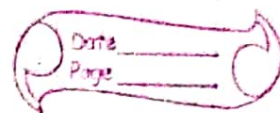


CHROMATOGRAPHY



Chromatography is a physical method for separation of compounds. Tsvet Russian Botanist (referred as the father of chromatography) is credited for the development of Chromatography.

He employed the technique to separate various plant pigments such as chlorophylls and xanthophylls by passing solution of these compounds through a glass column packed with finely divided Cal. carbonate. The separated species appeared as coloured bands on the column, which accounts for the name he chose for the method (Greek, Chroma meaning 'color' and graphian meaning 'writing').

Principles of Chromatography:-

Chromatography is based on very simple principle. The sample to be examined (called the solute or analyte) is allowed to interact with two immiscible phases - a mobile phase and a stationary phase. The two immiscible phase could be a solid and liquid, or gas and a liquid or a liquid and another liquid. The stationary phase (i.e, sorbent), which may be a solid or a liquid supported on solid, does not move.

The mobile phase moves the sample through the stationary phase. The mobile phase may be liquid (liquid chromatography) or gas (gas chromatography). All chromatographic methods involve passing a mobile phase through a stationary (immobile) phase. The two phases are chosen so that the components of the sample distribute themselves between the mobile and stationary phases to varying degrees.

Classification of Chromatography:.

Chromatographic method can be classified in three fundamental ways:

- 1. Based on the shape of chromatographic bed
- 2. Based on the physical state of the mobile & stationary phases.
- 3. Based on the mechanism of separation.

→ 1. Based on the shape of chromatographic bed
On this basis, there are two types of chromatography —
Planar Chromatography &
Column Chromatography

Planar Chromatography → the stationary phase is spread on flat, planar surface. The plane can be a paper impregnated by substance acting as stationary phase (Eg - Paper Chromatography, PC) or thin layer of substance acting as stationary phase spread on a glass, metal or plastic plate (eg - thin layer chromatography TLC).
Planar Chromatography is also termed as open-bed Chromatography.
~~Thin layer chromatography~~

Column Chromatography - In Column Chromatography, the stationary bed is within a tube. The particles of the solid stationary phase or support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated along the inside tube wall, leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open-tubular column). In gas chromatography the stationary phase is always packed in a column in order to contain the mobile phase, a gas.

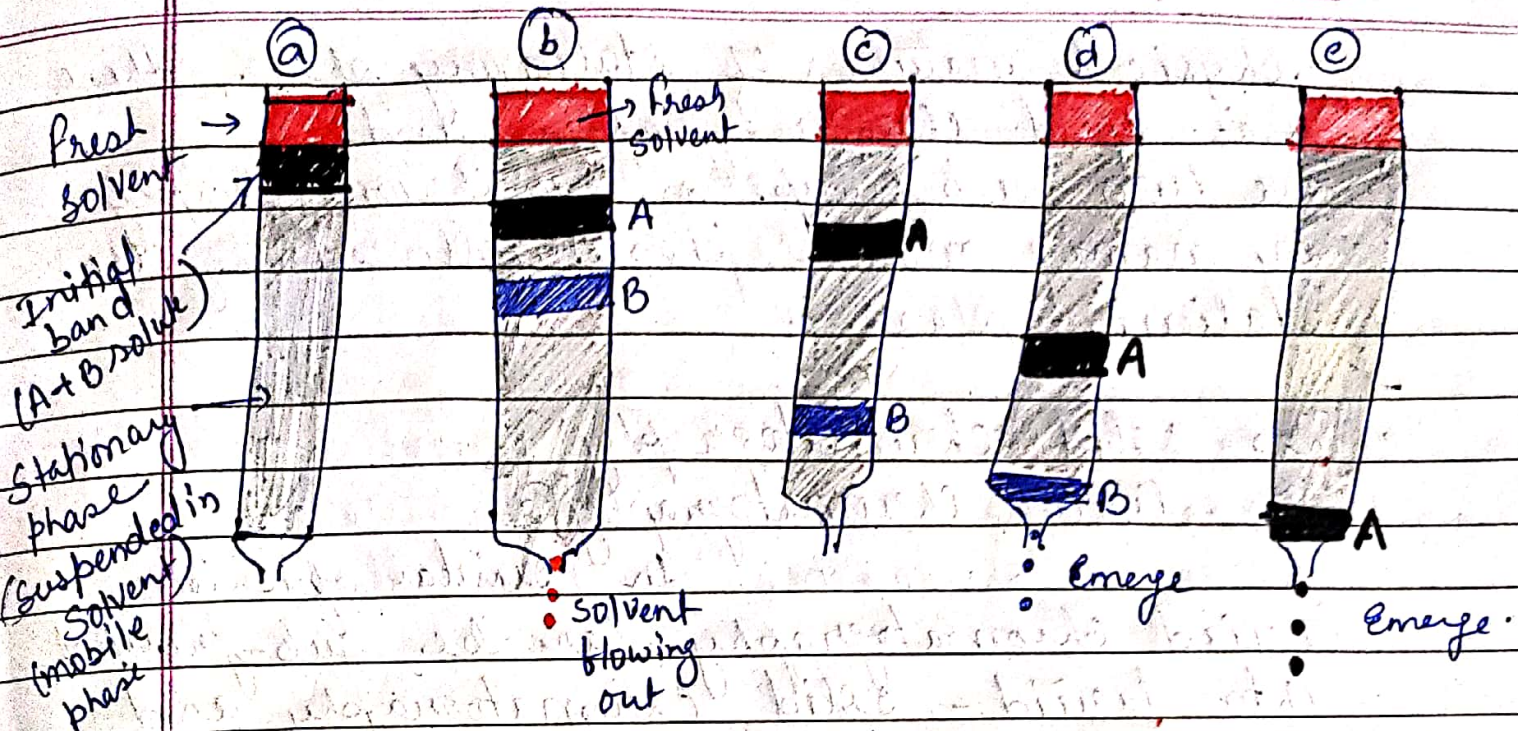


Fig: - The fig shows separation of solutes A and B present in a mixture by chromatography. A continuous flow of solvent carries a mixture of solutes A and B. (a) the solvent carries the two solutes down the column. (b) After some time, solute B is moving at a much faster rate than A. (c) Finally, solute B emerges first, while solute A finally emerges in (e)

2. Based on physical state of the mobile and stationary phases:
 Based on physical nature of mobile phase, there are two types of chromatography -
 → Gas chromatography
 → Liquid chromatography.
 further based on the

physical nature of stationary phase, — there
→ Gas-solid chromatography and Gas-liquid chromatography
are two sub groups of gas chromatography
by naming mobile phase followed by the
stationary phase —

- Gas-solid chromatography
- Gas-liquid chromatography

In similar fashion,
liquid chromatography can be sub-grouped
into liquid-solid chromatography and
liquid-liquid chromatography.

→ 3. Based on the mechanism of separation

This chromatographic techniques are used in
several types of mechanisms to separate
analytes. Based on the mechanism of
separation chromatographic techniques can
be of different types:

- (i) ~~Adsorption~~ Partition
- (ii) ~~Partition~~ Adsorption
- (iii) Size exclusion
- (iv) Affinity
- (v) Ion exchange chromatography