Chromatography

Advance Chemistry Lab 6

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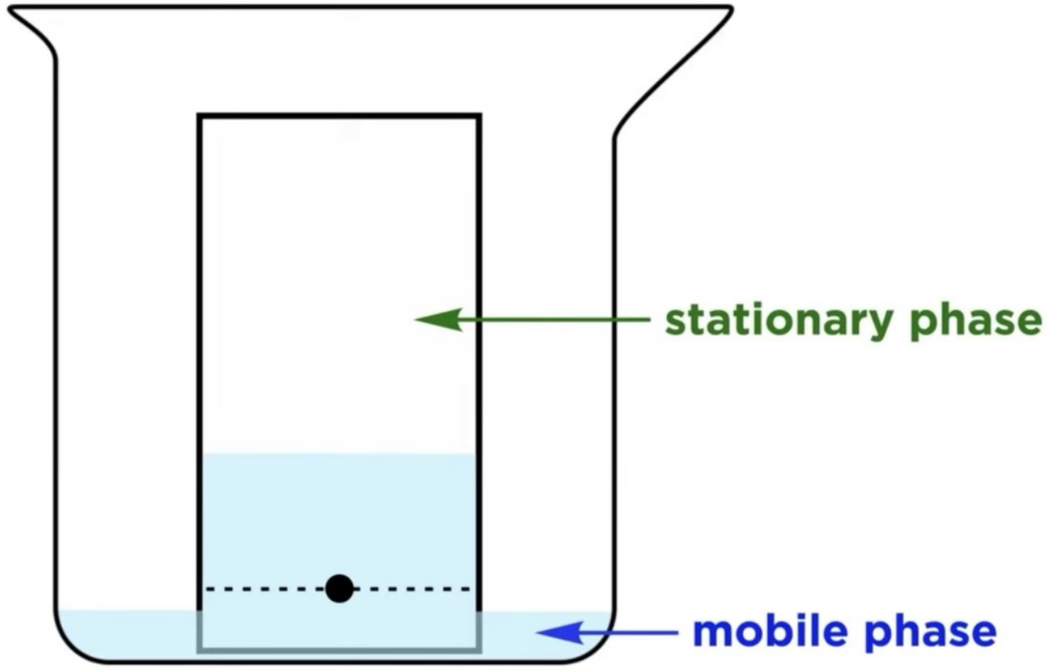
Chromatography

Chromatography is the technique for the separation, purification, and testing of compounds.

The term “chromatography” is derived from Greek, chroma meaning, “colour,” and graphein meaning “to write.”

Principle of Chromatography

In this process, apply the mixture to be separated on a stationary phase (solid/liquid) through a system (a column, a capillary tube, a plate, or a sheet) and a pure solvent (liquid/gas) mobile phase is allowed to move slowly over the stationary phase, carrying the components separately as per their solubility in the pure solvent.



Advantage of Chromatography

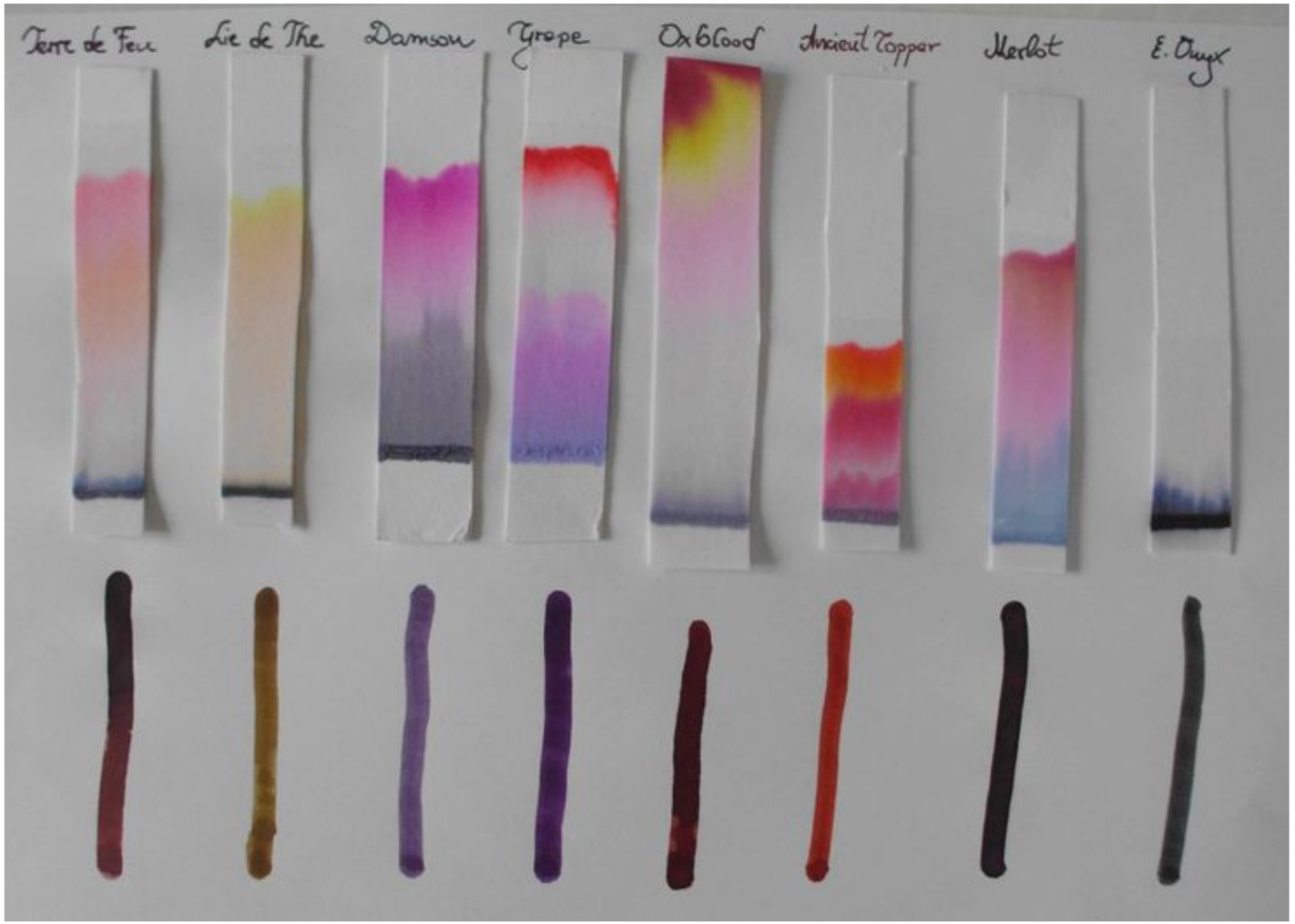
- It can separate all components of a chemical mixture without prior knowledge of their identity or amounts.
- Versatile, capable of handling molecular species of varying sizes from large viruses to small molecules like hydrogen.
- Works with large or small amounts of material.
- Some chromatography forms detect substances at the attogram level.

Types of Chromatography

- Column chromatography
- Ion-exchange chromatography
- Paper chromatography
- Thin-layer chromatography
- Gas chromatography

Paper Chromatography

Paper chromatography is a simple and widely used technique for separating mixtures into their individual components.



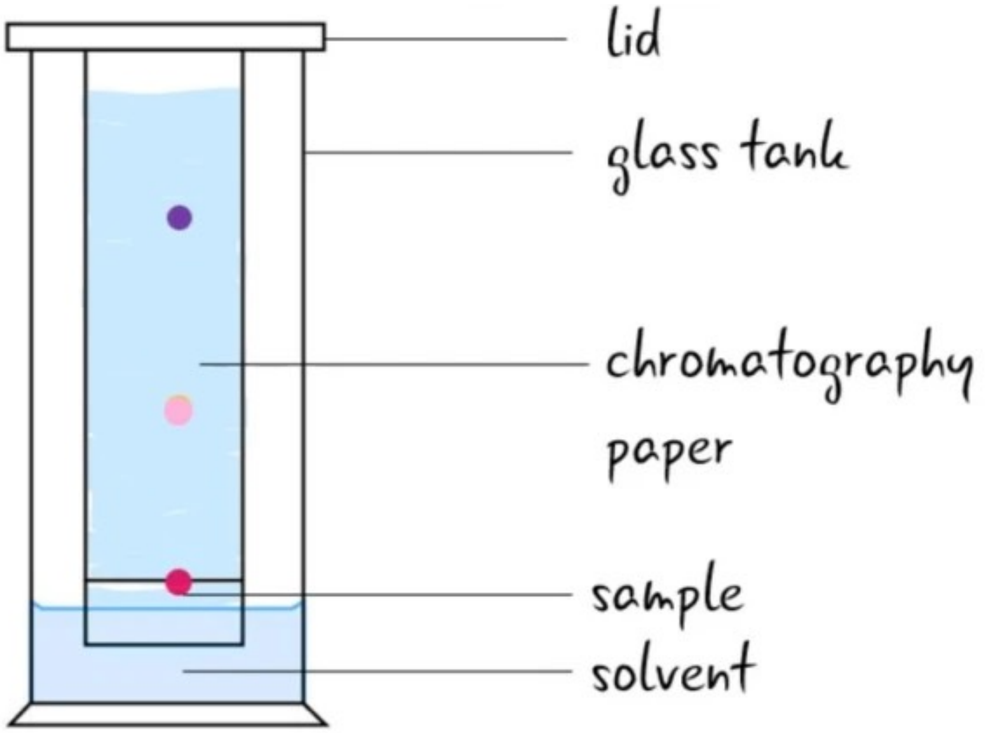
Materials needed:

1.chromatography paper: This is a special type of paper designed to absorb and separate the components of the mixture.

2.Solvent: A liquid that acts as the mobile phase, carrying the mixture components along the paper.

3.Sample: The mixture to be separated.

4.Beaker



Experiment Steps:

1.Cut a strip of chromatography paper and draw a pencil line near one end to mark the origin of the sample.

2.Using a pipette, apply a small spot of the sample mixture onto the pencil line.

3.Place the paper strip vertically in a container with a small amount of solvent (mobile phase). The solvent should be below the sample spot to avoid dissolving the sample directly. As the solvent travels up the paper, it carries the components of the mixture with it.

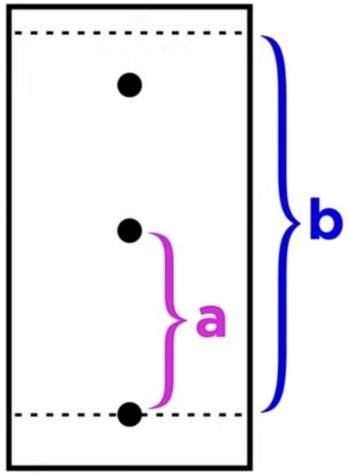
4.Once the solvent front nears the top of the paper, remove the paper and allow the paper to dry.

5.Different components of the mixture will have traveled different distances up the paper based on their affinity for the paper and the solvent. Compare the positions of the spots to determine the components present in the mixture.

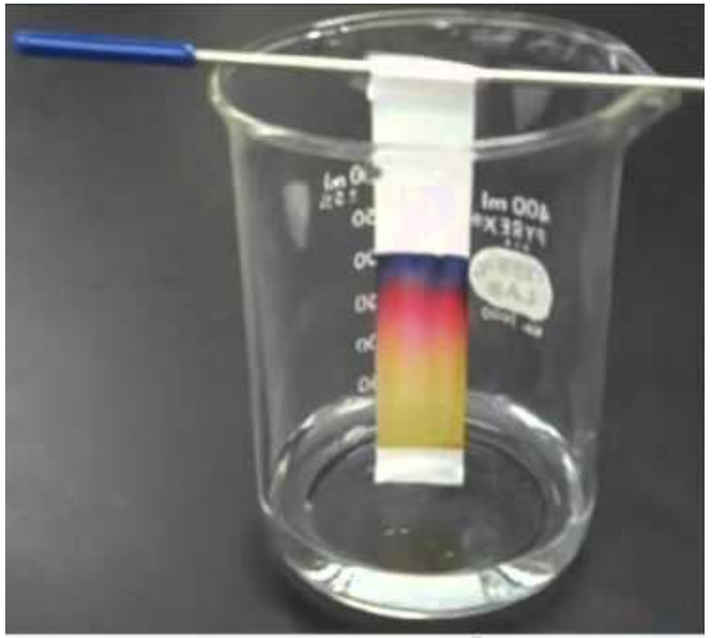
Paper Chromatography Test

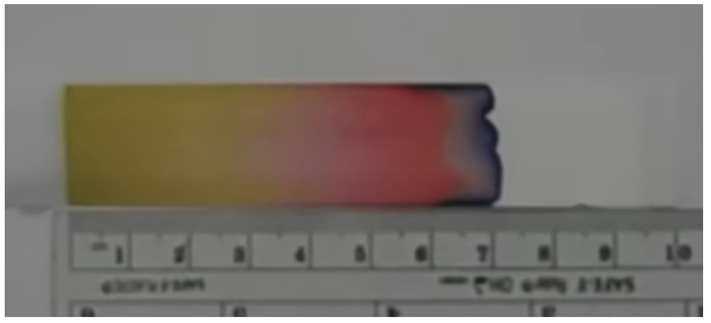
retention fraction/retention factor can be measured and compared to tabulated values

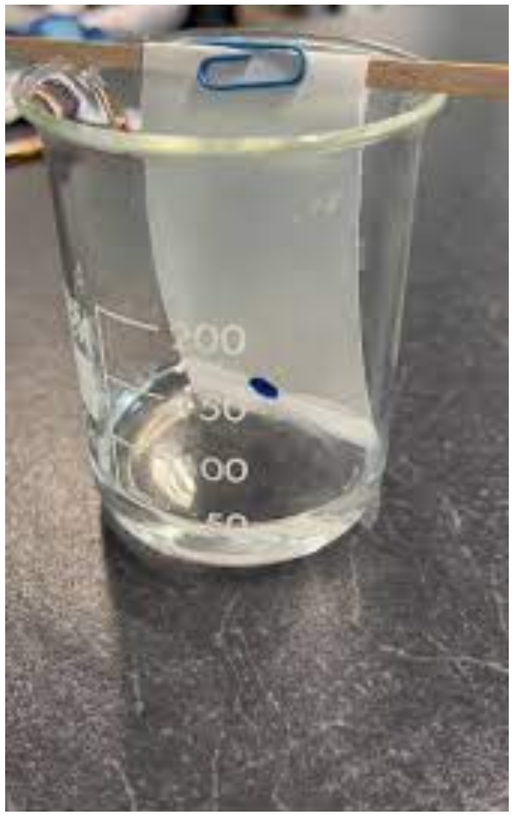
$$R_f = \frac{a}{b}$$



Paper Chromatography Test

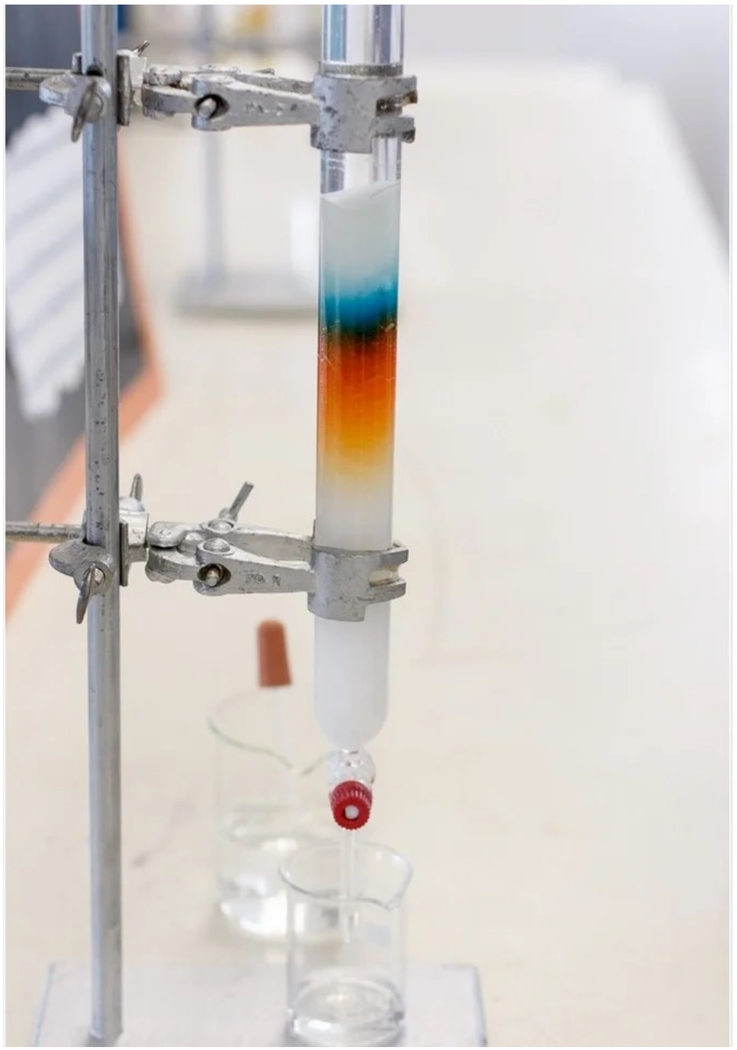






Column Chromatography

Column chromatography is a laboratory technique used to separate and purify individual components of a mixture based on their differential interactions with a stationary phase and a mobile phase as they flow through a column packed with a stationary material.



Equipments needed:

1.Column: A glass or plastic tube used to contain the stationary phase material.

2.Stationary phase material: Typically a solid support material like silica gel or alumina, packed into the column.

3.Sample: The mixture to be separated, dissolved in a suitable solvent.

4.Solvent reservoir: Containers to hold the solvents out of the column.

5.Fraction collector: A device used to collect the eluted fractions as they exit the column.

Experimental Steps:

1. Pack the column with stationary phase material.

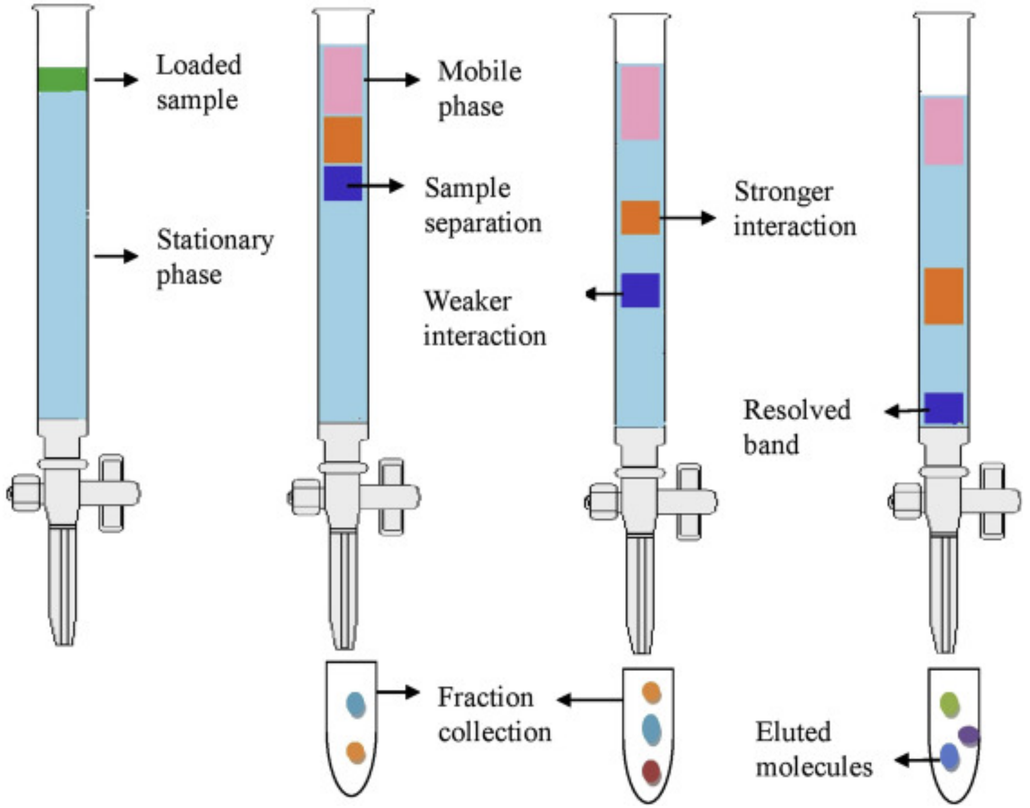
2. Dissolve the mixture in a suitable solvent.

3. Apply the sample evenly at the top of the column.

5. Start mobile phase flow to elute components at different rates.

6. Collect eluted fractions based on profile.

7. Analyze fractions for presence and purity.



Experimental Test

Experimental Test

Comparison between column chromatography and paper chromatography:

Column chromatography

- The stationary phase is packed inside a column
- The sample is applied at the top of the column
- Often used for larger-scale separation
- Slower than paper due to the longer separation times required for the components to travel through the column
- Higher resolution
- suitable for polar and non-polar compounds

Paper chromatography

- The stationary phase is a special paper strip
- The sample is applied as a spot on the paper
- Used for smaller-scale separations
- Faster separation due to the shorter distance traveled by components on the paper strip
- Lower resolution
- suitable for separating non-polar compounds