

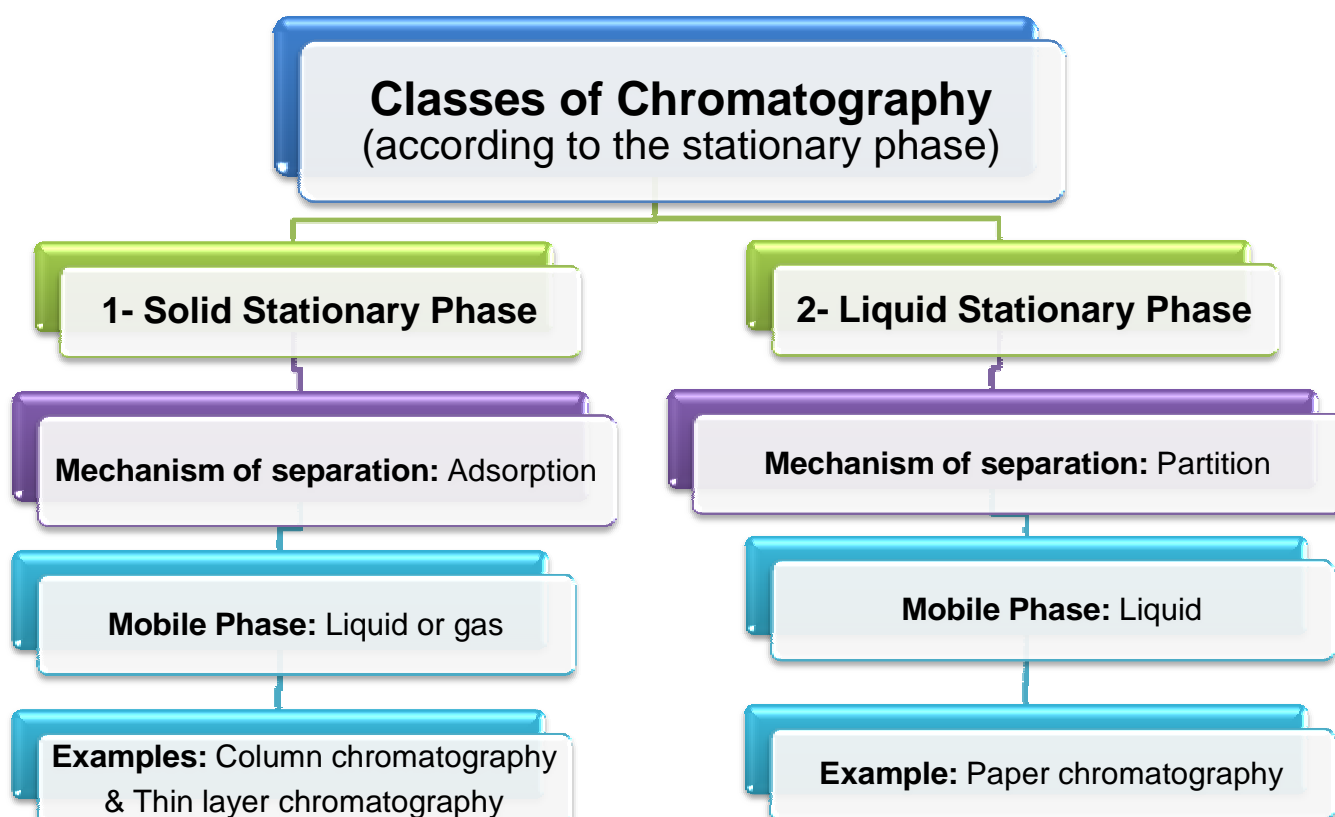


Chromatography

Lab No. 4

Definition of chromatography :

It is a technique used for the separation of a mixture of components, in which these components are distributed between two phases (stationary phase and mobile phase). The **stationary phase** remains fixed in the system, it is either solid or liquid supported on a solid, while the **mobile phase** moves between the particles, and it is either liquid or gas.



Examples of chromatography :

1- Column chromatography:

Column chromatography is a common separation technique; it can be applied to large and small scales.

Stationary phase: In this type of chromatography, the stationary phase is a solid material packed into the column, alumina and silica are the most common stationary phases

Mobile phase: The mobile phase is a liquid, it can be a single solvent or a mixture of solvents having different polarities

Methods of packing the column:

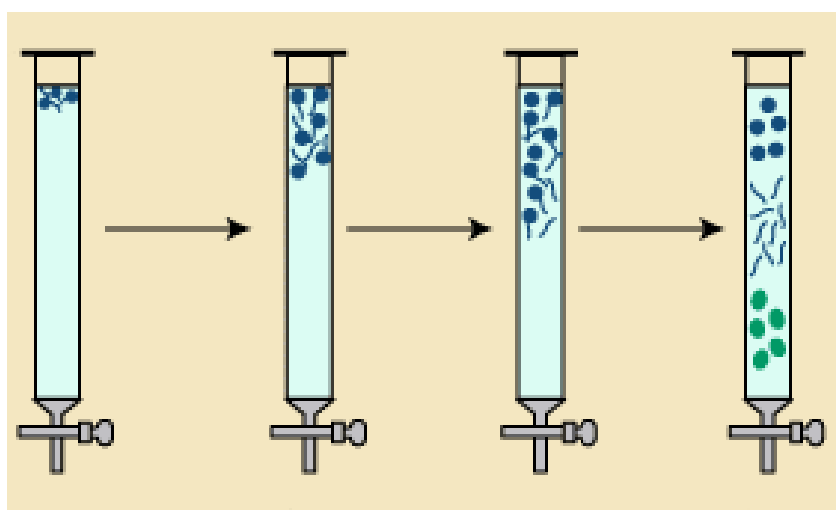
- Dry method: the stationary phase is added, then the column is filled with a solvent
- Wet method (slurry method): the solid stationary phase is added to a beaker & dissolved in a small amount of a solvent, until forming a paste and then poured into the column. Wet method is better since there is no entry of air bubbles

Loading the sample:

The sample (mixture) is loaded to the top of the column. If the mixture contains colored compounds, it will be separated in the form of colored bands, reaching the end of the column and then collected in suitable containers. Colorless compounds are monitored with TLC.

Application:

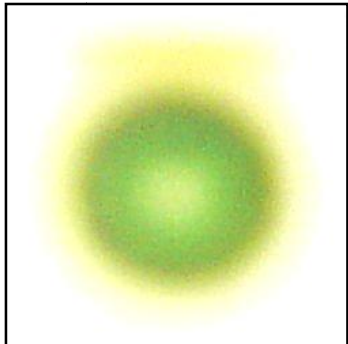
Stationary phase:	Alumina
Mobile phase:	Liquid (chloroform, ethanol and acidulated alcohol)
Mechanism of separation:	Adsorption
Sample:	Mixture of three dyes: Sudan III, Gentian violet and Dinitrophenol
Procedure:	<p>The column is packed with alumina using the wet method, the mixture is loaded and separated as following:</p> <ol style="list-style-type: none">1-Sudan III (red), separated with chloroform.2-Gentian violet (violet), separated with ethanol.3-Dinitrophenol (yellow), separated with acidulated alcohol.



Examples of chromatography :

2- Paper chromatography:

Application:

Stationary phase:	Moisture (liquid) adsorbed on a filter paper from the atmosphere
Mobile phase:	Chloroform (liquid)
Mechanism of separation:	Partition
Sample:	Mixture of three dyes: Sudan III, Gentian violet and Dinitrophenol
Procedure:	1-Cut a strip from the edge of a filter paper but keep it attached to the paper at the middle region. 2-Add chloroform to a petri dish, cover it with a fitting cover 3-Apply sample on the top of the strip of the filter paper using a capillary. 4-Immerse the strip inside the chloroform, cover and wait for separation.
Results:	The sample will be separated into three colors: 1-Red color indicates presence of Sudan III. 2-Violet color indicates presence of Gentian violet. 3-Yellow color indicates presence of Dinitrophenol. 

Examples of chromatography :

3- Thin Layer Chromatography (TLC):

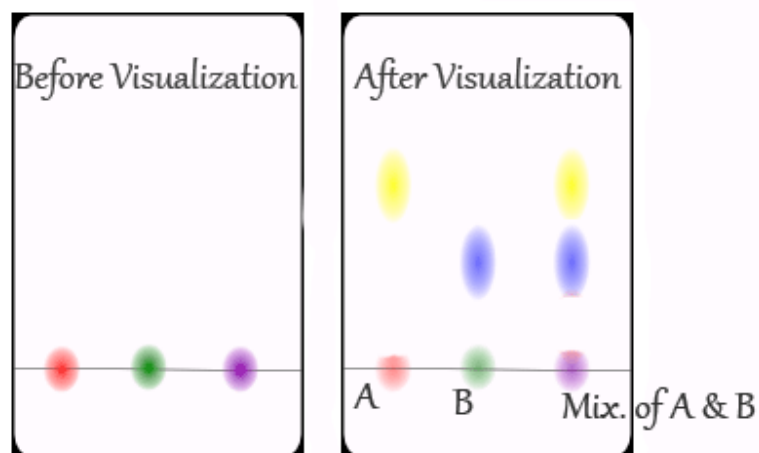
The technique of TLC is useful in determining the type and number of ingredients in a sample mixture. The stationary phase is solid (plate of silica gel), while the mobile phase is liquid. Each component will be separated as a spot, these spots are then visualized using UV lamp &/or different reagents, the results can be compared with a standard applied simultaneously with the sample.

Application:

- 1- On a TLC plate, a line is drawn 1cm from the lower edge.
- 2- The given unknown sample is spotted by a capillary, brucine and reserpine standards are also spotted on the same plate.
- 3- The solvent system is prepared and poured into a jar
- 4- The TLC is immersed in the jar and covered, until the mobile phase is developed (1 cm before the upper edge)
- 5- The plate is taken out, visualized under U.V. lamp and then sprayed with dragendorff's reagent
- 6- The rate of flow (R_F value) is calculated for each spot
- 7- The resulted spot/s of the sample and the R_F values are compared with those of the standard

Solvent system: chloroform: benzene: methanol: ammonia

(30 ml : 15 ml : 5 ml : 2 drops)



$$R_F = \frac{\text{Distance travelled by substance}}{\text{Distance travelled by solvent}}$$

RF of brucine:

RF of reserpine:

RF of your unknown: