



GEL FILTRATION: PRINCIPLE, METHODOLOGY & APPLICATIONS

Dr. Rashmi. R
Asst. Professor
UG Dept. of Biochemistry
JSSCACS, Ooty road, Mysuru

GEL CHROMATOGRAPHY

- Gel filtration chromatography is used to **separate proteins, peptides and oligo-nucleotides** on the basis of their size only.
 - It is also known as **gel permeation, molecular sieving or size exclusion** chromatography.
 - In gel filtration, as **sample moves through a bed of porous beads**, molecules of different size diffuse into the beads to greater or lesser extent.
 - Small molecules enter further into the beads and thus, **elute slowly**.
 - In comparison to that, large molecules enter less or move directly through the void space and thus, **eluting fast**.
- 

- Gel Filtration Chromatography is widely applied for molecular size analysis, separation of components of a mixture, as well as for desalting.

1. PRINCIPLE OF SEPERATION

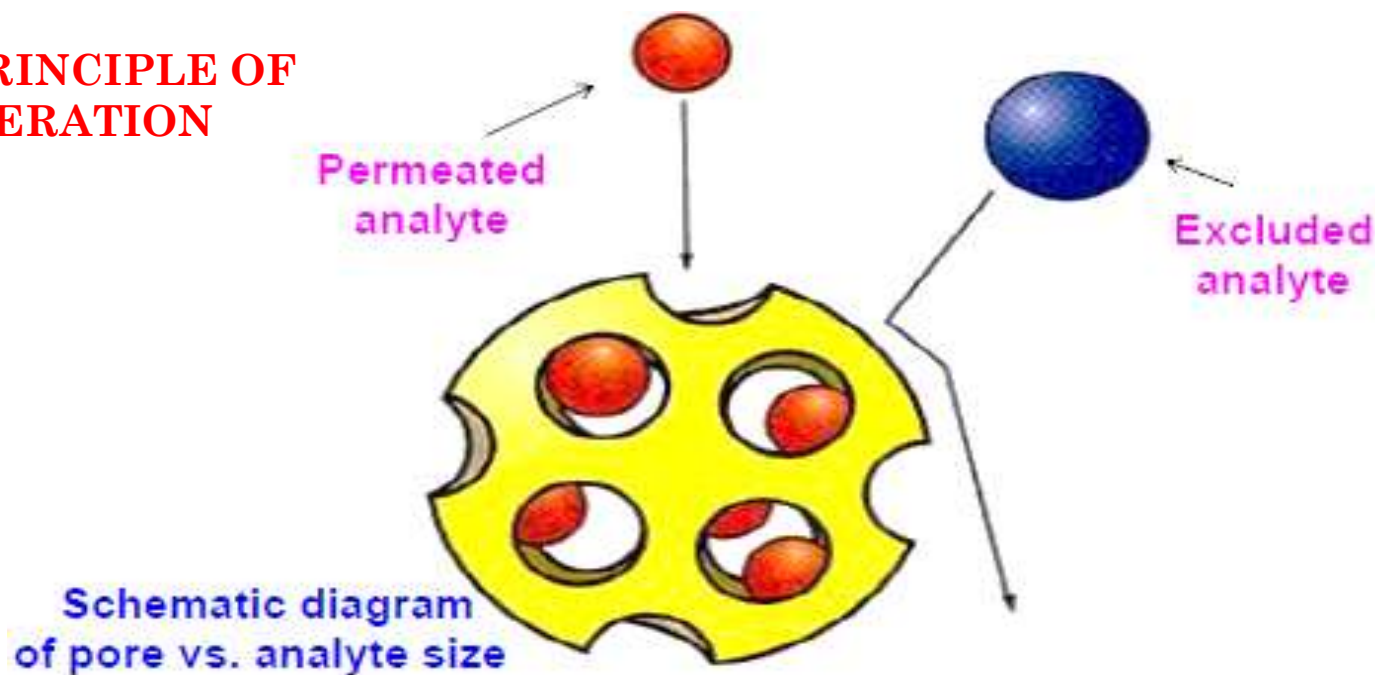


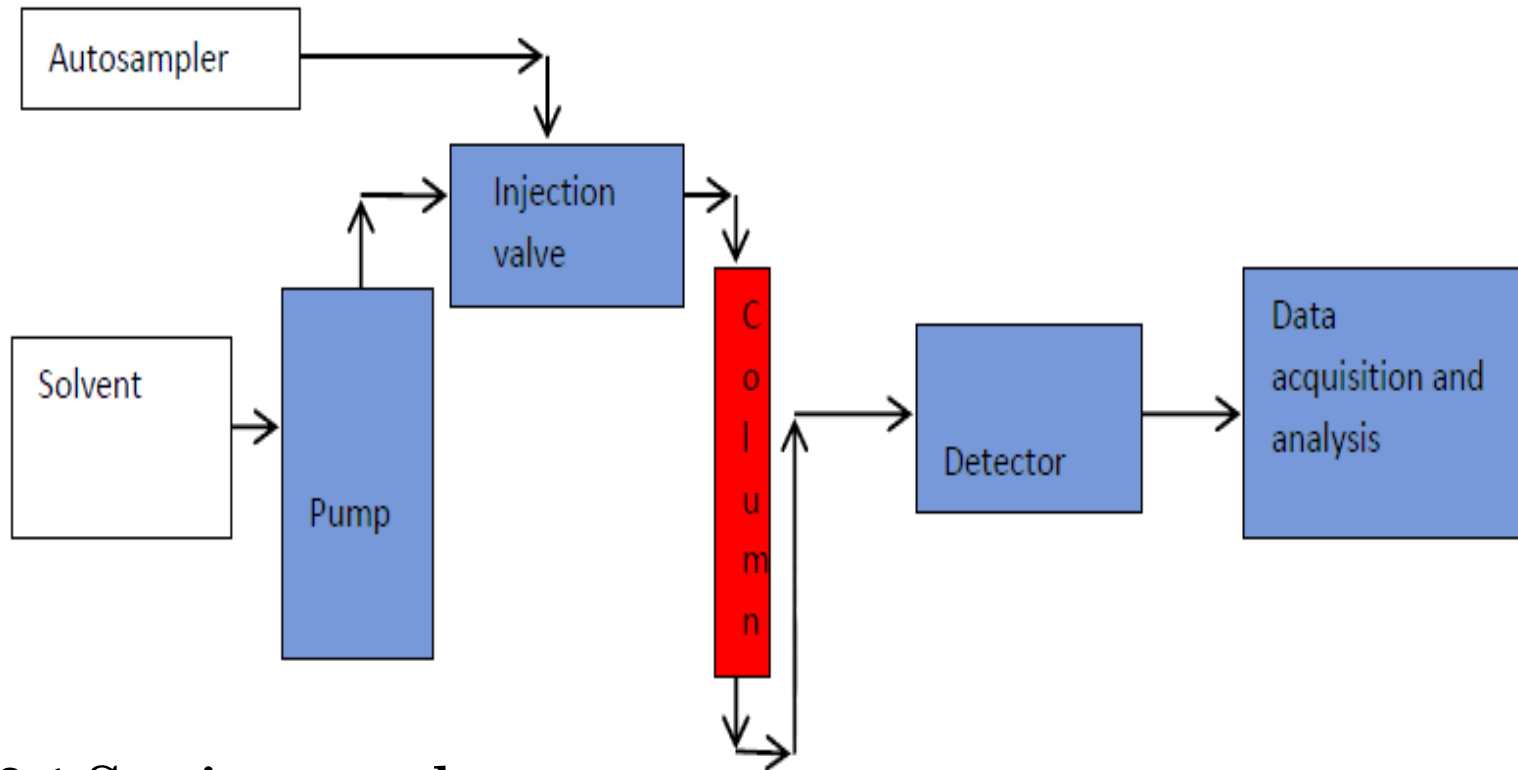
Figure Gel filtration chromatography. (a) Principle of the method. A resin bead is schematically represented as a “whiffle ball” (yellow). Large molecules (blue) cannot fit into the beads, so they are confined to the relatively small buffer volume outside the beads. Thus, they emerge quickly from the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

SEPARATION PROCESS OF COMPONENTS OF SAMPLE DURING GEL FILTRATION

- Trapping of the mobile phase inside the beads solely depends upon the porosity of the resin beads and the size of the molecules to be separated.
- Thus, the distribution of a molecule in a gel filtration column of beads is determined by the total volume of mobile phase, both inside and outside the beads.



INSTRUMENTATION OF GEL FILTRATION CHROMATOGRAPHY



- 3.1 Stationary phase
- 3.2 Mobile phase
- 3.3 Preparation of sample
- 3.4 Column (for separation)
- 3.5 Pump (maintain constant flow rate)
- 3.6 Detector (Quantify the result)



PRINCIPLE

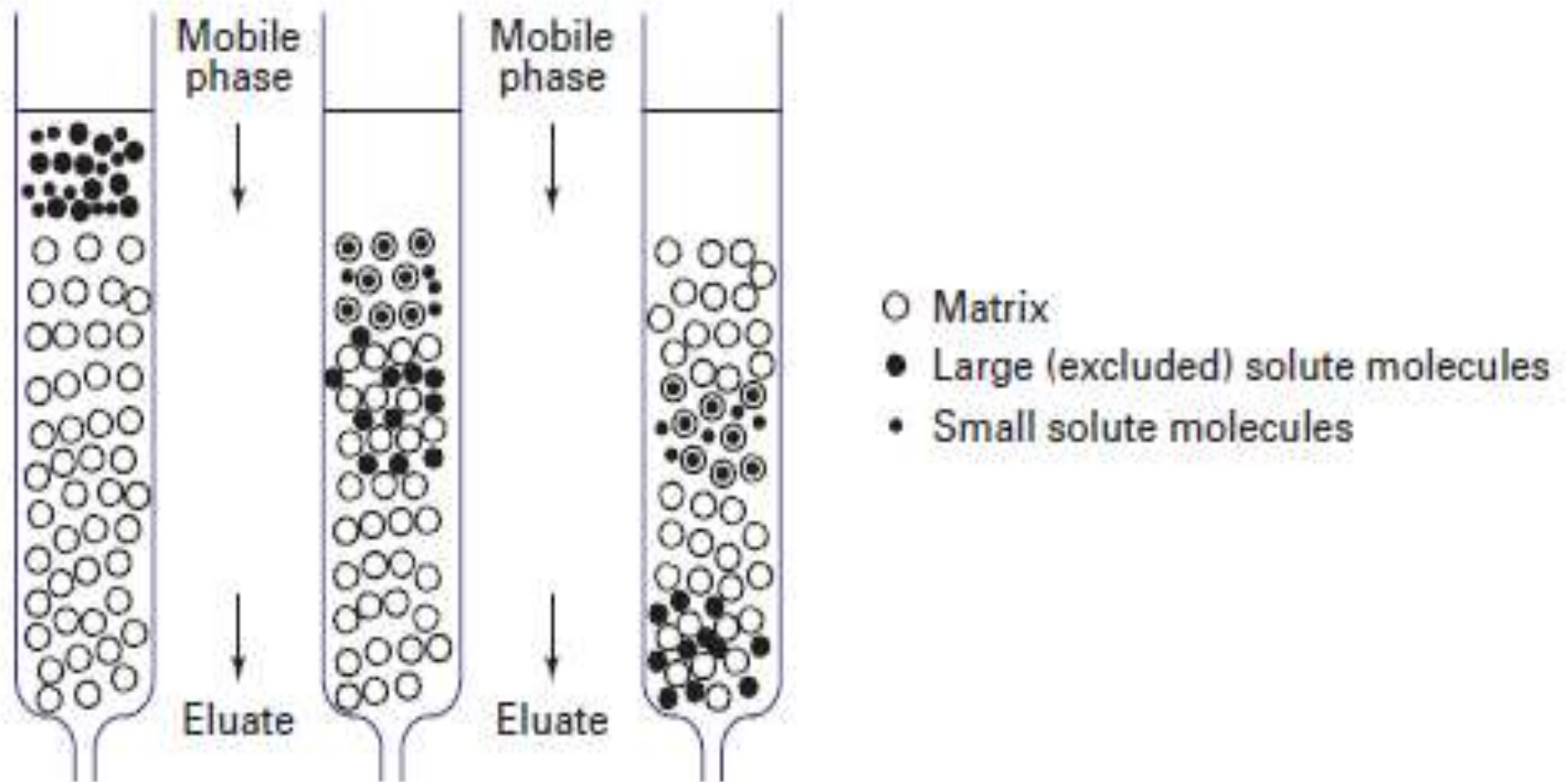
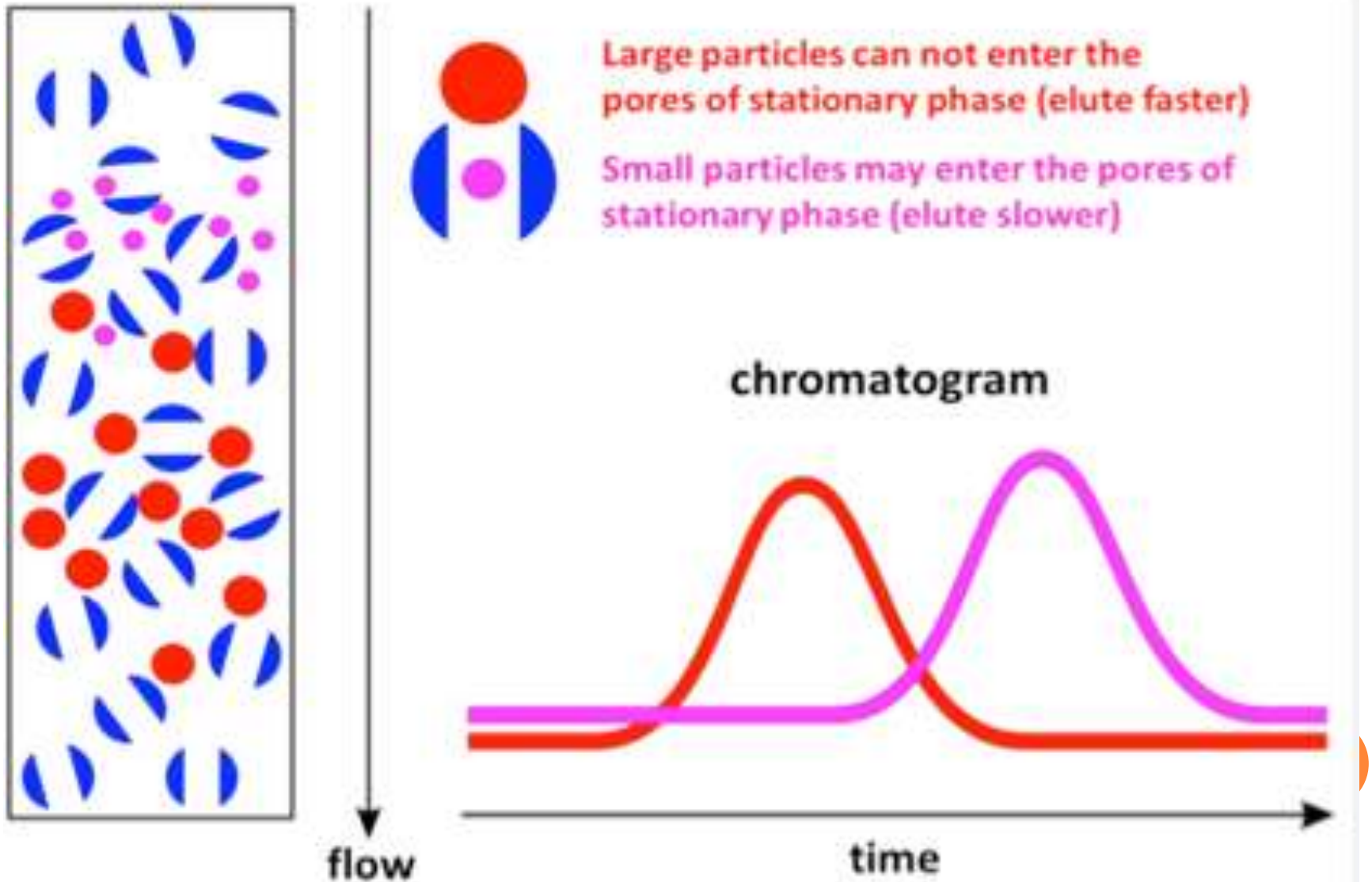
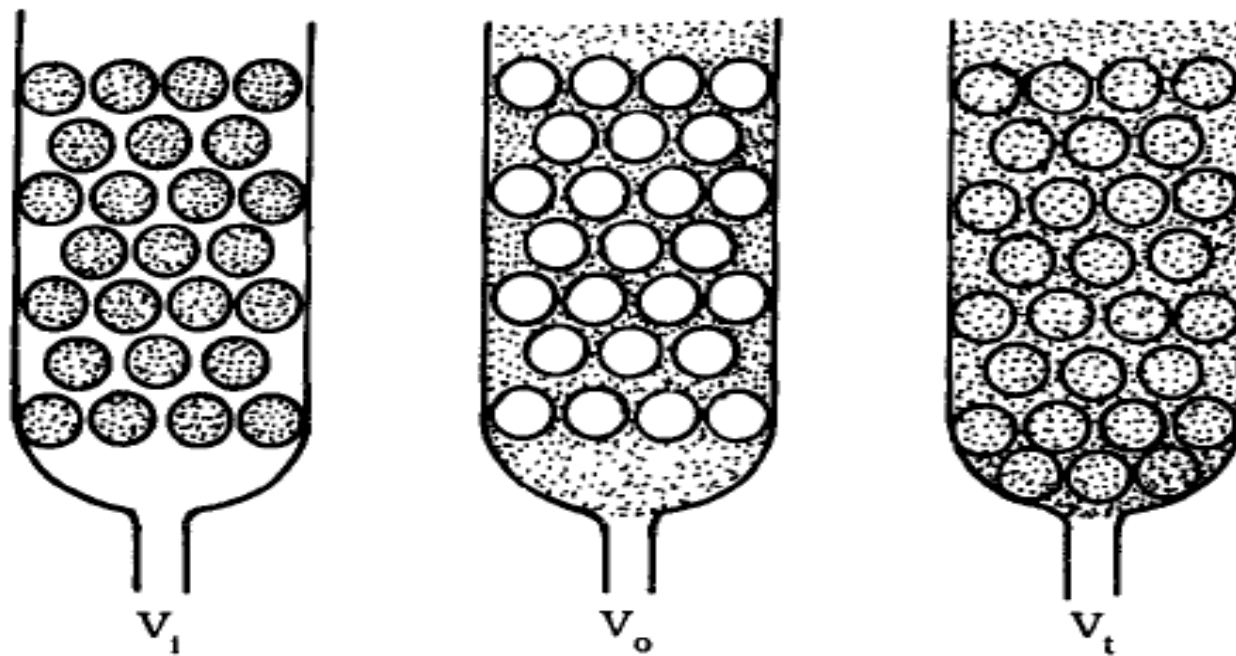


Fig. 11.14. Separation of different size molecules by exclusion chromatography. Large (excluded) molecules are eluted first in the void volume.

RESULT GRAPH



- In case of gel filtration chromatography, the **distribution coefficient of a molecule (K_d)** between the **inner and outer mobile phase** is a function of its molecular size.
 - If a **molecule is sufficiently large** to completely exclude from the mobile phase **between the beads**, then **$K_d = 0$** .
 - On the other hand, if a molecule is so **small that it accesses to the innermost mobile phase** deep in the beads, then **$K_d = 1$** .
 - For others, value of K_d will vary between **0 and 1**.
 - This variation of K_d between 0 and 1 makes it possible to separate various molecules present in a mixture within a narrow molecular size range.
- 



Diagrammatic representation of –

- **Solvent inside the gel bead (V_i - internal volume),** the volume inside the gel beads accessible to smaller molecules.
- **Void volume (V_o)** –volume of solvent outside the bead
- **total volume (V_t)**- In gel filtration chromatography, " V_t " represents the total volume of the column, which includes both the liquid volume between the gel beads (void volume) and the volume occupied by the gel beads themselves; essentially, it's the entire volume of the packed gel bed within the column.
- **Knowing the total volume is crucial for determining the elution volume of a molecule on the column and for accurately interpreting the results of a gel filtration experiment.**

3.1 STATIONARY PHASE

- There are **five principal types** of media that fulfill the criteria to quite some extent.

They include

- (i) cross-linked **dextrans** (trade name **Sephadex**).
 - (ii) **agarose** (Sephарose, Bio-Gel A,),
 - (iii) **polyacrylamide** (Bio-Gel P),
 - (iv) **porous glass and silica granules** (Bio-Glas Porasil), and
 - (v) **polystyrene** (Styragel, Bio-Beads S).
- 

TABLE : DIFFERENT COMMERCIALLY AVAILABLE MEDIA FOR GEL FILTRATION CHROMATOGRAPHY

Sl No.	Type of media	Commercial name	Molecular weight range (kDa)
1	Dextran	Sephadex G-50	1.5-30
		Sephadex G-75	3-80
		Sephadex G-100	4-150
		Sephadex G-200	5-600
2	Polyacrylamide	Bio-Gel P-10	1.5-20
		Bio-Gel P-30	2.5-40
		Bio-Gel P-100	5-100
		Bio-Gel P-150	15-150
		BioGel P-200	30-200
3	Dextran-polyacrylamide gels	Sephacryl S-200	5-250
		Sephacryl S-300	10-1500
		Sephacryl S-400	20-8000
4	Agarose	Sepharose 6B	10-4000
		Sepharose 4B	60-20,000
		Bio-Gel A-0.5	10-500
		Bio-Gel A-1.5	10-1500
		Bio-Gel A-5	10-5000

3.2 MOBILE PHASE OR BUFFER SELECTION

- Most commonly **Chloroform, toluene, dimethyl formamide** and mixed solvents are used to separate various components.
- In case of **proteins and polysaccharides**, **aqueous buffers** are used as mobile phase.



3.3 PREPARATION OF SAMPLE

- A sample is dissolved in an appropriate solvent to get its dilute solution usually **less than 1mg/mL**.
- The samples are **filtered before loading** into the instrument to prevent the clogging.



3.4 COLUMNS

- The columns of various lengths and diameters are available commercially.
- **Small columns are recommended to save the time and solvent.**



3.5 DETECTOR

- The detectors used with GFC are either concentration sensitive detectors like,
- **UV-Vis detectors** and
- **Evaporative light scattering detectors**, or
- **Molar mass sensitive detectors** like low angle light scattering detectors, multiangle light scattering detectors and **viscosity detectors**.



5. APPLICATION OF GEL FILTRATION

- *i) Molecular weight determination* –
 - The molecular weight of a given protein is estimated.
- *(ii) Separation of macromolecules* – Gel filtration chromatography is commonly applied in research laboratories for **separation of proteins and peptides**. It is applied for detection and **separation of oligomers**.
- *(iii) Group separation* - Gel filtration chromatography is also applied for **fractionation of crude samples** into low and high molecular weight protein groups.

1. Desalting: Remove salts from samples
2. Contaminant removal: Remove small molecules like nucleotides, primers, dyes, and other contaminants
3. Protein purification: Purify enzymes and other proteins
4. Nucleic acid purification: Purify nucleic acids
5. Polysaccharide purification: Purify polysaccharides



Thank You

