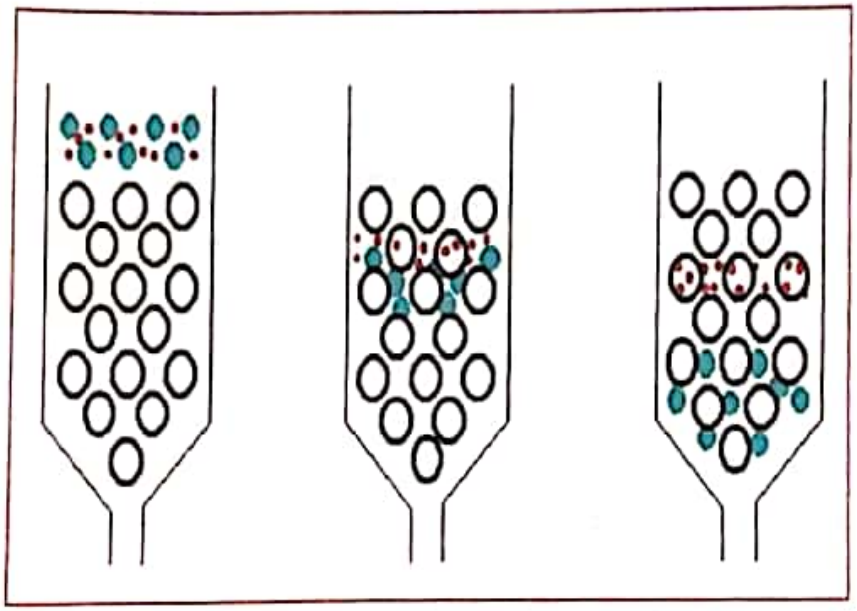
Basic principle

- **Gel permeation chromatography (GPC)** is a type of size exclusion chromatography (SEC), that separates analytes on the basis of size; large molecules emerge first from the bed, while smaller molecules are retarded. The separation of the components of a mixture by gel chromatography is based on the differences in the molecular sizes of the components. Small molecules tend to diffuse into the interior of the porous particles so that their flow is restricted, while large molecules are unable to enter the pores and tend to flow unhindered. Thus, the components of highest molecular weight leave the bed first, followed by successively smaller molecules.



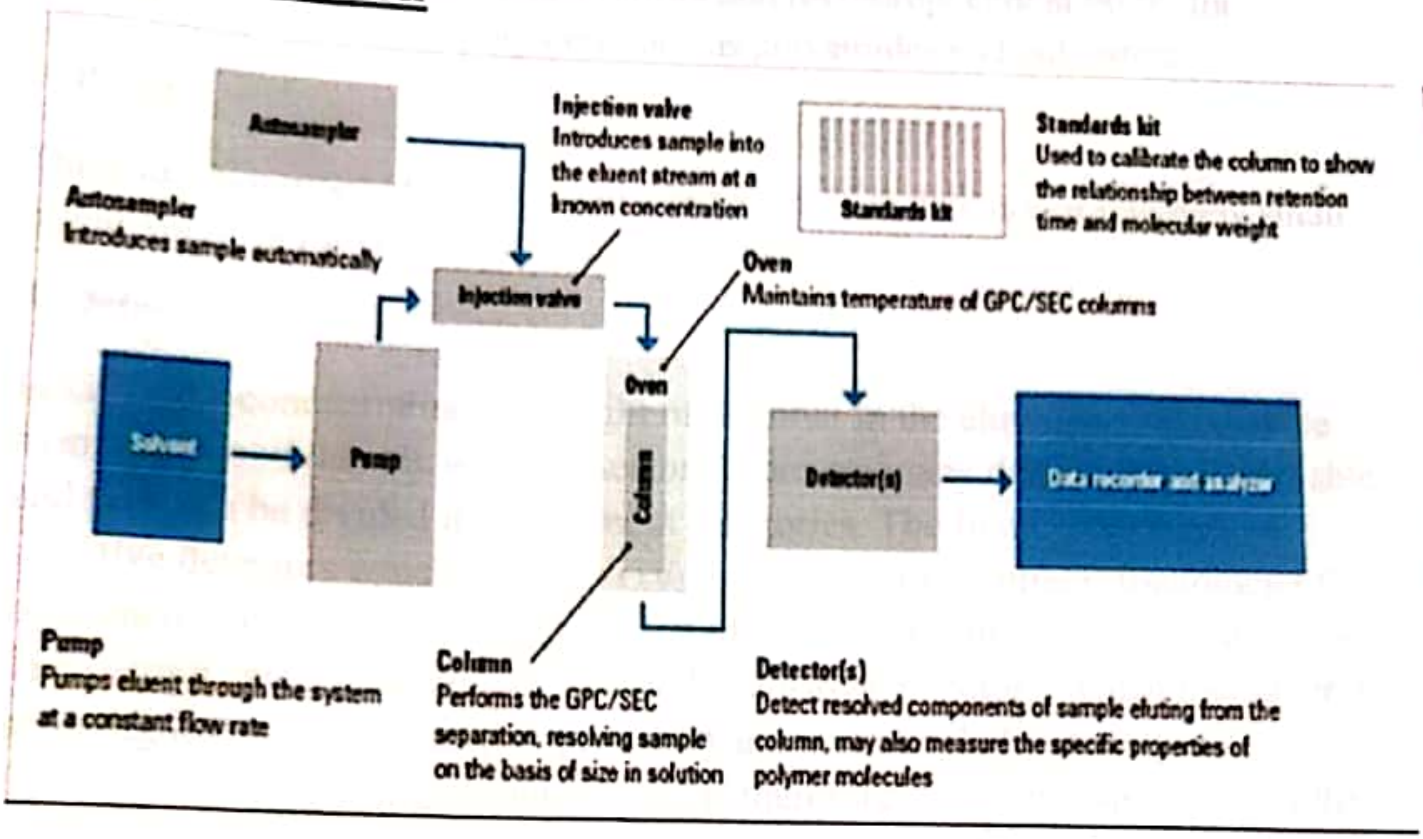
STATIONARY PHASE

It consists of beads of porous poylmeric material. The materials most extensively used are polyacrylamide and a polymer prepared from dextran and epichlorohydrin.

MOBILE PHASE

Organic Solvent is used as the mobile phase

Instrumentation



1. Gel

Gels are used as stationary phase for GPC. The pore size of a gel must be carefully controlled in order to be able to apply the gel to a given separation. Other desirable properties of the gel forming agent are the absence of ionizing groups and, in a given solvent, low affinity for the substances to be separated. Commercial gels like PLgel, Sephadex, Bio-Gel (cross-linkedpolyacrylamide), agarose gel and Styragel are often used based on different separation requirements.

2. Column

The column used for GPC is filled with a microporous packing material. The *column is filled with the gel.*

3. Eluent

The eluent (mobile phase) should be a good solvent for the polymer, should permit high detector response from the polymer and should wet the packing surface. The most common eluents in for polymers that dissolve at room temperature GPC are tetrahydrofuran (THF), *o*-dichlorobenzene and trichlorobenzene at 130–150 °C

for crystalline polyalkynes and *m*-cresol and *o*-chlorophenol at 90 °C for crystalline condensation polymers such as polyamides and polyesters.

4. Pump

There are two types of pumps available for uniform delivery of relatively small liquid volumes for GPC: piston or peristaltic pumps.

5. Detector

In GPC, the concentration by weight of polymer in the eluting solvent may be monitored continuously with a detector. There are many detector types available and they can be divided into two main categories. The first is concentration sensitive detectors which includes UV absorption, differential refractometer (DRI) or refractive index (RI) detectors, infrared (IR) absorption and density detectors. The second category is molecular weight sensitive detectors, which include low angle light scattering detectors (LALLS) and multi angle light scattering (MALLS).The resulting chromatogram is therefore a weight distribution of the polymer as a function of retention volume.

Working

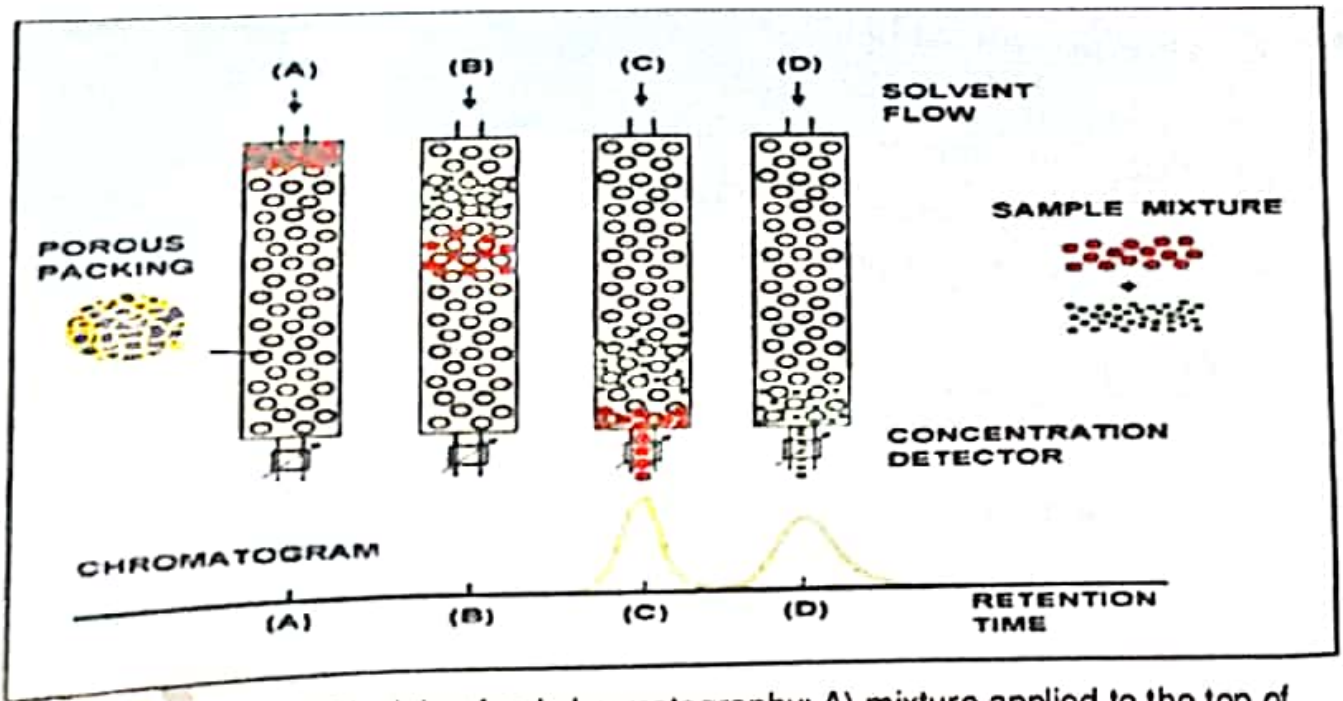
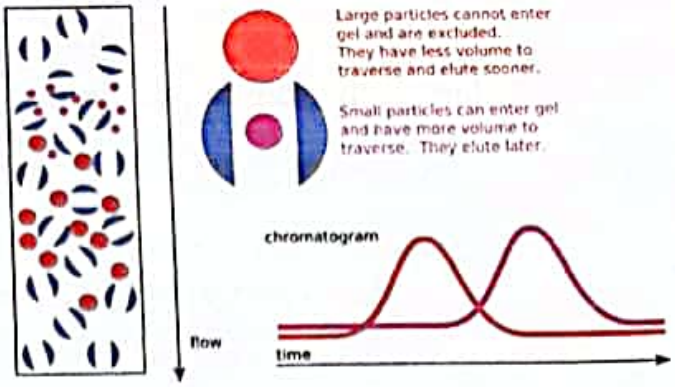


Figure : Principle of gel chromatography: A) mixture applied to the top of the column; B) partial separation; C) complete separation; D)excluded substance emerges from the column.

Gel filtration chromatography is a separation based on size. It is also called molecular exclusion or gel permeation chromatography. Solvent is pumped by a pump into the column. On its way injection valve adds sample into the eluent stream and this mixture then moves to the column. The column is placed inside the oven that maintains the temperature. The column is the responsible for the separation of the molecules on the basis of their size. The larger molecules are filtered quicker then the smaller molecules. The separation of these molecules on the basis of size is detected by a detector and the data is the recorded and analyzed.



Applications

- Separation of large molecular weight compound as protein, carbohydrate, peptides, nucleic acids
- Desalting colloids.
  Small size of contaminating salt will allow them to diffuse inside the gel particles e.g desalting of albumin from 25% ammonium sulfate.
- Molecular weight determination.
  A linear relationship exists between the logarithm of the molecular weight and the elution volume
- It can be used to either fractionate molecules and complexes in a sample into fractions with a particular size range, to remove all molecules larger than a particular size range from the sample, or a combination of both operations.
- Gel filtration chromatography can be used to separate compounds such as small molecules, proteins, protein complexes, polysaccharides, and nucleic acids when in aqueous solution.
- Gel filtration chromatography is used fractionation of molecules and complexes within a predetermined size range
- It is used for size analysis and determination
- Removal of large proteins and complexes
- Buffer exchange

- For Desalting
- Removal of small molecules such as nucleotides, primers, dyes, and contaminants
- Assessment of sample purity
- Separation of bound and unbound radioisotopes
- Separation of sugars polypeptides, proteins, lipids, butyl rubbers, polystyrenes, silicon polymers
- Sephadex G-25
  For separation of salts and amino acids from proteins
- Sephadex G-75
  Fractionation and purification of proteins polysaccharides and nucleic acid

Advantages

1. Has well defined separation time
2. Can provide narrow bands
3. Low chance for analyte loss
4. Determination of molecular weight of polymers
5. Less time of analysis

Disadvantages

1. Requires at least 10% difference in molecular weight for reasonable resolution of peaks
2. Pre-filtration of sample