

Gel-filtration chromatography

Size-exclusion chromatography (SEC) is a chromatographic method in which molecules in solution are separated by their size, and in some cases molecular weight. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers. Typically, when an aqueous solution is used to transport the sample through the column, the technique is known as **gel-filtration chromatography**, versus the name **gel permeation chromatography**, which is used when an organic solvent is used as a mobile phase. SEC is used primarily for the analysis of large molecules such as proteins or polymers. SEC works by trapping smaller molecules in the pores of the adsorbent materials adsorption ("stationary phases"). The larger molecules simply pass by the pores because those molecules are too large to enter the pores. Larger molecules therefore flow through the column more quickly than smaller molecules, that is, the smaller the molecule, the longer the retention time.

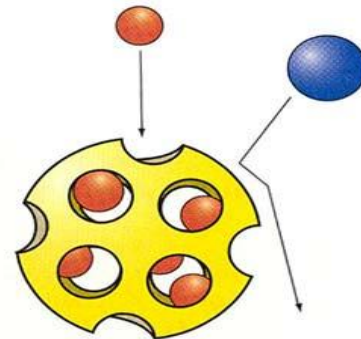
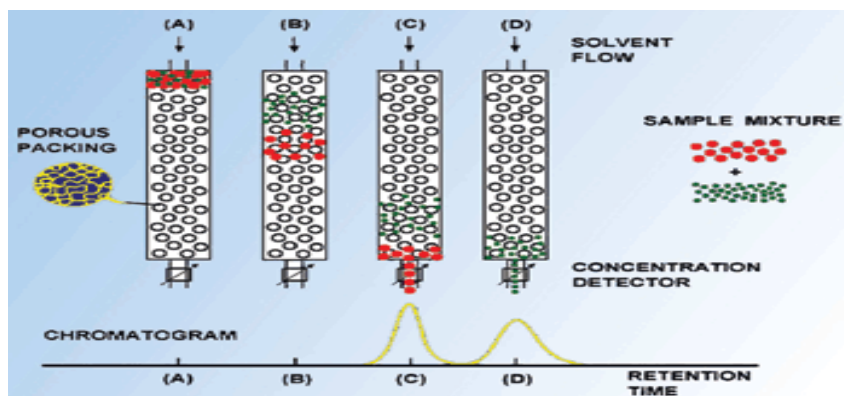


Figure Gel filtration chromatography. (a) Principle of the method. A resin bead is schematically represented (yellow). Large molecules (blue) cannot fit into the beads and are confined to the relatively small buffer volume outside the beads; they emerge quickly from the column. Small molecules, in contrast, can fit into the beads and so have a large



- The gels used as molecular sieves are cross linked polymers. They are uncharged and inert. don't bind or react with the materials being analyzed.

The types of gels are used:

1. *Dextran*: is a homopolysaccharide of glucose residues.
 - it's prepared with various degrees of cross-linking to control pore size.
 - It's bought as dry beads, the beads swell when water is added.
 - The trade name is sephadex.

It's mainly used for separation of small peptides and globular proteins with small to average molecular mass

2. *Agarose*: linear polymers of D-galactose and 3,6 anhydro-1-galactose.

It forms a gel that's held together with H bonds. It's dissolved in boiling water and forms a gel when it's cold. The concentration of the material in the gel determines the pore size. The pores of agarose gel is much larger than these of sephadex . It's useful for analysis or separation of large globular proteins or long linear molecules such as DNA

Advantages of Gel filtration

1. It's the best method for separation of molecules differing in molecular weight because:
2. It doesn't depend on temperature, pH, ionic strength and buffer composition. So separation can be carried out under any conditions.
3. There is very little adsorption
4. The elution volume is related to the molecular weight

Application of gel filtration

Purification of enzymes and other proteins.

Gel Electrophoresis

- Electrophoresis is a method whereby charged molecules in solution, chiefly proteins and nucleic acids, migrate in response to an electrical field. Their rate of migration through the electrical field, depends on the strength of the field, on the net charge, size, and shape of the molecules, and also on the ionic strength, viscosity, and temperature of the medium in which the molecules are moving. As an analytical tool, electrophoresis is simple, rapid and highly sensitive. It can be used analytically to study the properties of a single charged species or mixtures of molecules. It can also be used preparatively as a separating technique . Electrophoresis is the movement of molecules by an electric current. Nucleic acid moves from a negative to a positive pole. The movement of molecules is impeded in the gel so that molecules will collect or form a band according to their speed of migration. The concentration of gel/buffer will affect the resolution of fragments of different size ranges. Electrophoresis is usually done with gels formed in tubes, slabs, or on a flat bed. In many electrophoresis units, the gel is mounted between two buffer chambers containing separate electrodes, so that the only electrical connection between the two chambers is through the gel.

Electrophoresis Buffers

- **Tris Borate EDTA (TBE),**
- **Tris Acetate EDTA (TAE),**
- **Tris Phosphate EDTA (TPE)**
used most often for DNA.
- **sodium phosphate buffer used for RNA.**