सत्यमेव जयते

GOVERNMENT OF MAHARASHTRA

# RAJARAM COLLEGE

## VIDYANAGAR, KOLHAPUR – 416 004 (MS)

B. Sc. Part-I, Semester-II

# CHROMATOGRAPHY


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Assistant Professor

Department of Chemistry


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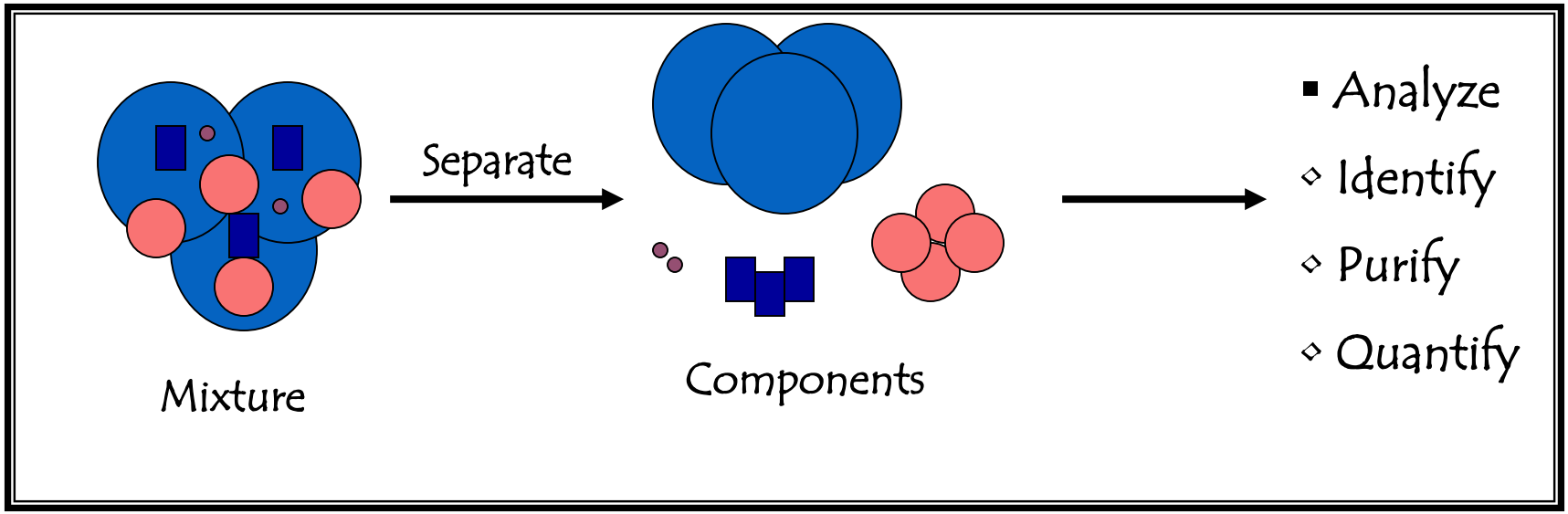
# CHROMATOGRAPHY

**It is a technique used to separate and identify the components of a mixture.**

**Chromatography** is a combination of two words:

* **Chromo –** Meaning **color**
* **Graphy** – Representation of something on paper



# HISTORY

## Mikhail Tswett, Russian Botanist

In 1906, Tswett used to chromatography to separate plant pigments.

He called the new technique chromatography because the result of the analysis was 'written in color' along the length of the adsorbent column.

Chroma means “**color**” and

Graphy means to “write”

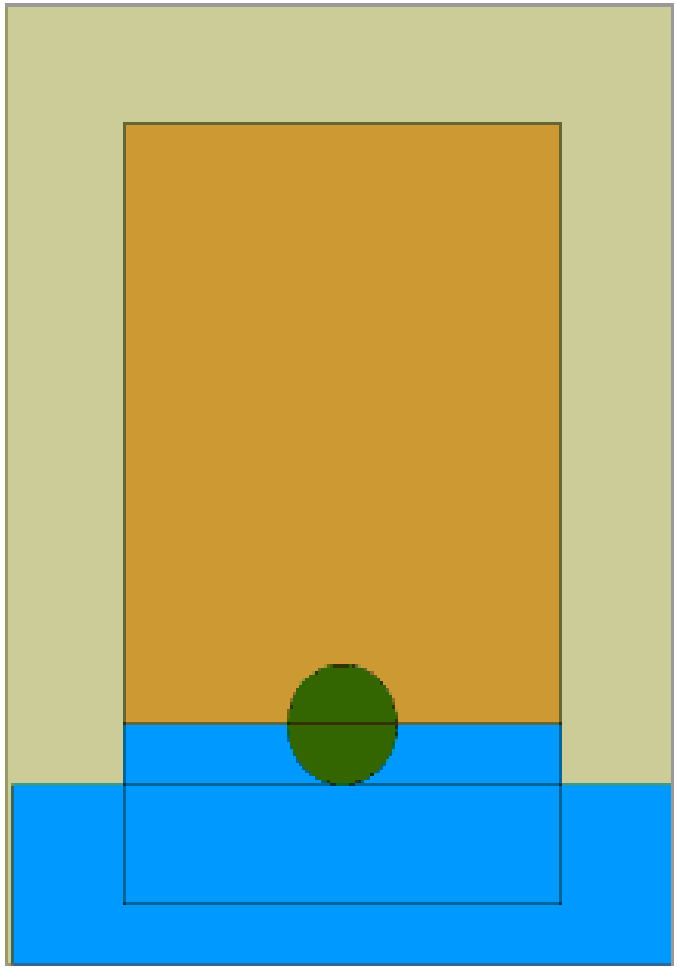
# DEFINATION

*It is a physical separation method of separation in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction.*

# COMPONENTS OF CHROMATOGRAPHY

Mobile Phase – gas or liquid that carries the mixture of components through the stationary phase.

Stationary Phase – the part of the apparatus that holds the components as they move through it, separating them.



# TERMS USED IN CHROMATOGRAPHY

Analyte – It is the substance to be separated during chromatography.

Chromatogram – Visual output of the chromatography.

Detector – It is the part of the chromatographic instrument used for detection of analyte.

# TYPES OF CHROMATOGRAPHY

## A] Based on phases:

I. Solid - Liquid
II. Solid - Gas
III. Liquid-Liquid
IV. Liquid - Gas

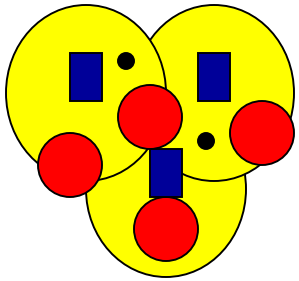
## B] Based on shape of chromatography bed:

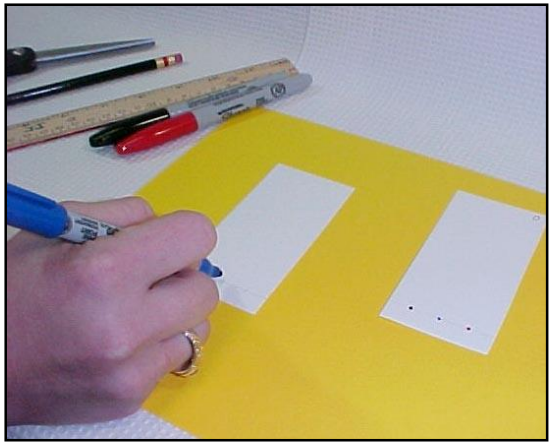
I. Planar Chromatography: e.g. Paper, TLC
II. Column Chromatography

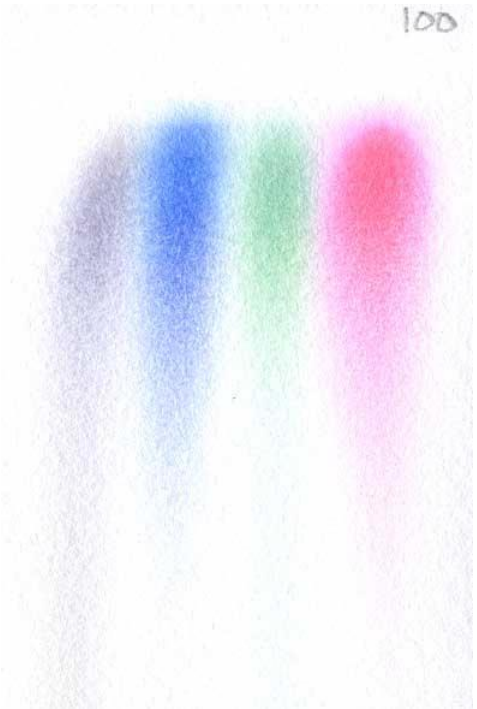
## C] Based on mechanism

I. Adsorption chromatography
II. Partition chromatography
III. Ion-exchange chromatography
IV. Gel-Permeation chromatography

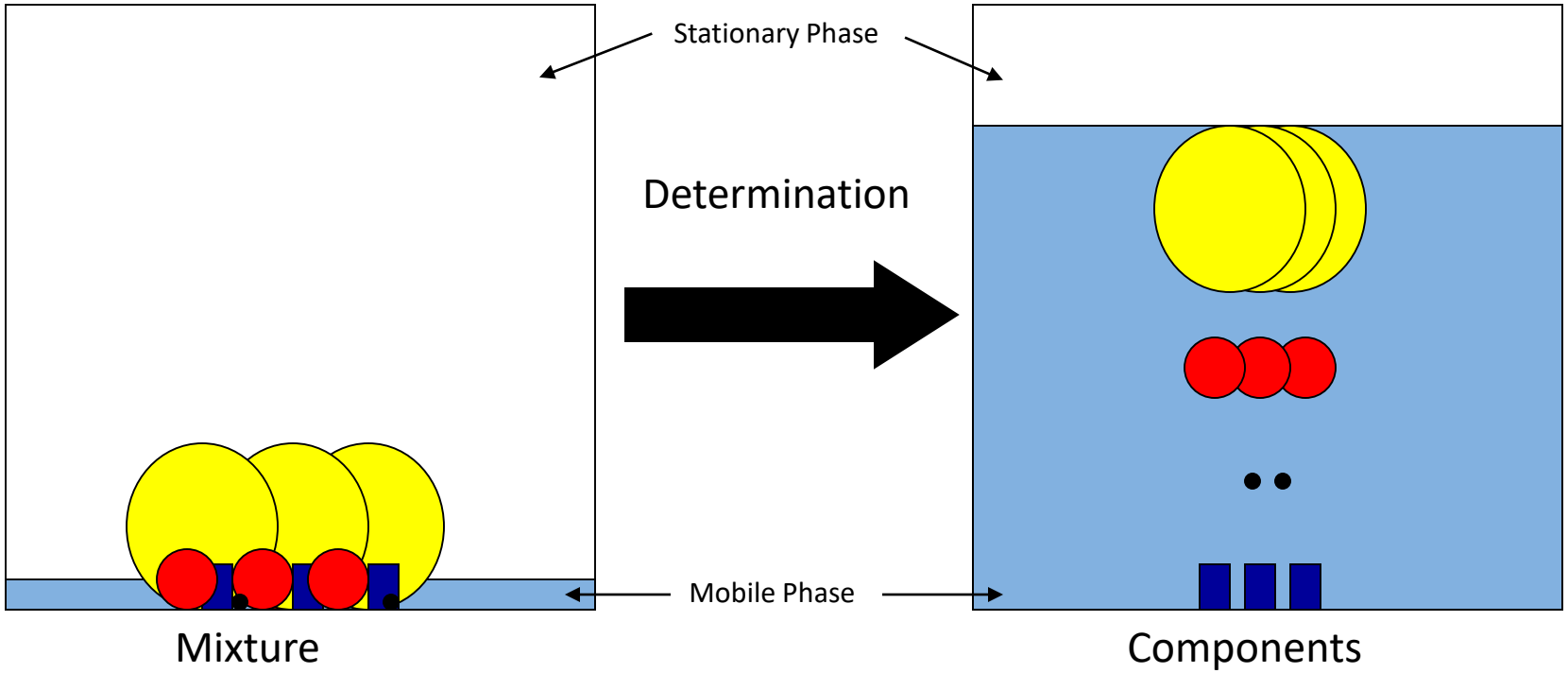
# Paper Chromatography







# ILLUSTRATION OF CHROMATOGRAPHY



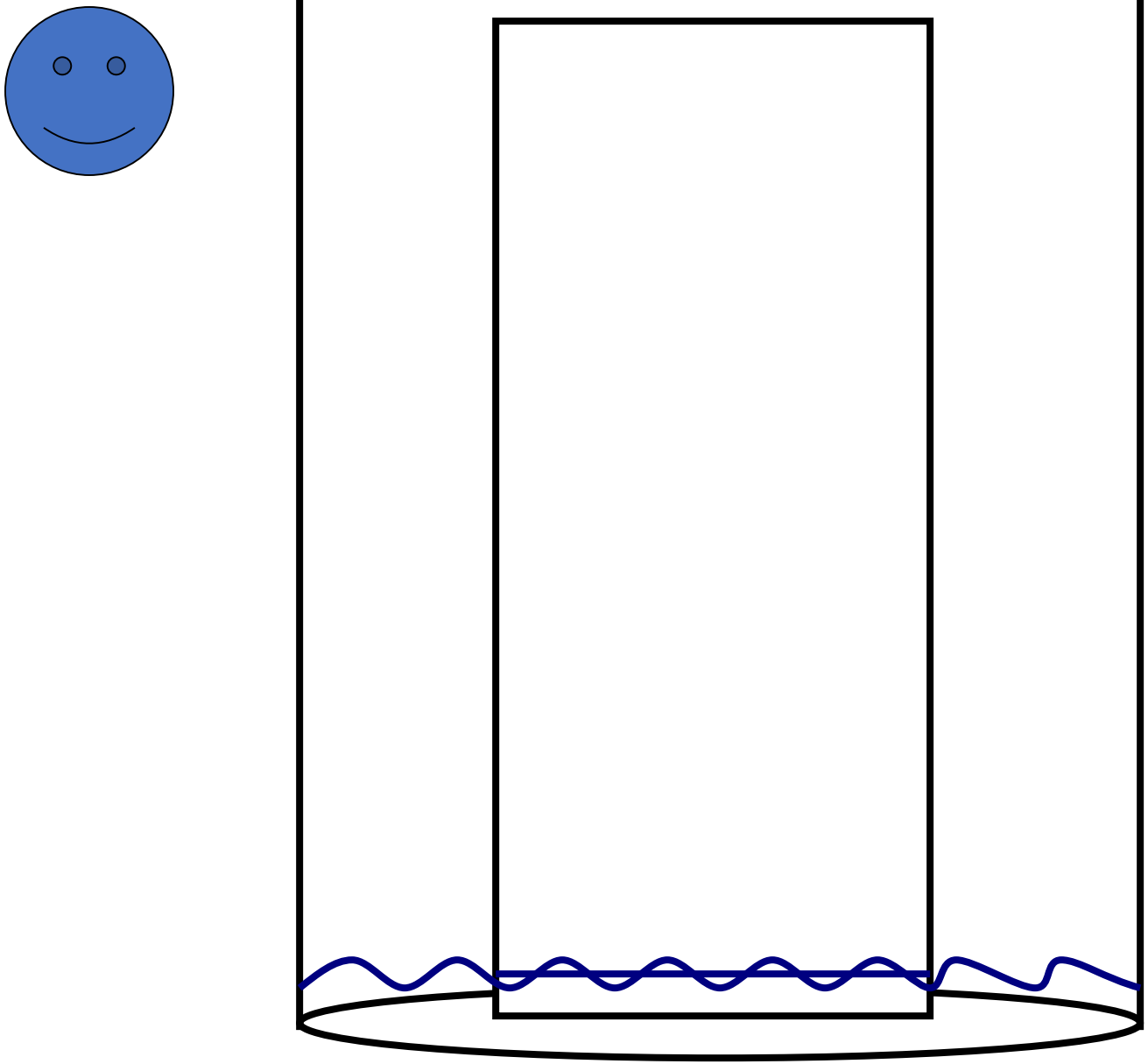

| Components | Affinity to Stationary Phase | Affinity to Mobile Phase |
|---|---|---|
| Blue | ---------- | Insoluble in Mobile Phase |
| Black | ✓ ✓ ✓ ✓ ✓ ✓ | ✓ ✓ |
| Red | ✓ ✓ | ✓ ✓ ✓ ✓ ✓ |
| Yellow | ✓ | ✓✓✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ |

# PRINCIPLES OF PAPER CHROMATOGRAPHY

- **Capillary Action** – the movement of liquid within the spaces of a porous material due to the forces of adhesion, cohesion, and surface tension. The liquid is able to move up the filter paper because its attraction to itself is stronger than the force of gravity.

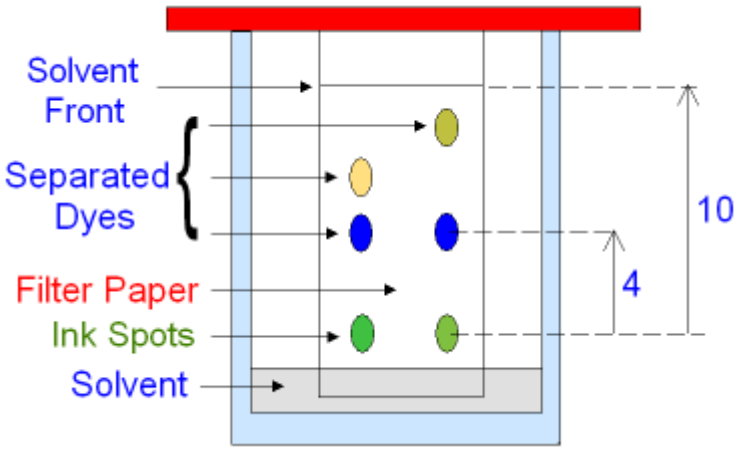
- **Solubility** – the degree to which a material (solute) dissolves into a solvent. Solutes dissolve into solvents that have similar properties. (Like dissolves like) This allows different solutes to be separated by different combinations of solvents.

Separation of components depends on both their solubility in the mobile phase and their differential affinity to the mobile phase and the stationary phase.



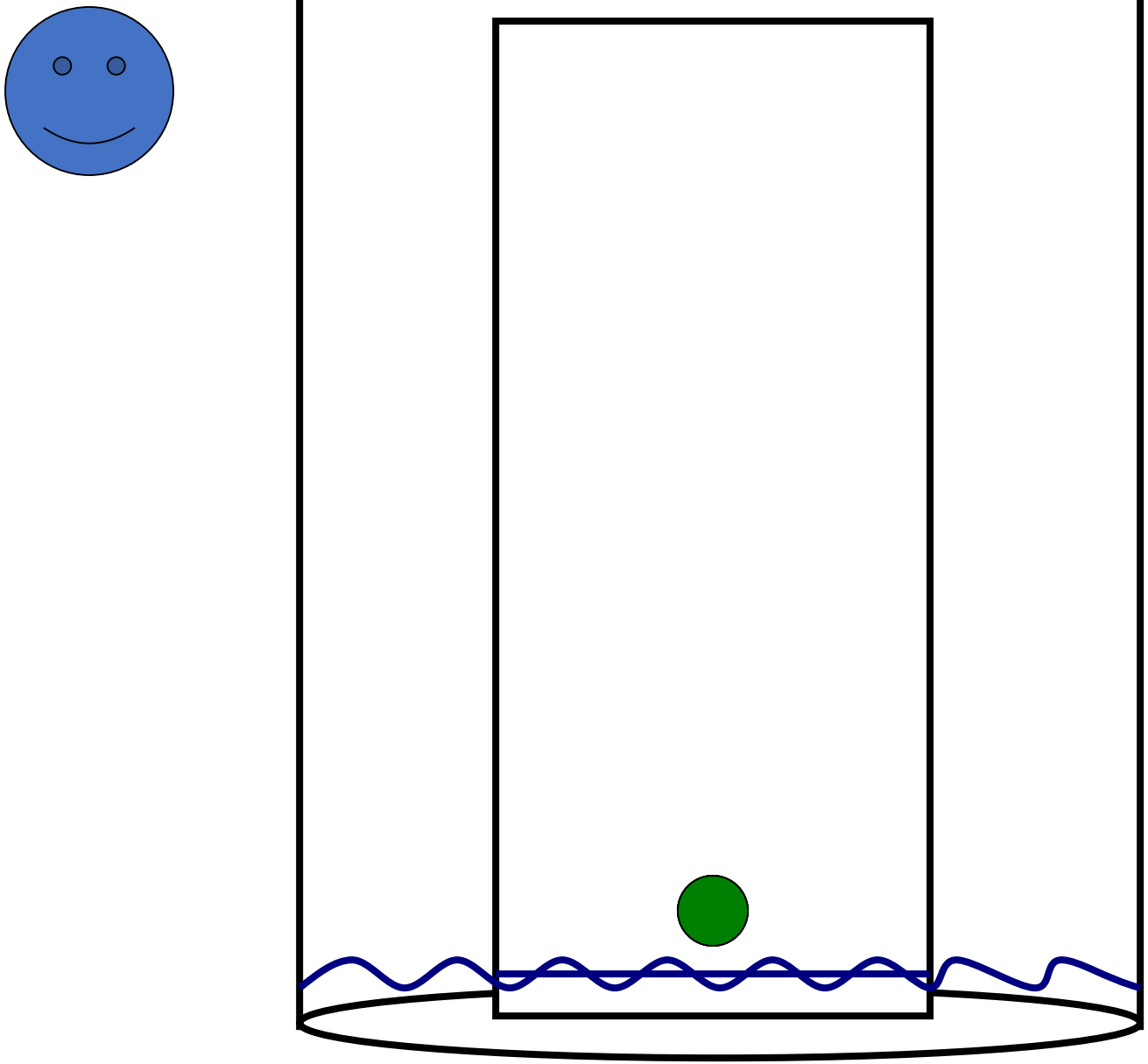
# PRINCIPLES OF PAPER CHROMATOGRAPHY

## Separation of Ink components




**Activity**:

- Ink is placed on filter paper
- Solvent(mobile phase) is soaked up & passes through the filter paper
- Mobile phase carries the ink components and causes separation based on the difference in migration rates.

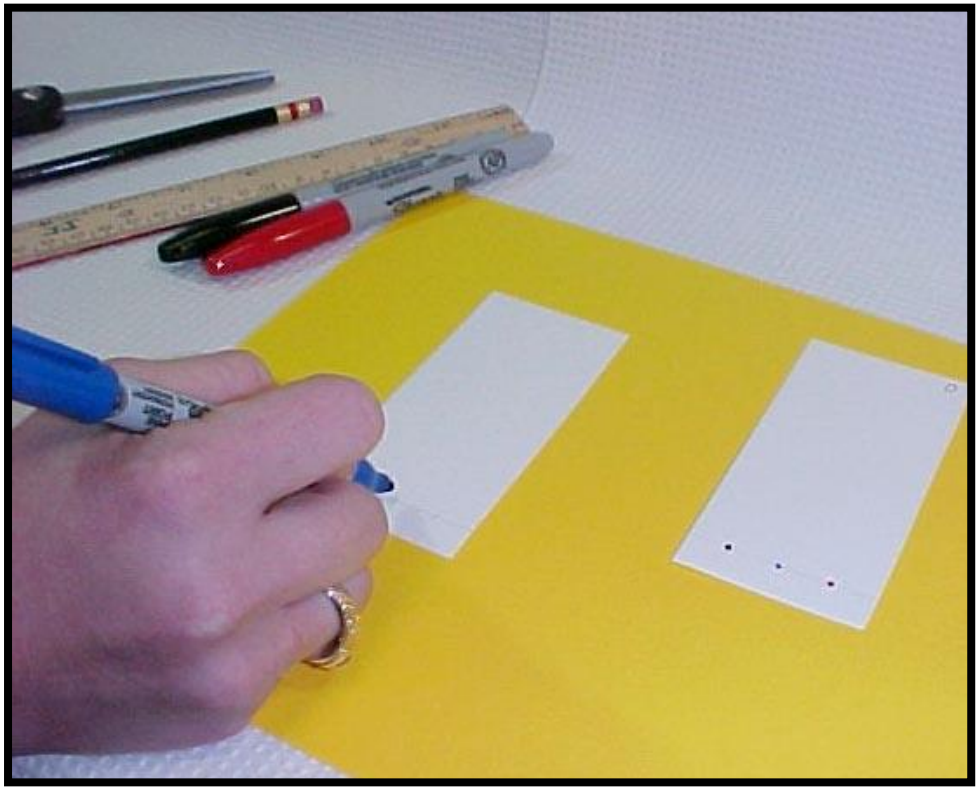


# MATERIALS LIST

- 1 beaker or jar
- 1 cover or lid
- Distilled $H_2O$
- Isopropanol
- Graduated cylinder
- strips of filter paper
- Pencil
- Ruler
- Scissors
- Tape

# PREPARING THE CHROMATOGRAPHY STRIPS

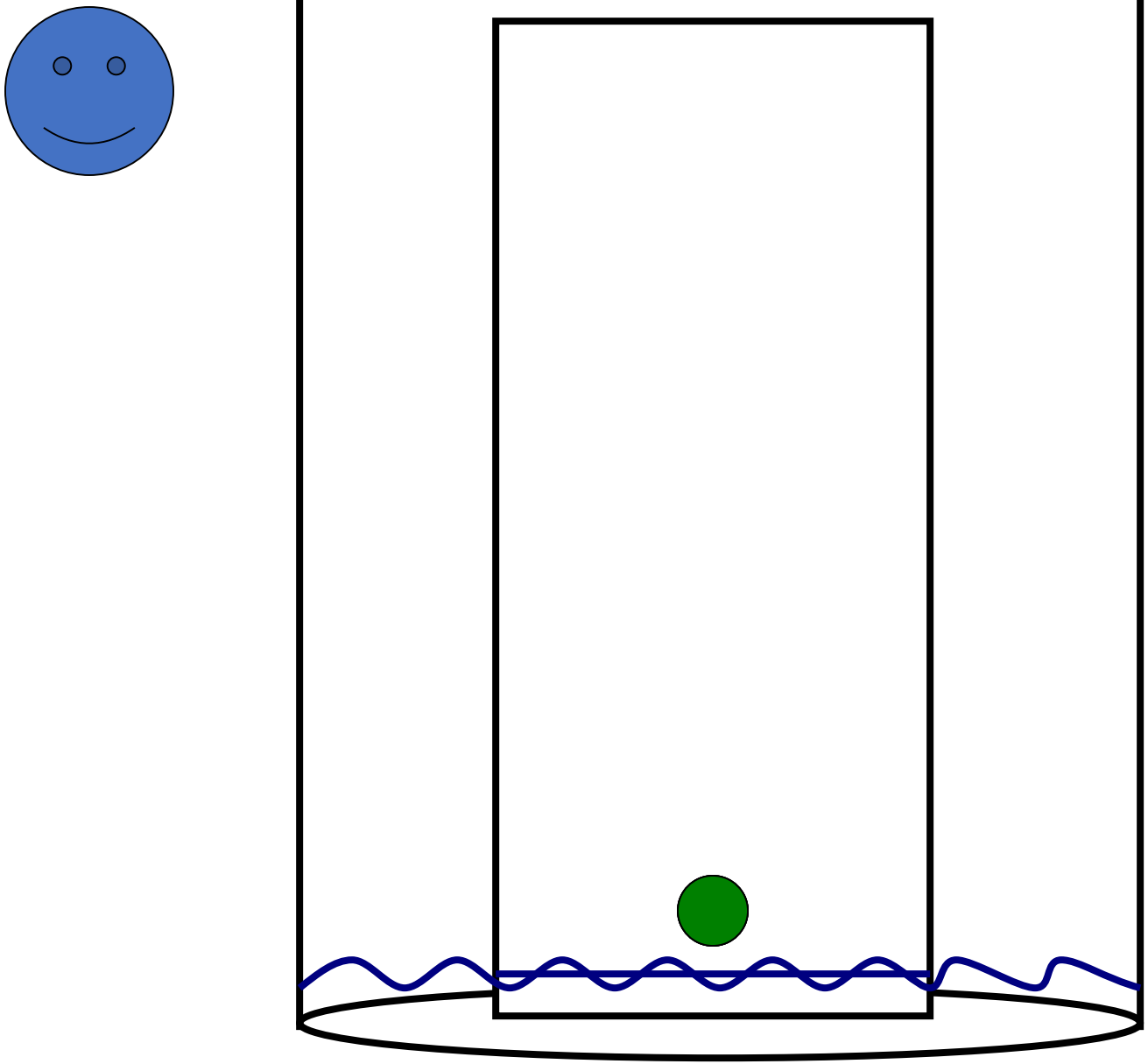
- Cut 1 strip of filter paper
- Draw a line 1 cm above the bottom edge of the strip with the pencil
- Label each strip with its corresponding solution
- Place a spot from of analyte.



# *PREPARING THE ISOPROPANOL SOLUTIONS*

- Prepare 15 ml of the following isopropanol solutions in appropriately labeled beakers:

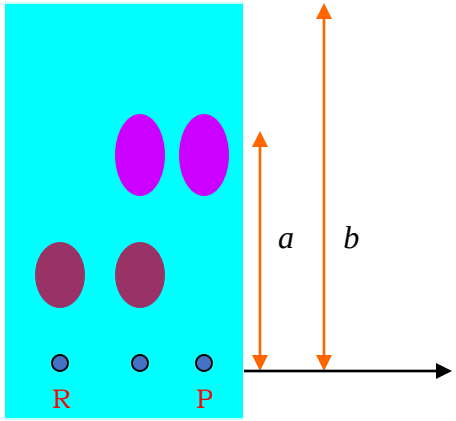
  - 0%, 5%, 10%, 20%, 50%, and 100%



# PAPER CHROMATOGRAPHY

## Interpretation and Calculation of $R_f$ values

Calculate $R_f$ values for various compounds




$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}} = \frac{a}{b}$$

Measure the distance from baseline upto centre of the spot, say a = 4cm.

Measure the distance traveled by the solvent, say b = 7cm.

Hence $R_f$ for that compound is 4/7 = 0.57, $R_f$ value for any compound is always less than 1

# *PAPER CHROMATOGRAPHY*

## Factors which will affect $R_f$ value

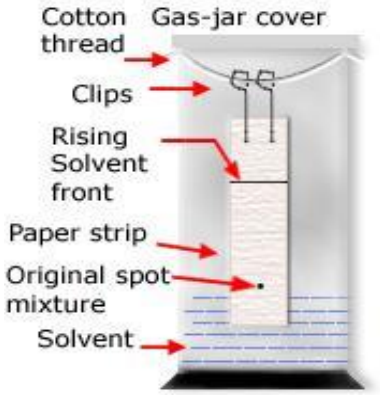
1. **Type of paper**
2. **Solvent composition**
3. **Temperature**
4. **Chamber saturation**

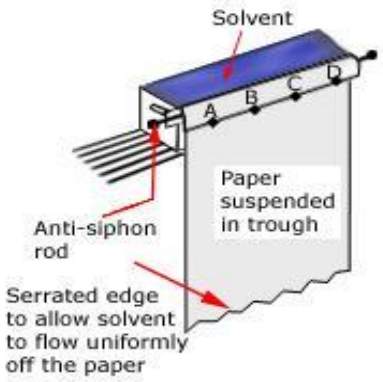
## Factors which will NOT affect $R_f$ value

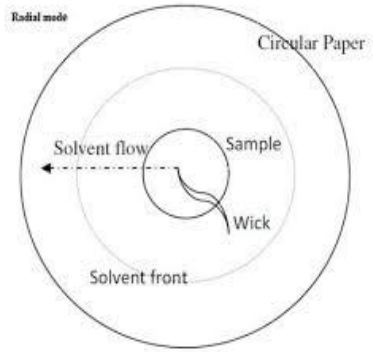
1. **Solvent volume**
2. **Size of paper**
3. **Sample size**

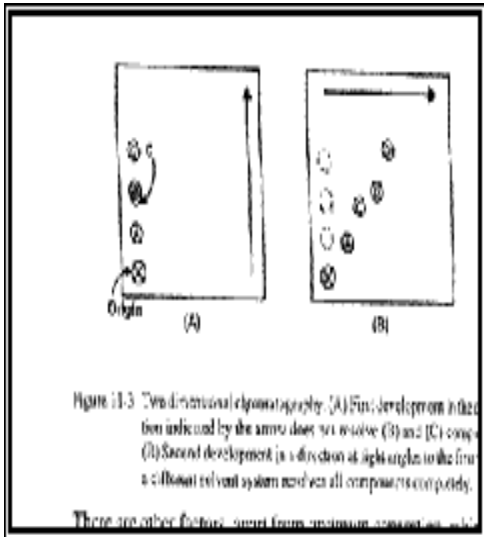
# TYPES OF PAPER CHROMATOGRAPHY

1. Ascending Paper Chromatography
2. Descending Paper Chromatography
3. Circular / Radial Paper Chromatography
4. Multi-dimentional




Ascending Descending Circular Multi-dimentional

# APPLICATIONS OF PAPER CHROMATOGRAPHY

- Separation of Mixture of polar and non-polar molecules.
- Separation of amino acid.
- Analysis of biochemical sample like Urine, blood etc
- Pharmaceutical Company - determine amount of each chemical found in new product
- Law Enforcement – to compare a sample found at a crime scene to samples from suspects
- Environmental Agency - determine the level of pollutants in the water supply
- Agriculture field – Fermentation, ripening

# THIN LAYER CHROMATOGRAPHY

# THIN LAYER CHROMATOGRAPHY

- TLC is a Chromatography technique used to **separate mixtures**.

- Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose. This layer of adsorbent is known as the **stationary phase**.

- After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

# THIN LAYER CHROMATOGRAPHY

**Thin layer chromatography can be used to:**

- Monitor the progress of a reaction.
- Identify compounds present in a given substance.
- Determine the purity of a substance.

# PRINCIPLES OF THIN LAYER CHROMATOGRAPHY

The molecules of substances present in the mixture moves between the stationary and the moving phase and the equilibria is continuously changing.

The mobile phase by capillary action begins to move in upward direction.

The mobile phase carries the molecules to the new area of the adsorbent where they are adsorbed.

Extremely soluble molecules in the solvent and having almost no affinity for the adsorbent will move faster and a greater distance than those having reverse properties.

# PROCEDURE OF THIN LAYER CHROMATOGRAPHY

## Preparation of the plate:

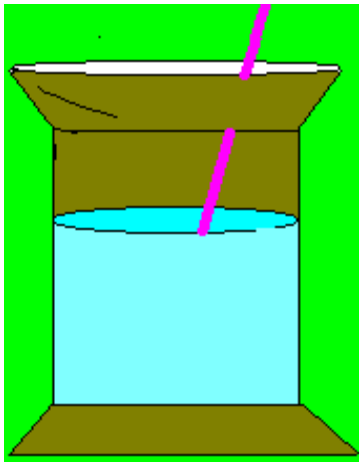
> Slurry is made by adding silica gel in solvent like $CHCl_3$,$CCl_4$ etc.

> Glass plate is dipped in silica gel jar.

> Remove silica gel from one side and also remove edges from right and left side of TLC plate.

## Activation of the plate:

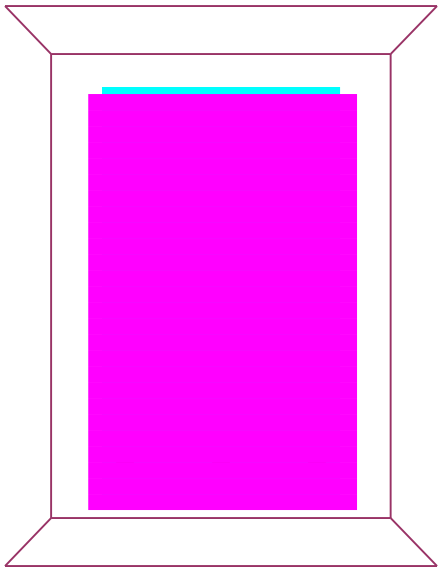
> In order to remove moisture and adsorb solvent on the adsorbent it is heated in an oven around 80-100$^0$c for about 5-10 minutes.



# PROCEDURE OF THIN LAYER CHROMATOGRAPHY

**Spotting on TLC Plate**

Load the samples with fine capillary and put the TLC plate in a jar containing suitable solvent.

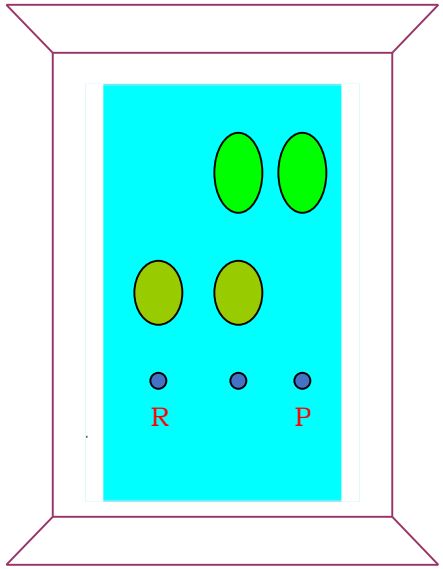


**Development of TLC plate**

Remove the plate, blow the solvent and keep in the jar containing iodine crystals. We can use different staining agents.

**Identification of compounds**

**a.** Iodine for most organic compounds.
**b.** 2,4 -Dinitrophenylhydrazine for aldehydes and ketones).
**c.** sulfuric acid for carbohydrates
**d.** ninhydrin for amino acids



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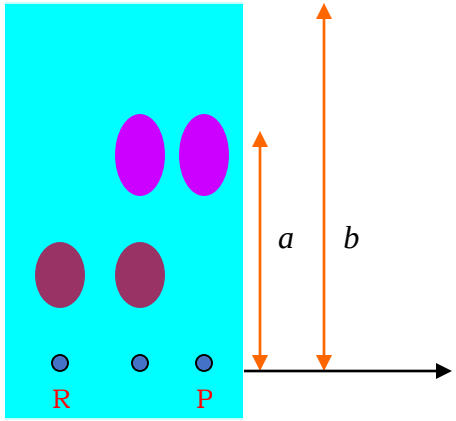


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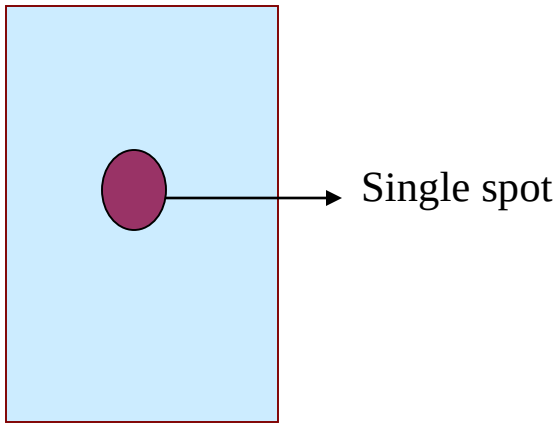
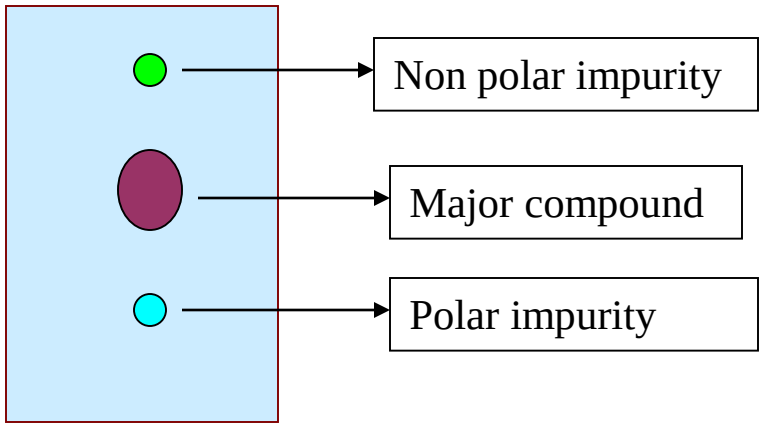
# THIN LAYER CHROMATOGRAPHY

## To check purity of organic compound

Pure Compound



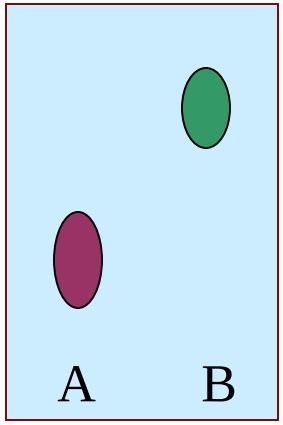

Impure Compound



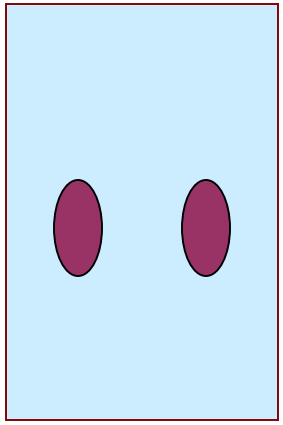

# THIN LAYER CHROMATOGRAPHY

## To compare two compounds

Different compounds




Same compounds

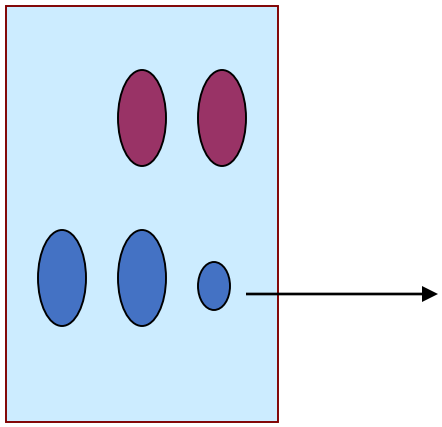


# THIN LAYER CHROMATOGRAPHY

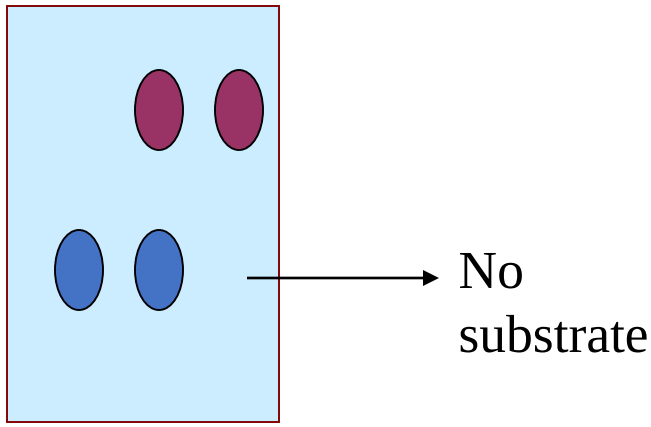
## To monitor organic reactions

(To check the completion of reaction)

Incomplete reaction




Complete reaction




# Thank You...