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Study of Chromatography and its Principle

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Introduction : Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. Chromatography may be defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phase.

Key Words : Chromatography

The name chromatography (Greek chroma color and Graphy writing) means color writing. Essentially, the technique of chromatography is based on the difference in the rate at which the components of a mixture move through a porous medium (called stationary phase) under the influence of some solvent or gas (called moving phase). The chromatography method of separation in general involves the following steps :

1. Adsorption or retention of a substance or substances on the stationary phase.
2. Separation of the adsorbed substances by the mobile phase.
3. Recovery of the separated substances by a continuous flow of the mobile phase, the method being called elution.
4. Qualitative and quantitative analysis of the eluted substances.

Chromatography Principle

All chromatographic method require one static part (the stationary phase) and one moving part (the mobile phase). The most logical way of classification is based upon the mechanism of solute or analyte in the stationary phase. The techniques rely on one of the following phenomena :

- Adsorption
- Partition
- Ion exchang
- Molecular exclusion.

Adsorption Chromatography : Adsorption chromatography was developed first. It has solid stationary phase and a liquid or gaseous mobile phase. Each solute has its own equilibrium between adsorption onto the surface of the solid and solubility in the solvent, the least soluble or best adsorbed ones travel more slowly. The result is a separation into bands containing different solutes. So this type of chromatography is based on exploitation of the difference in adsorbility solute and stationary phase. Example : LC, GC.

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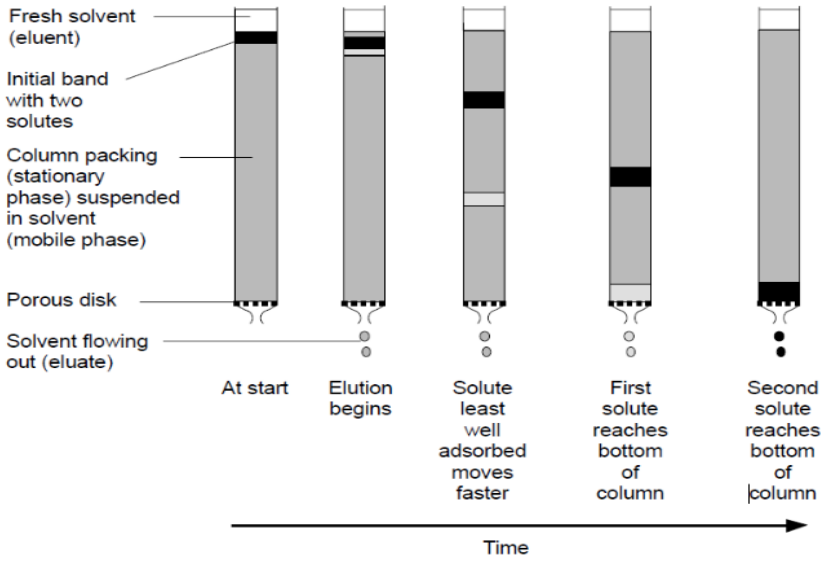
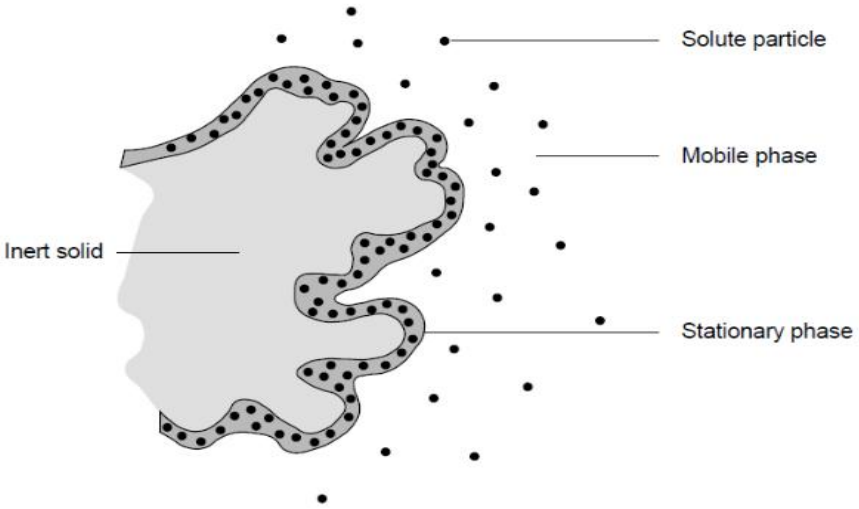


Partition Chromatography : In partition chromatography the stationary phase is a non-volatile liquid which is held as a thin layer (or film) on the surface of an inert solid. The mixture to be separated is carried by gas or a liquid as the mobile phase. The solutes distribute themselves between the moving and the stationary phases, with the more soluble component in the mobile phase reaching the end of the chromatography column first. So this type of chromatography is based on difference in the partition coefficient or distribution ratio of individual species in the mobile and stationary phase. Example : TLC, PC.



Each solute partitions itself between the stationary phase and the mobile phase

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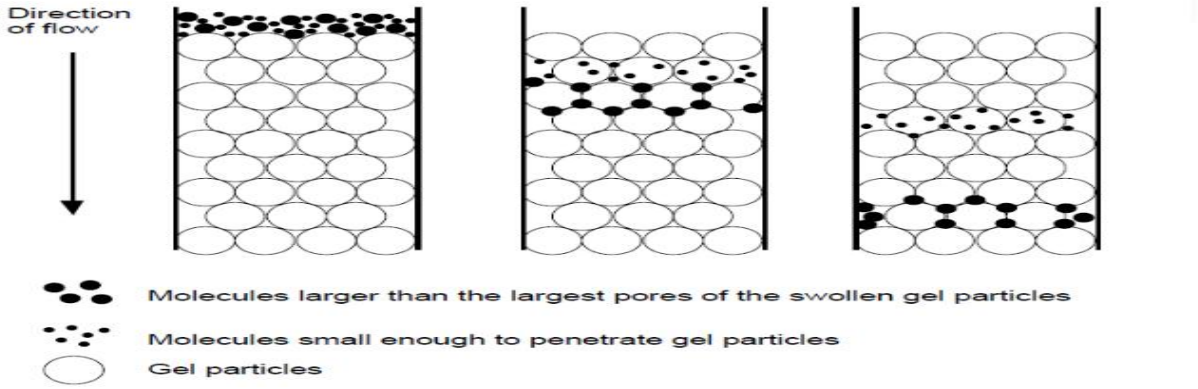
Ion Exchange Chromatography : Ion exchange chromatography is similar to partition chromatography in that it has a coated solid as the stationary phase. The coating is referred to as a resin, and has ions (either cations or anions, depending on the resin) covalently bonded to it and ions of the opposite charge are electrostatically bound to the surface. When the mobile phase (always a liquid) is eluted through the resin the electostatically bound ions are released as other ions are bonded preferentially. Domestic water softeners also work on this principle. So this type of chromatography is based on difference in the exchange potential between various ions for an ion exchange resin packed in column. Example : Ion exchange chromatography, Ion Chromatography.

Molecular Exclusion Chromatography : Molecular exclusion differs from other types of chromatography in that no equilibrium state is established between the solute and the stationary phase. Instead, the mixture passes as a gas or a liquid through a porous gel. The pore size is designed to allow the large solute particles to pass through uninhibited. The small particles, however, permeate the gel and are slowed down so the smaller the particles, the longer it takes for them to get through column. Thus separation is according to particle size. So this type of chromatography is based on exploitation of the difference in size or molecular geometry of compound. Example : Gel permeation chromatography, Ion exclusion chromatography.



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