

DSF-2T,
Analytical Methods in Chemistry

Chromatography

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For BSC (Hons) 5th Sem

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In 1901, a Russian botanist Mikhail Tswett, during separation of coloured Chlorophyll and other plant pigments sent the leaf extract through a column packed with CaCO_3 , Al_2O_3 and sucrose eluting them with Petroleum ether / ether mixture.

He coined the term Chromatography in his research paper in 1906. Chromatography comes from Greek words, "Chroma" means colour and "graphos" means to write.

Generally chromatography refers to separation of components of a mixture by distribution of the components ~~between~~ between two phases — one stationary and the other mobile (moving phase) usually in a column.

② A definition recommended by IUPAC: "Chromatography is a physical method of separation in which two components to be separated are distributed between two phases, one of which is stationary while the other (~~is~~ ~~is~~ mobile phase) moves in a definite direction."

~~The principle of~~ In any chromatographic separations one sample free phase, called a mobile phase, is continuously passed over another sample free phase, ~~that~~ that remains fixed or stationary. The sample is injected or placed into the mobile phase. As it moves with the mobile phase, the sample's components partition themselves between the two phases. Components ~~whose~~ whose distribution ratio favours the stationary phase requires a longer time to pass through the system.

Types and classification

Classification by three ways.

(A) The physical state of ~~mobile~~ mobile phase and stationary phase:

Classified as Gas Chromatography or Gas liquid Chromatography and Liquid Chromatography.

(B) By the method of contact between the mobile phase and stationary phase: Two approaches are there to bring mobile and stationary phase to come into contact.

One is Column Chromatography ~~and~~ in which stationary phase is placed in a narrow column and the mobile phase is passed through it under the influence of gravity or pressure.

In planar chromatography (also called thin layer chromatography or TLC) the stationary phase coats a glass plate, or plastic plate in a developing chamber and mobile phase is placed in a reservoir in contact with the stationary phase which moves up by capillary action.

③ Mechanism responsible for separation of sample's constituents:

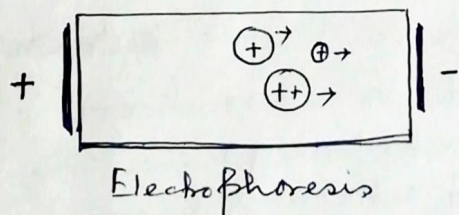
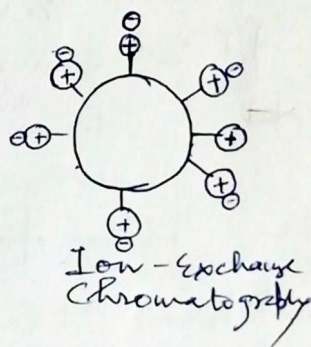
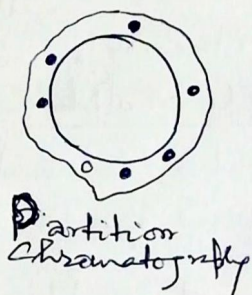
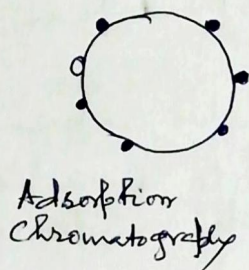
1) Adsorption chromatography: - Solutes separate based on their ability to adsorb to a solid stationary phase followed by desorption process.

2) Partition chromatography: - The stationary phase here is usually a liquid ~~adsorbed~~ supported on a solid or a network of molecules. Again, the mobile phase may be a liquid (liq-liq partition chromatography) or a gas (gas-liq chromatography or GLC).

In normal mode of operations, of liq-liq partition chromatography, a polar stationary phase (e.g. cyanogen bonded to silica gel) is used with a non polar mobile phase (e.g. hexane). When analyte (i.e. sample under investigation) dissolved in mobile phase is introduced into the system, retention increases with increasing polarity. This is known as Normal-phase-chromatography.

But when the ~~stationary~~ stationary phase is nonpolar, increasing polarity of analytes decrease the retention of solutes. This is known as reversed-phase chromatography.

Normal-phase chromatography is used to separate water insoluble compounds while water soluble compounds can be separated by reversed-phase chromatography ~~that~~ based on their varying degree of hydrophobicity.



(iii) Ion-exchange Chromatography—

In ion exchange chromatography stationary phase consisting of a solid support with covalently bonded cationic or anionic functional groups (e.g. SO_3^- or $\text{N}^+(\text{Me}_3)$) are used. Ionic solutes are attracted towards the stationary phase by electrostatic force of attraction.

(iv) Size Exclusion Chromatography— In size exclusion chromatography porous gels are used as stationary phase.

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Large molecules (solutes) are unable to penetrate the pores of the stationary phase and simply pass through the column while smaller materials spend much time passing through the pores.

Electrophoresis : Charged solutes migrate under the influence of an applied potential field. The solutes are separated based on their different mobilities.

Some definitions

- * Counter current extraction → A technique in which solutes are extracted back and forth between two fresh portions of two extracting phase.
- * Mobile phase → In chromatography the extracting phase that moves through the system.
- * Stationary phase → In chromatography the extracting phase that remains in a fixed position.
- * Column chromatography → A form of chromatography in which the stationary phase is retained in the column.
- * Planar chromatography → A form of chromatography in which the stationary phase is immobilized on a flat surface.