# SEPARATION TECHNIQUES: CHROMATOGRAPHY

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Chromatography

• Is a separation technique used to separate different components in a liquid mixture.

• All chromatography uses two phases to separate a mixture. These are the:

mobile phase, which is a solvent, that moves over or through the stationary phase

Chromatography principle

- it is based on the **difference in the movement** of individual components of a mixture through the **stationary phase (stable)** under the influence of **mobile phase (moving)**. the various components of the mixture travels at different speeds, causing them to seperate.
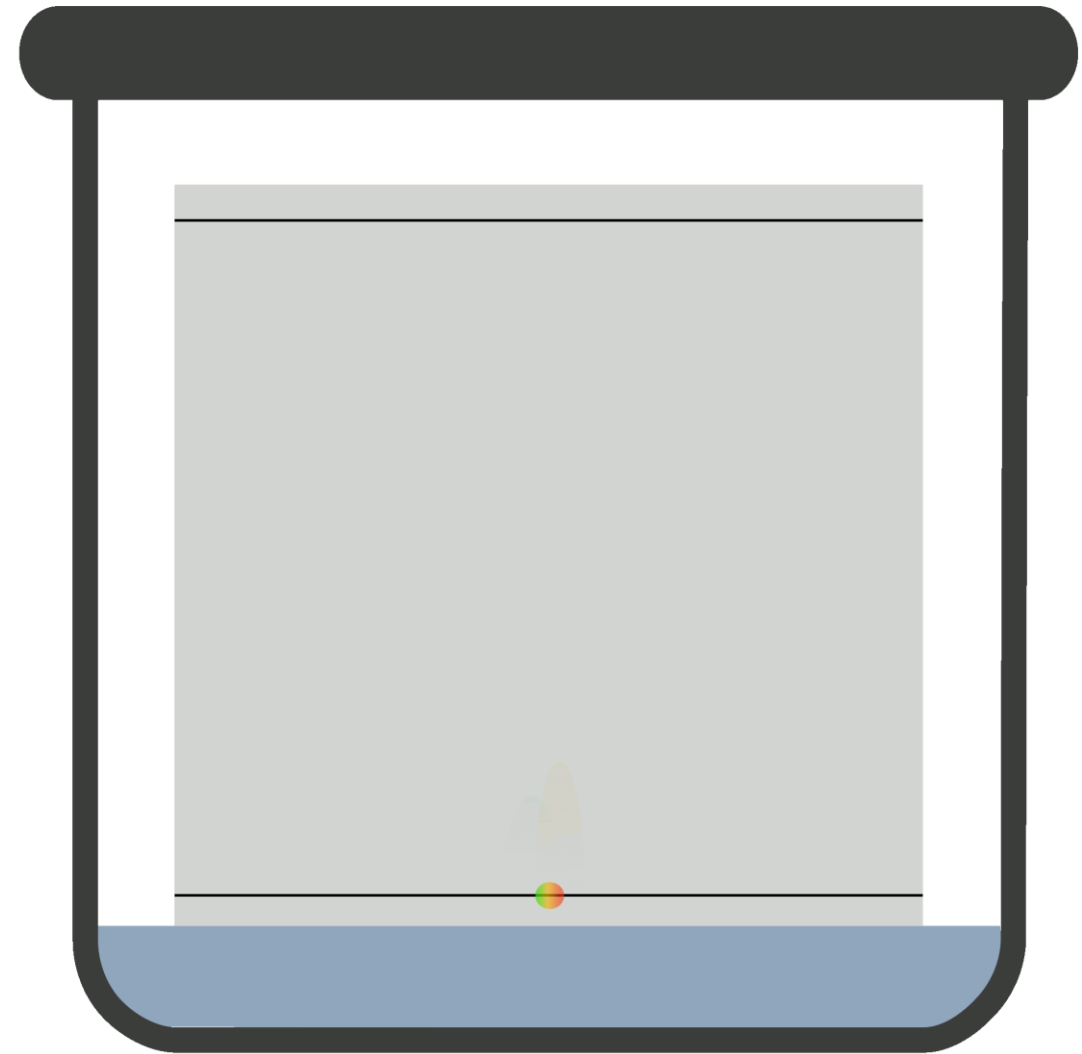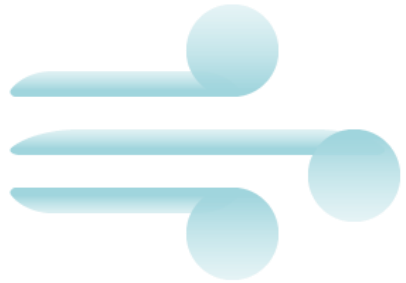
Why do mixture components travels at different speeds?
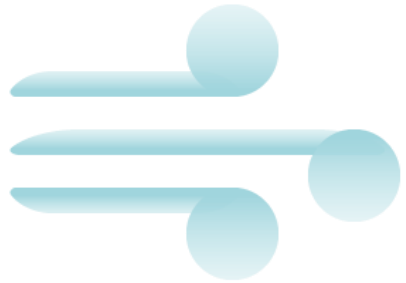
Why do mixture components travels at different speeds?

# Why do mixture components travels at different speeds?

Factors effecting chromatography separation

1- Affinity of the sample to the mobile phase and stationary phase: the sample components generally are more attracted to the mobile phase or stationary phase that share similar properties with them, like polarity, charge.

2-molecular weight or size of the sample components: if we have a mixture of components with same affinity properties to the mobile phase or stationary phase, then the smaller size or weight molecules will elute faster.

Which component has the lowest molecular weight? Blue, red or yellow?

Chromatography paper

Solvent rises

Yellow

Chromatography paper

Solvent rises
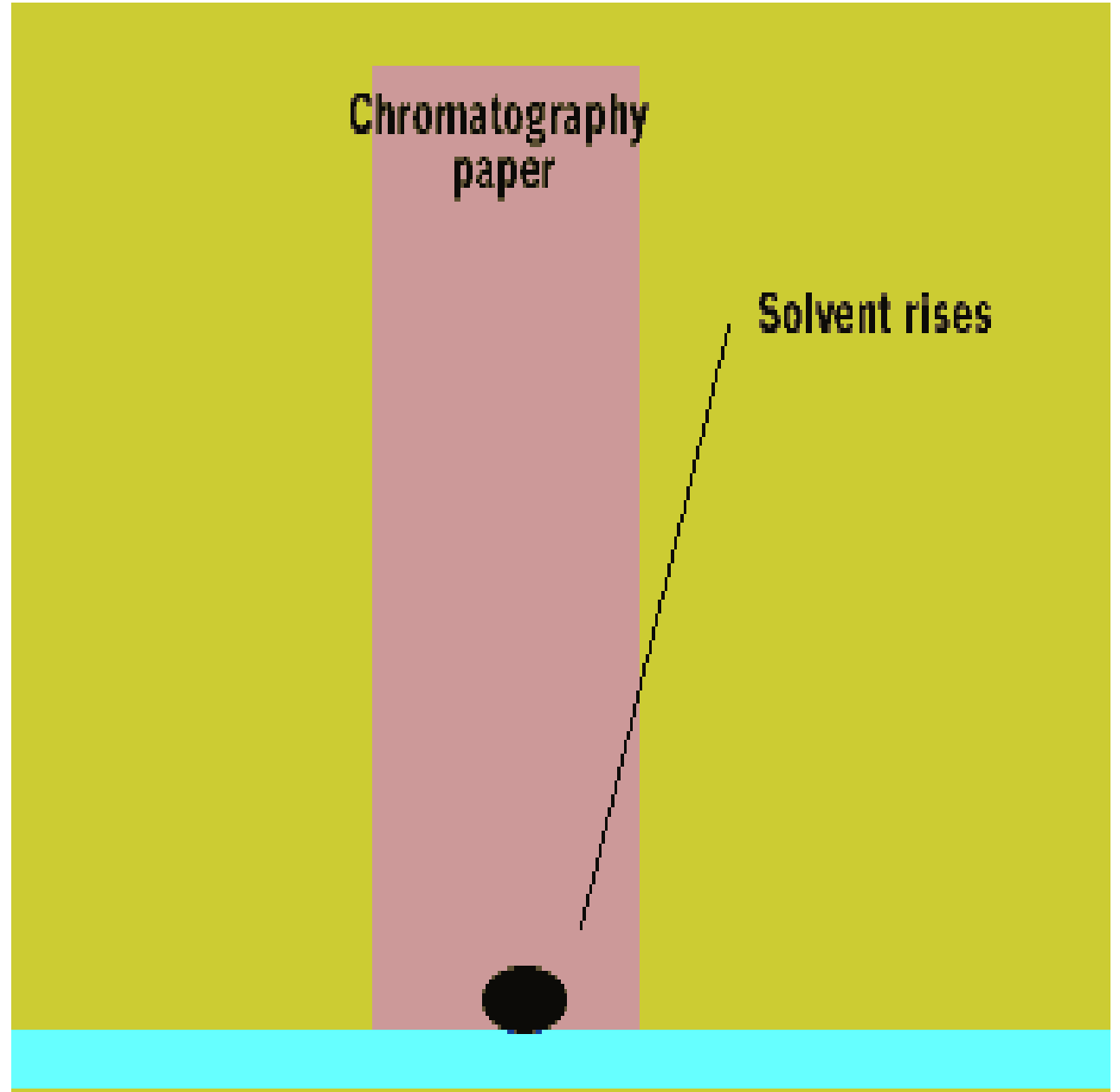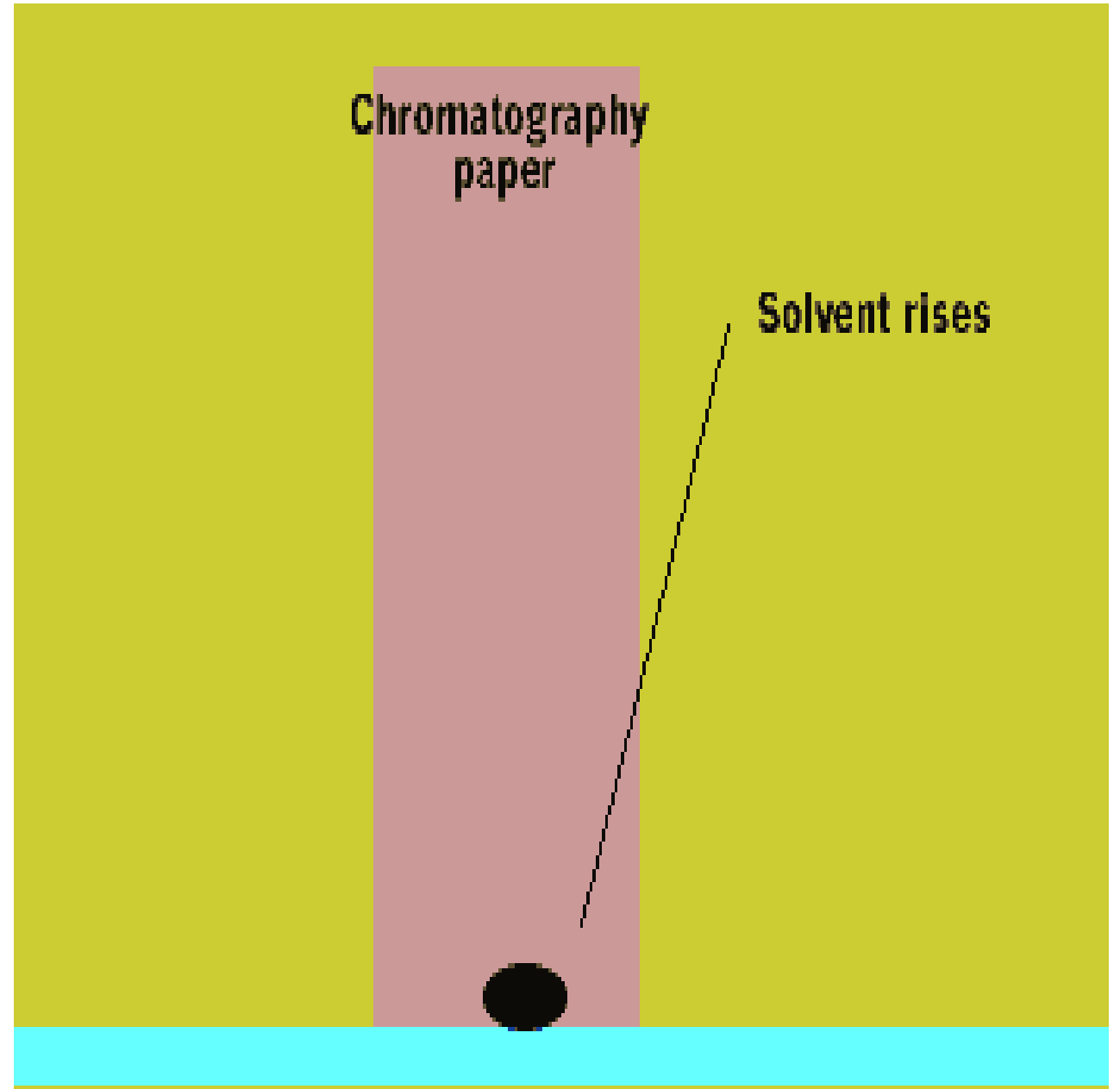
Paper chromatography

- **Mobile phase:** liquid solution

- **Stationary phase** : cellulosic (paper)

- **Interaction type between the mobile and stationary phase:** partitioning

- The sample is placed as a spot on the paper, and the paper is carefully dipped into a solvent. The solvent rises up the paper due to capillary action and the components of the mixture rise up at different rates.
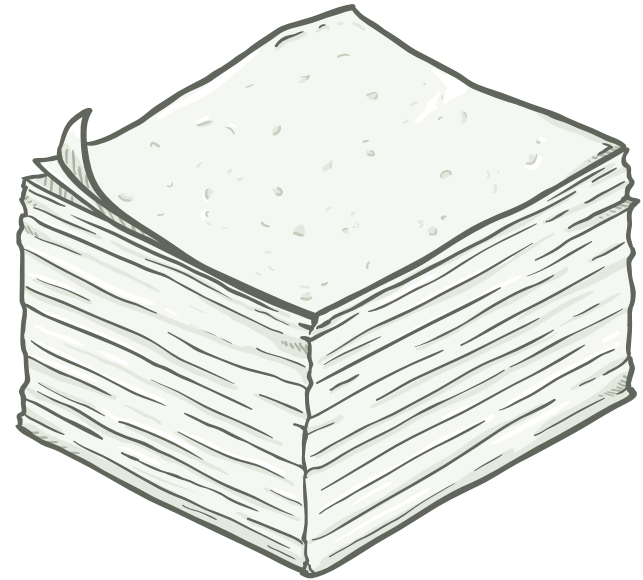
Thin Layer Chromatography

- **Mobile phase:** liquid solution
- **Stationary phase:** <u>thin layer</u> of silica or alumina or cellulose supported by a piece of glass or aluminum.
- **Interaction type between the mobile and stationary phase:** partitioning
- It is faster than paper chromatography, and the spots could be visualized by acids for example, which would destroy the paper chromatography.
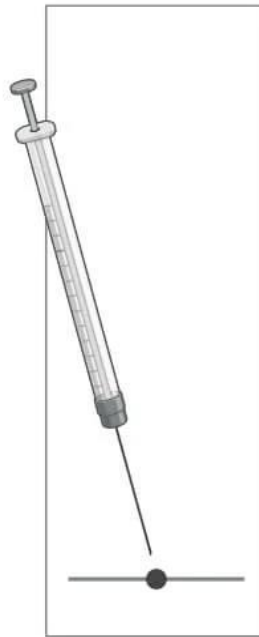
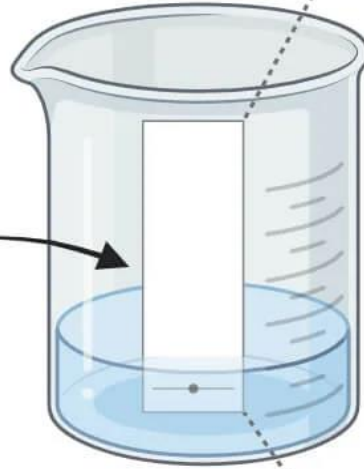# Thin Layer Chromatography

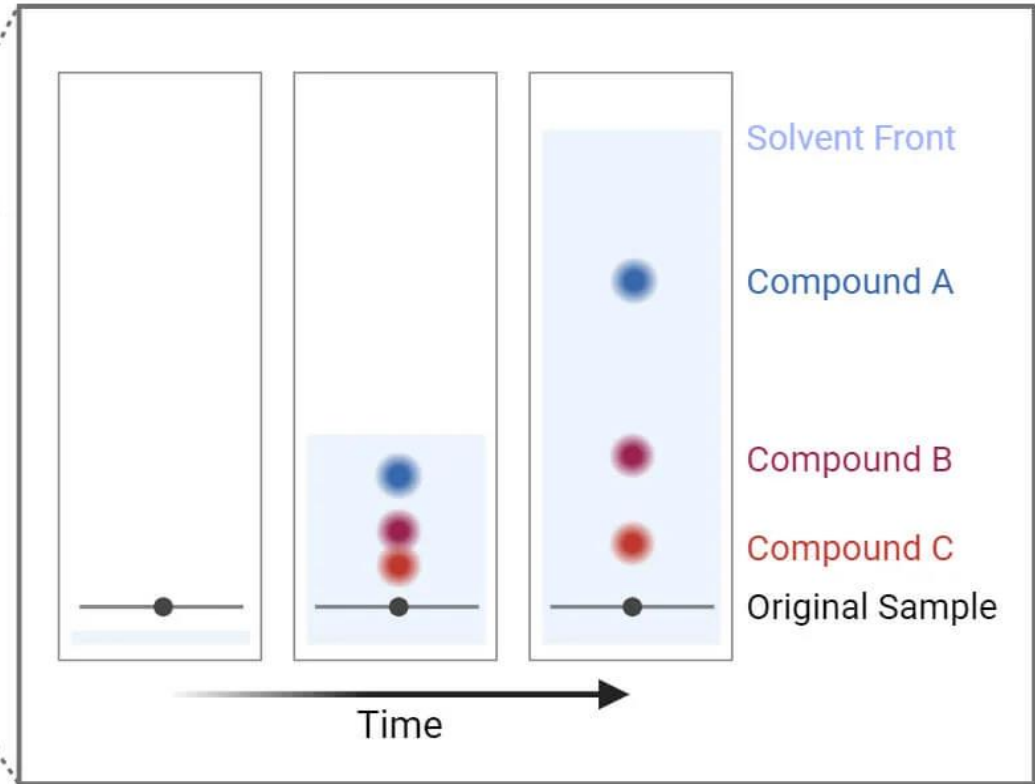**Stationary Phase**
Sample spotted on TLC plate

Mobile Phase
Compounds separated based on their polarity over time

Solvent Front

Compound A

Compound B

Compound C

Original Sample

TLC Plate

TLC Chamber

Time

Rf : retardation factor

- Each atom, iOn or mOlecule has a unique Rf
- All Rf values must be expressed as a decimal and cannot be greater than or equal to One.
- An Rf of "**1**" indicates that the substance has not separated frOm the mobile phase and therefore cannot be identified…(what to do??)
- ✓ The analysis must be run again under different mobile or statiOnary phase conditiOns.

DISTANCE OF SOLVENT FRONT

DISTANCE TRAVELLED BY THE DYE
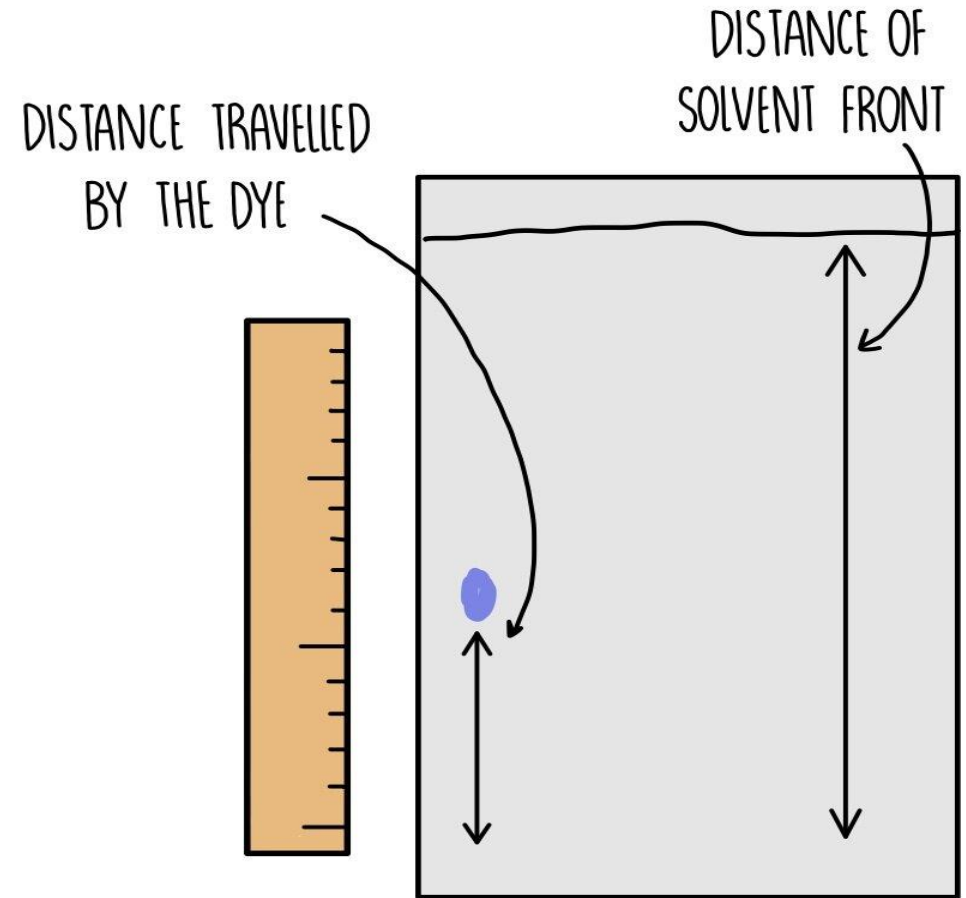
$$R_f \text{ VALUE} = \frac{\text{DISTANCE TRAVELLED BY THE DYE}}{\text{DISTANCE TRAVELLED BY THE SOLVENT}}$$

Procedure

1- Use pencil (not a pen…why??) to draw base line and top line (1 cm from each end of the plate)

2- Put spot of the sample (quercetin flavonoid) and <u>let it dry completely </u>(why??).

3- Put the mobile phase within the jar and <u>cover it </u>(why??)(mobile phase within this lab. is Chloroform: Methanol 95:5)

4- Put the plate inside jar that contain mobile phase and re-cover it. The mobile phase should be <u>below the line drawn at the bottom </u>(why??). Let the mobile phase separate sample until reach to the end line which you drew. Let the TLC dry.

5- Visualize spot by using iodine then calculate Rf.

Important notes

- Note 2: its also used in identification and purification.

- Note 1: chromatography Applications: To separate colors in a dye. To separate pigments from natural colors. To separate drugs from blood.

- Note 3: Rf value represent the distance traveled by sample divided by the distance traveled by solvent, and its useful for the identification of separated compounds.

Are there any Questions?

Yes ?

Use Google