

LECTURE 1

Introduction to Chromatography

Chromatography is a modern technique used for the examination and separation of mixture of chemical substances. It facilitates the purification, isolation and comparison of compounds. It may be employed with all kinds of volatile and soluble substances, organic and inorganic, polar and non-polar, and it may be adopted to use with various quantities under many different conditions. Chromatography is taken from the Greek word 'chroma' for color and 'graphy' to write.

Chromatography is non-destructive procedure for resolving a multi-component mixture of trace, minor or major constituents into its individual fraction. Chromatography can be used for the quantitative and qualitative analysis.

History

Scientist	Year	Work
Mikhail Tswett	1906	Separation of pigments based on selective adsorption
Zechmeister	1937	Published first book on chromatography (paper and thin layer chromatography)

Martin	1941	Idea of partitioning
Synge	1944	Replaced silica gel by strip of paper
James and Martin	1952	Idea of gas liquid chromatography. Got nobel price. The liquid liquid partition chromatography was developed and it was based on a HPLC
Porath and Flodin	1959	Used gels of cross linked dextran for separating the biological materials and this leads to electrophoresis.

1. Defination

According to the IUPAC in 1993, Method for the separation of components of a sample, in which the components are distributed between two phases, one of which is stationary phase, while the other moves in definite direction. The stationary phase may be solid or liquid supported on a solid or gel. The mobile phase may be gaseous or liquid.

2. Chromatographic Terminology

Chromatograph

Instrument employed for a chromatography.

Stationary phase

The phase which remains fixed by a support or does not move by the incoming mobile phase is called stationary phase.

Mobile phase

It is the solvent moving through the column which may be a liquid in liquid chromatography or gas in gas chromatography.

Support

An inert material having tendency to retain the liquid stationary phase on it is called support.

Eluent

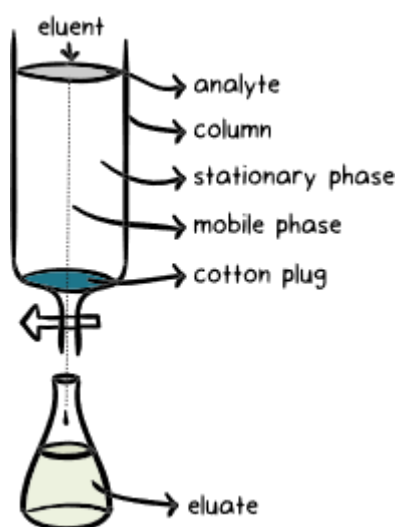
Fluid entering a column is called eluent.

Eluate

Fluid exiting the column is called eluate.

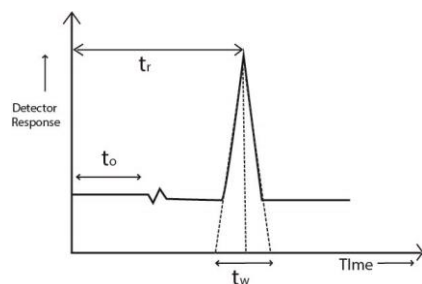
Elution

The process of passing the mobile phase through the column is called elution.



Chromatogram

Graph showing detector response as a function of time is chromatogram.



Flow rate

How much mobile phase passed per minute (ml/min) is flow rate.

Linear velocity

Distance passed by mobile phase per minute in the column.

Retention time (t_R)

Time required for the mobile phase to sweep a component from the stationary phase is called retention time.

Retention volume (V_R)

Volume of the mobile phase required to sweep a component from the stationary phase.

Resolution

The degree of separation of compounds of similar characteristics is called resolution.

Distribution co-efficient (D)

It is the concentration of a component in or on the stationary phase divided by the concentration of the component in the mobile phase in equilibrium condition.

3. Steps of chromatographic method

- Adsorption or retention of substances by the mobile phase.
- Separation or the retention of a substance by the mobile phase.
- Recovery of the adsorbed substances by a continuous flow of the mobile phase, the method being called elution.
- Qualitative and quantitative analysis of the eluted substances.

4. Classification of chromatographic methods

Its classification is based on following ways;

1. Based on mechanism of separation
 - Adsorption chromatography
 - Partition chromatography
 - Ion exchange chromatography
 - Size exclusion chromatography or gel permeation
2. On the basis of mobile phase
 - Liquid chromatography (normal phase, reverse phase, hydrophobic interaction, hydrophilic interaction, ion exchange, affinity chromatography).
 - Gas chromatography
 - Super critical fluid chromatography

3. Based on shape of chromatographic bed

- Planer chromatography (paper or thin layer chromatography)
- Column chromatography (gravity or flash column chromatography).