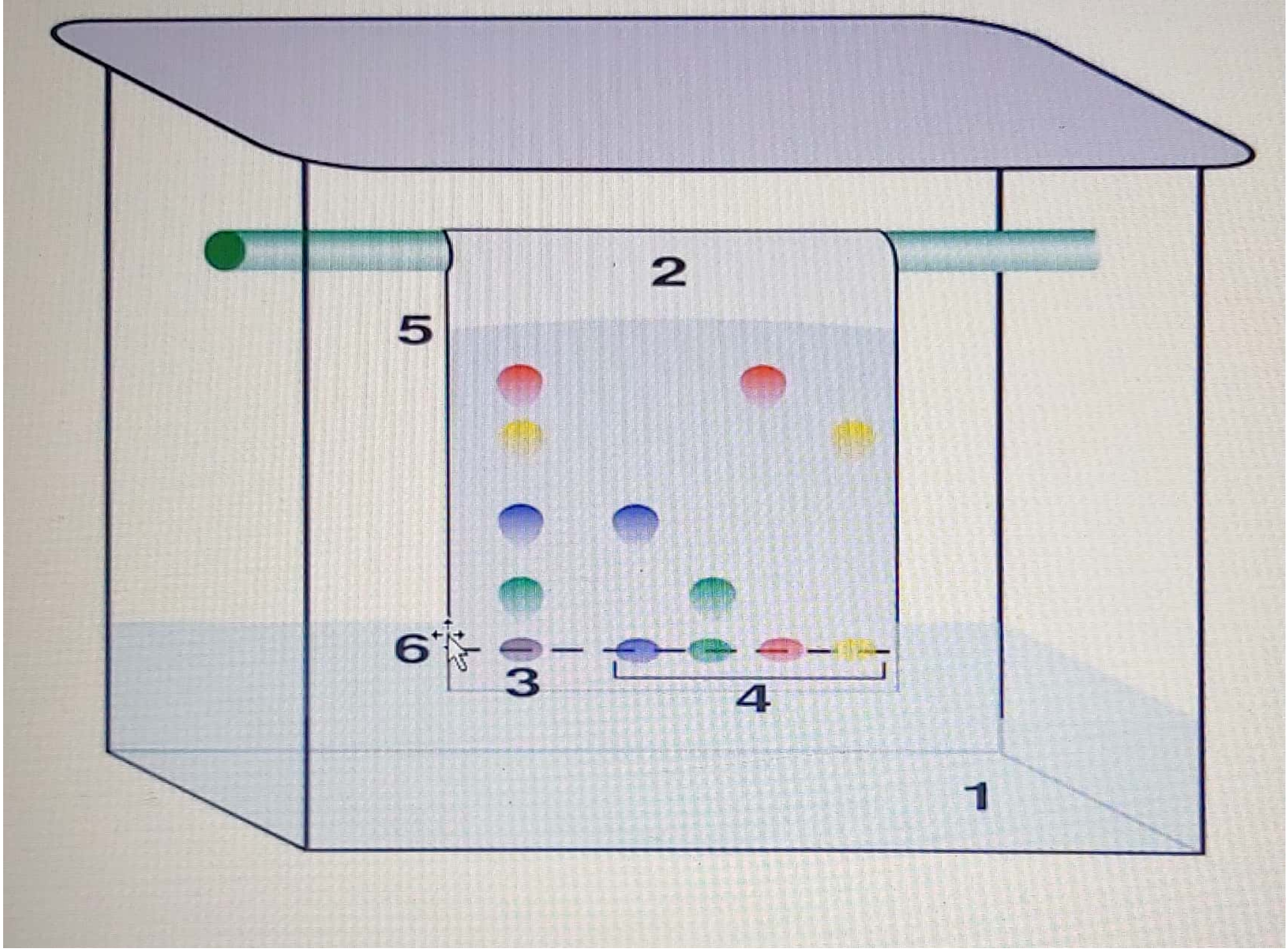
- Stationary phase: water in cellulose or silica gel
- Mobile phase : organic solvent

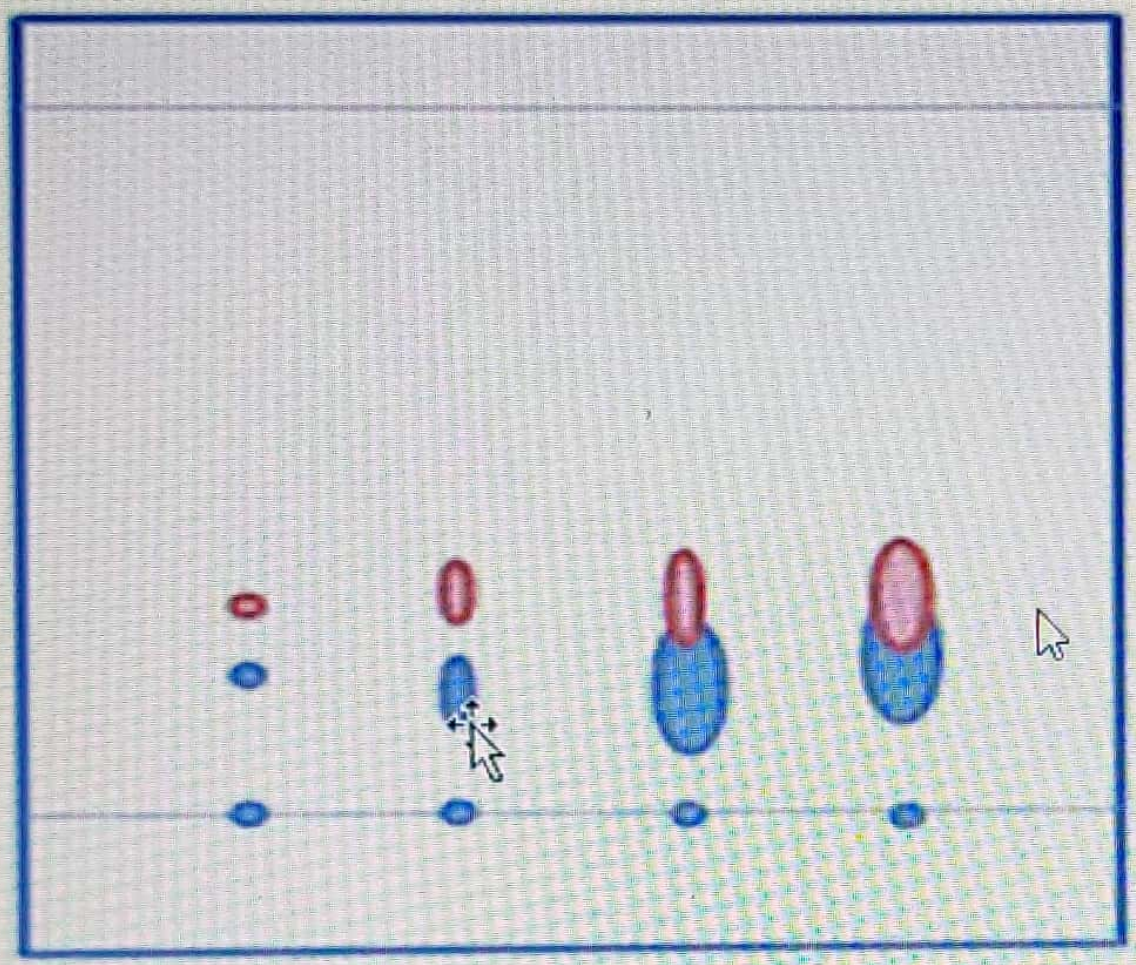
Steps:

1- Sampling: spotting mixture on the paper by capillary tube resulting in a chromatogram

2- Developing: immersing paper in mobile phase

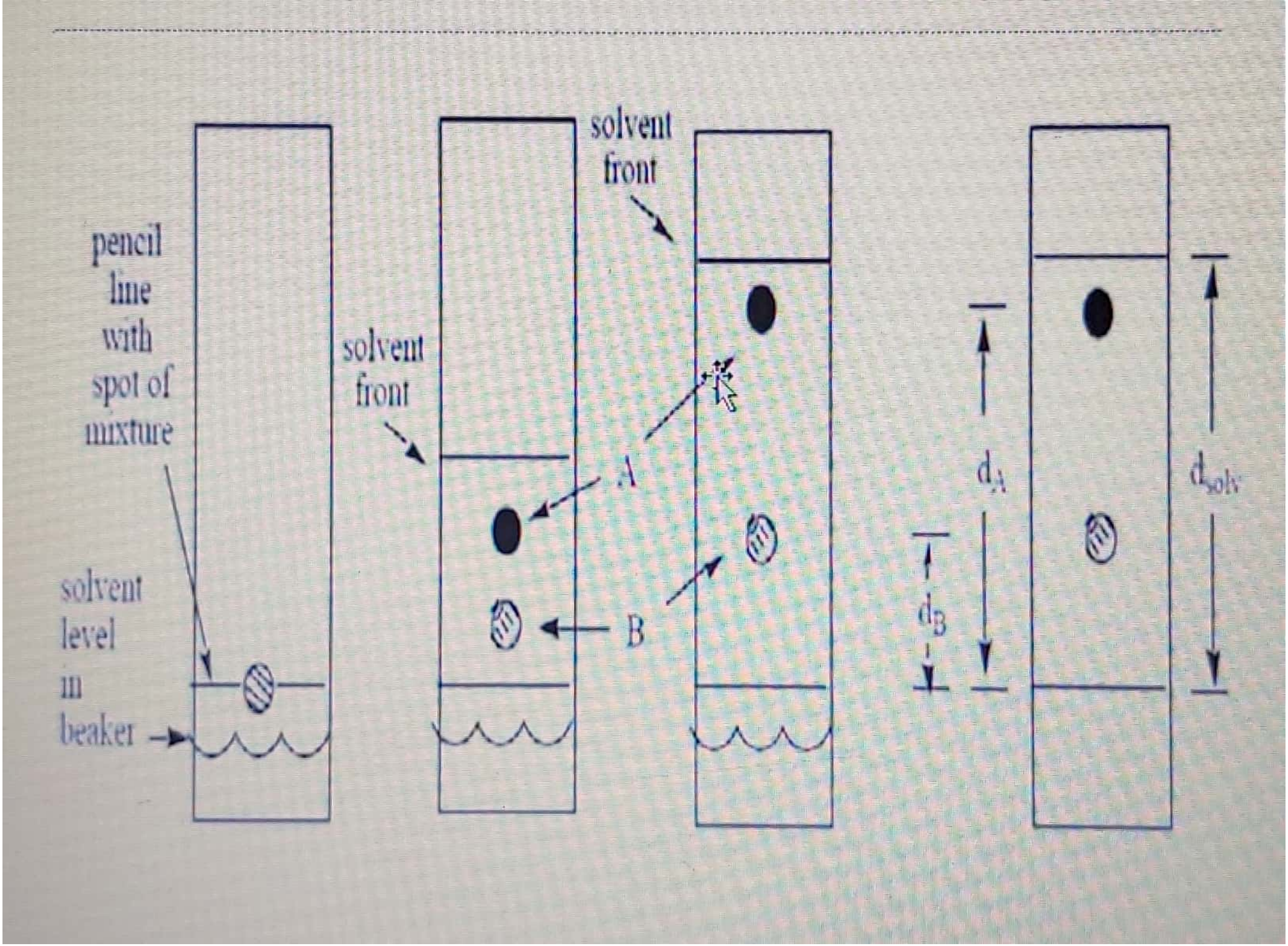






1 2 3 4

Ideal shape → Bad tailing



Characterization of a compound

- Using retardation factor (Rf).
- (Rf) = distance travelled by substance
- distance travelled by solvent
- Note:
- • The analyte with the largest Rf value is less polar because it interacts weakly with stationary phase (it's polarity similar to mobile phase)
- • The analyte with the lowest Rf value is more polar because it interacts strongly with stationary phase (it's polarity similar to stationary phase)

Notes:

- Rf = 0 → solute remains in stationary phase
- Rf = 1 → no affinity of solute in the stationary phase thus it travels with the eluent

- spots of analytes on cellulose paper must be small and far from each other to avoid tailing and overlapping