

Chromatography

Chromatography is usually introduced as a technique for separating and/or identifying the components in a mixture. The basic principle is that components in a mixture have different tendencies to adsorb onto a surface or dissolve in a solvent. It is a powerful method in industry, where it is used on a large scale to separate and purify the intermediates and products in various syntheses.

Types of Chromatography

There are several different types of chromatography currently in use such as;

- Paper chromatography
- Thin layer chromatography (TLC)
- Gas chromatography (GC)
- Liquid chromatography (LC)
- High performance liquid chromatography (HPLC)
- Ion exchange chromatography &
- Gel permeation/Gel filtration chromatography.

Basic Principles

All chromatographic methods require one static part (the stationary phase) and one moving part (the mobile phase). The techniques rely on one of the following phenomena:

- Adsorption
- Partition
- Ion exchange &
- Molecular exclusion.

1. Adsorption

Adsorption chromatography was developed first. It has a solid stationary phase and a liquid or gaseous mobile phase. (Plant pigments were separated at the turn of the 20th century by using a calcium carbonate stationary phase and a liquid hydrocarbon mobile phase. The different solutes travelled different distances through the solid, carried along by the solvent.) 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. Liquid chromatography using a column containing silica gel or alumina is an example of adsorption chromatography (Fig. 1). The solvent that is put into a column is called the eluent, and the liquid that flows out of the end of the column is called the eluate.

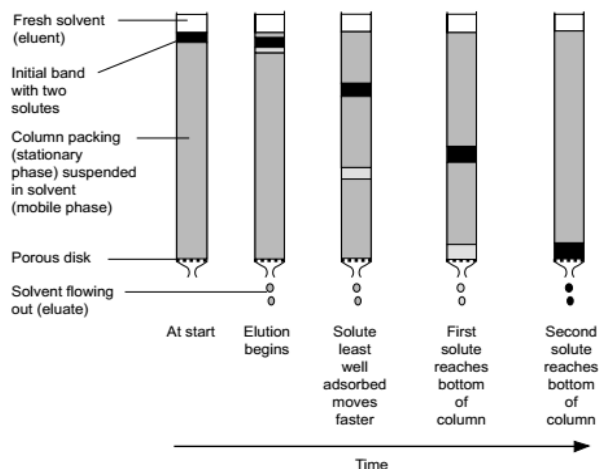


Figure 1 Adsorption chromatography using a column

2. Partition

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 a 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 (Fig. 2). Paper chromatography is an example of partition chromatography.

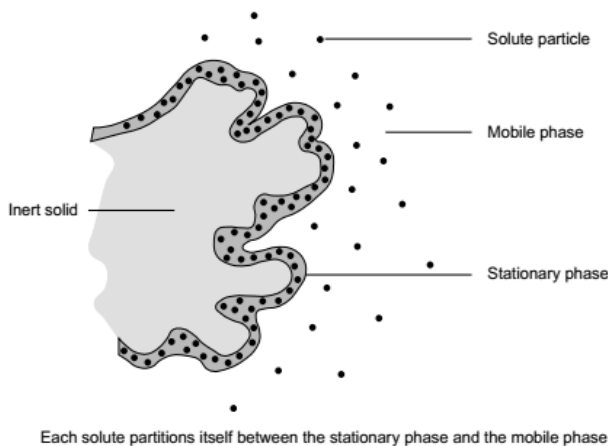


Figure 2 Partition chromatography

3. Ion exchange

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 electrostatically bound ions are released as other ions are bonded preferentially (Fig. 3). Domestic water softeners work on this principle.

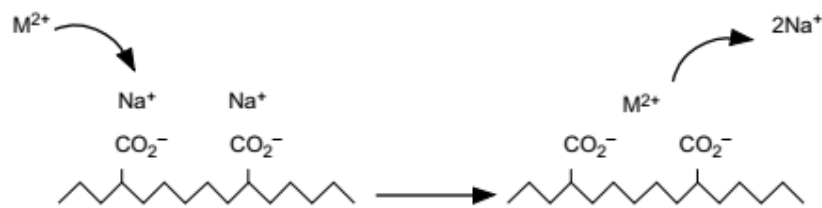


Figure 3 Ion exchange chromatography

4. Molecular exclusion

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 the column. Thus separation is according to particle size (Fig. 4).

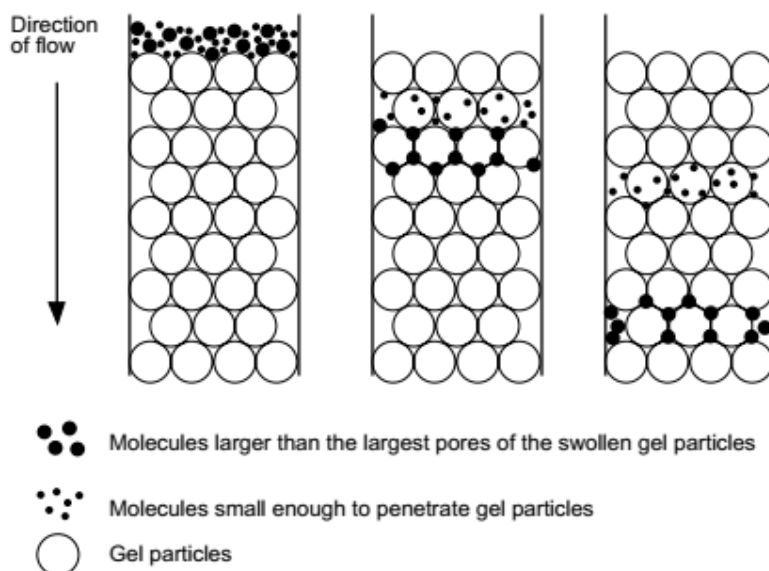


Figure 4 Gel permeation chromatography