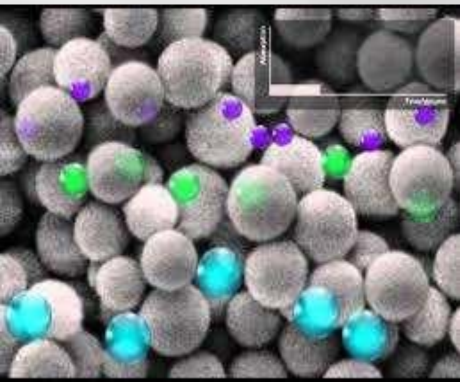
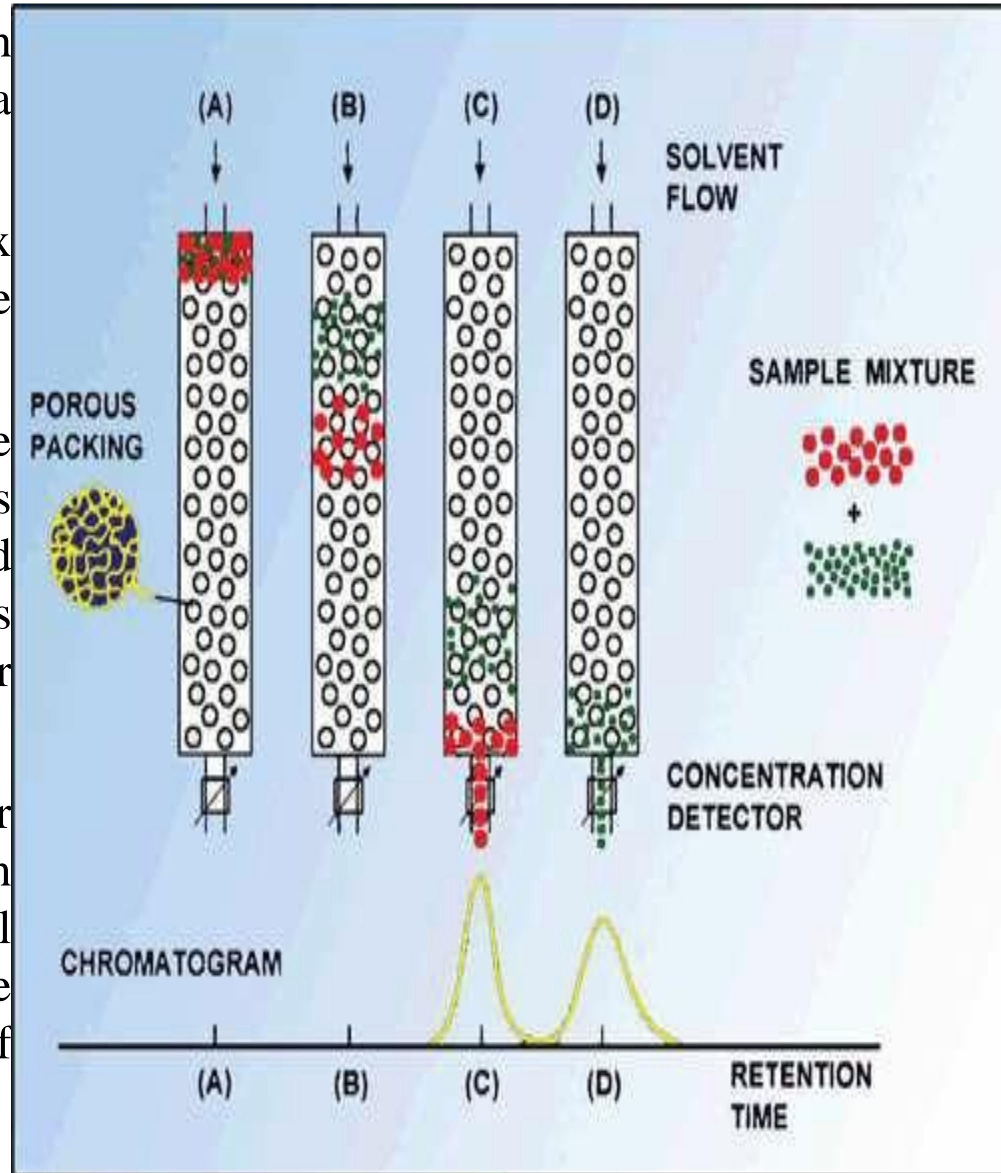
GEL PERMEATION CHROMATOGRAPHY (GPC)



Principle of separation

- It's a technique that separates dissolved molecules on the basis of their size by pumping these molecules through specialized columns containing a microporous packing material(gel).
- Stationary phase is a porous polymer matrix whose pores are completely filled with the solvent to be used as the mobile phase.
- The pore size is highly critical, since the basis of the separation is that molecules above a certain size are totally excluded from the pores, and the interior of the pores is accessible, partly or wholly, to smaller molecules.
- The flow of mobile phase will cause larger molecules to pass through the column unhindered, without penetrating the gel matrix, whereas smaller molecules will be retarded according to their penetration of the gel.



Theory of separation

A column is made up of swollen gel particles and the solvent used to swell the gel in a suitable tubular container.

An equation is given below:

$V_t = V_0 + V_i + V_m$

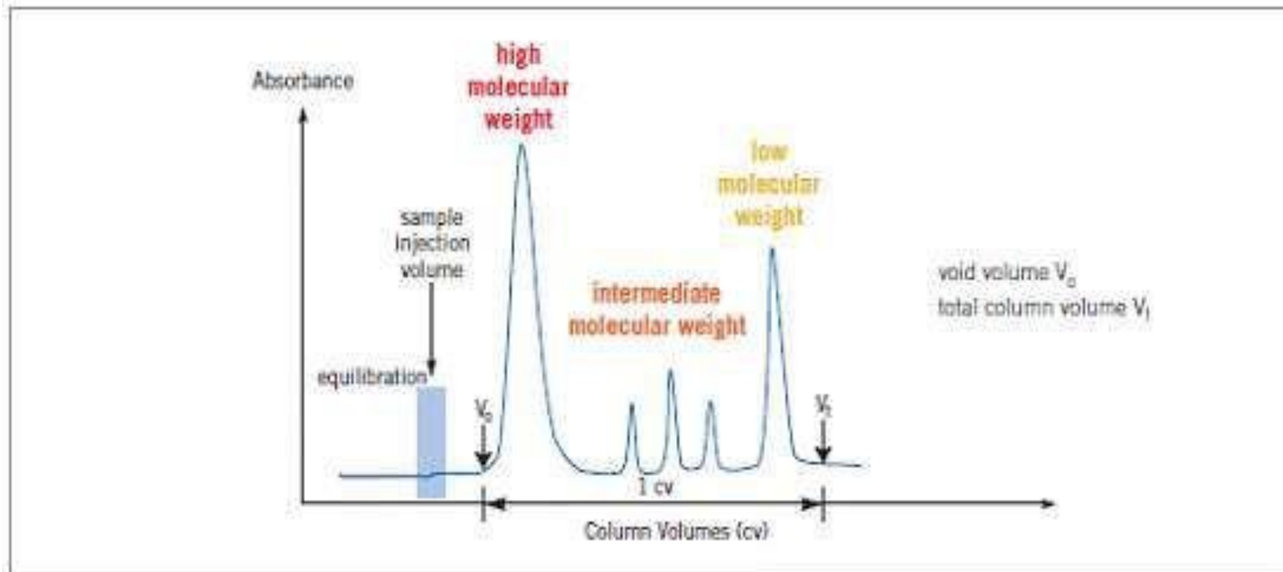
where,

V_t = the total volume of the column (which can be measured),

V_0 = the volume of liquid outside the gel matrix (known also void or dead volume),

V_i = the volume of liquid inside the matrix,

V_m = the volume of the gel matrix



GPC components

1. Stationary Phase
2. The Mobile Phase
3. The Columns
4. The Pump
5. Detectors

1. Stationary phase:

Composed of semi-permeable, porous polymer gel beads with well defined range of pore sizes .

Properties of gel beads:

1 Chemically inert

2 Mechanically stable

3 Has ideal and homogeneous porous structure (wide pore size give low resolution). 4- Uniform particle and pore size.

5- The pore size of the gel must be carefully controlled.

Examples of gel

- Dextran(Sephadex) gel: An α 1-6-polymer of glucose natural gel
- Agarose gel: A 1,3 linked β-D-galactose and 1,4 linked 3,6-anhydro-α, L-galactose natural gel
- Acrylamide gel: A polymerized acrylamide, a synthetic gel

2. The Mobile Phase

Composed of a liquid used to dissolve the biomolecules to make the mobile phase permitting high detection response and wet the packing surface.

Material	Solvent
Synthetic elastomers (polybutadiene , polyisoprene)	Toluene
PS, PVC, Styrene-Butadiene Rubber , Epoxy resins	Tetrahydrofuran (THF)
Polyolefins	Tri- chloro -benzene
Polyurethane	Di- methylformamide (DMF)
Proteins, polysaccharides	Water / Buffers

3. Columns

Commercially Available Columns include

- Analytical column- 7.5–8mm diameters.
- Preparative columns-22–25mm
- Usual column lengths-25, 30, 50, and 60 cm.
- Narrow bore columns- 2–3mm diameter have been introduced

4. The pump

Are either syringe pumps or reciprocating pumps with a highly constant flow rate.

5. Detectors

Concentration sensitive detectors

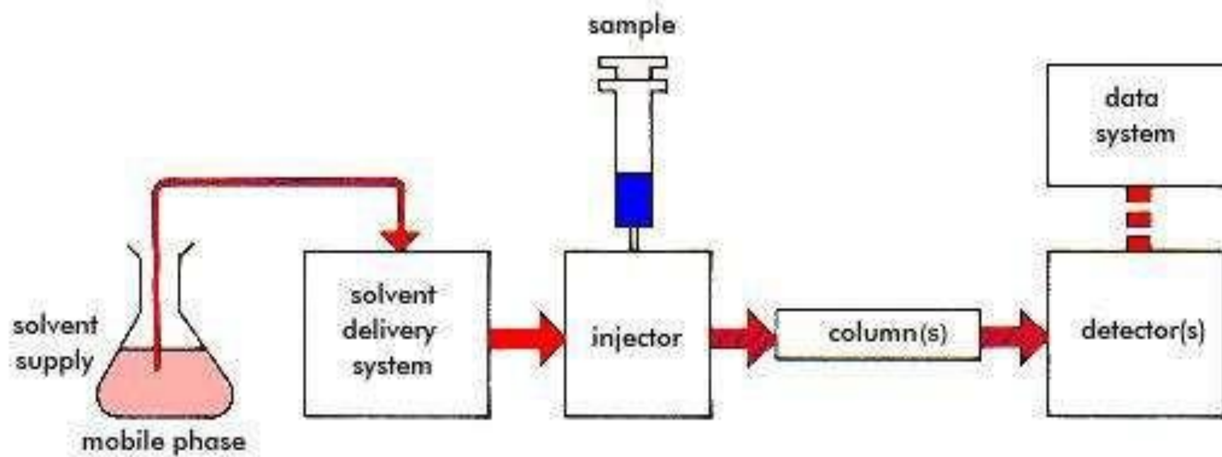
- Bulk Property Detectors- Refractive Index (RI) Detector
- Solute Property Detectors- Ultraviolet (UV) Absorption Detector
- Evaporative Detectors- Evaporative Light Scattering Detector (ELSD)

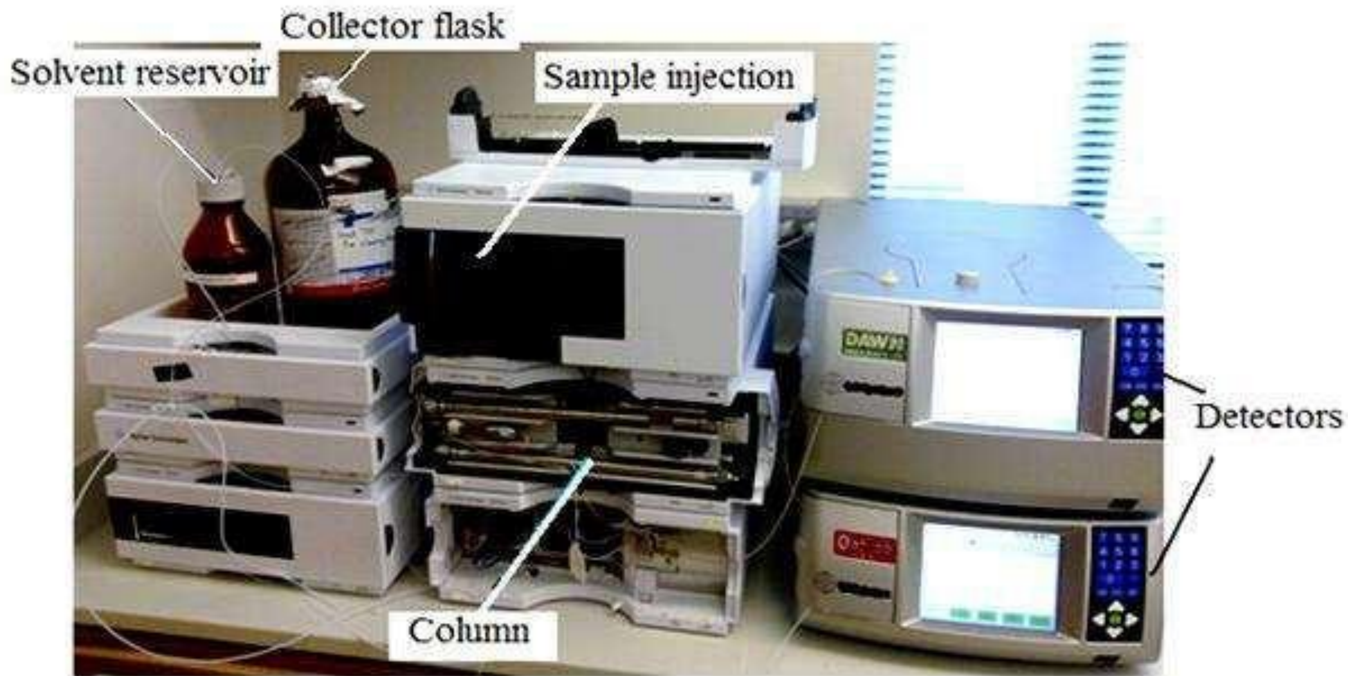
Molar mass sensitive detectors

1. Light Scattering Detectors

- Low Angle Light Scattering (LALS) Detectors
- Multiangle Light Scattering (MALS) detectors

2. Viscosity Detectors- Differential Viscometers


sample
data
system
solvent
supply
solvent
delivery
system
injector
column(s)
detector(s)
mobile phase


Collector flask
Solvent reservoir
Sample injection
Detectors
Column

Advantages and disadvantages

Advantages:

- Short analysis time.
- Well defined separation.
- Narrow bands and good sensitivity.
- There is no sample loss.
- Small amount of mobile phase required.
- The flow rate can be set.

Disadvantages:

- Limited number of peaks that can be resolved within the short time scale of the GPC run.
- Filtrations must be performed before using the instrument to prevent dust and other particulates from ruining the columns and interfering with the detectors.
- The molecular masses of most of the chains will be too close for the GPC separation to show anything more than broad peaks.