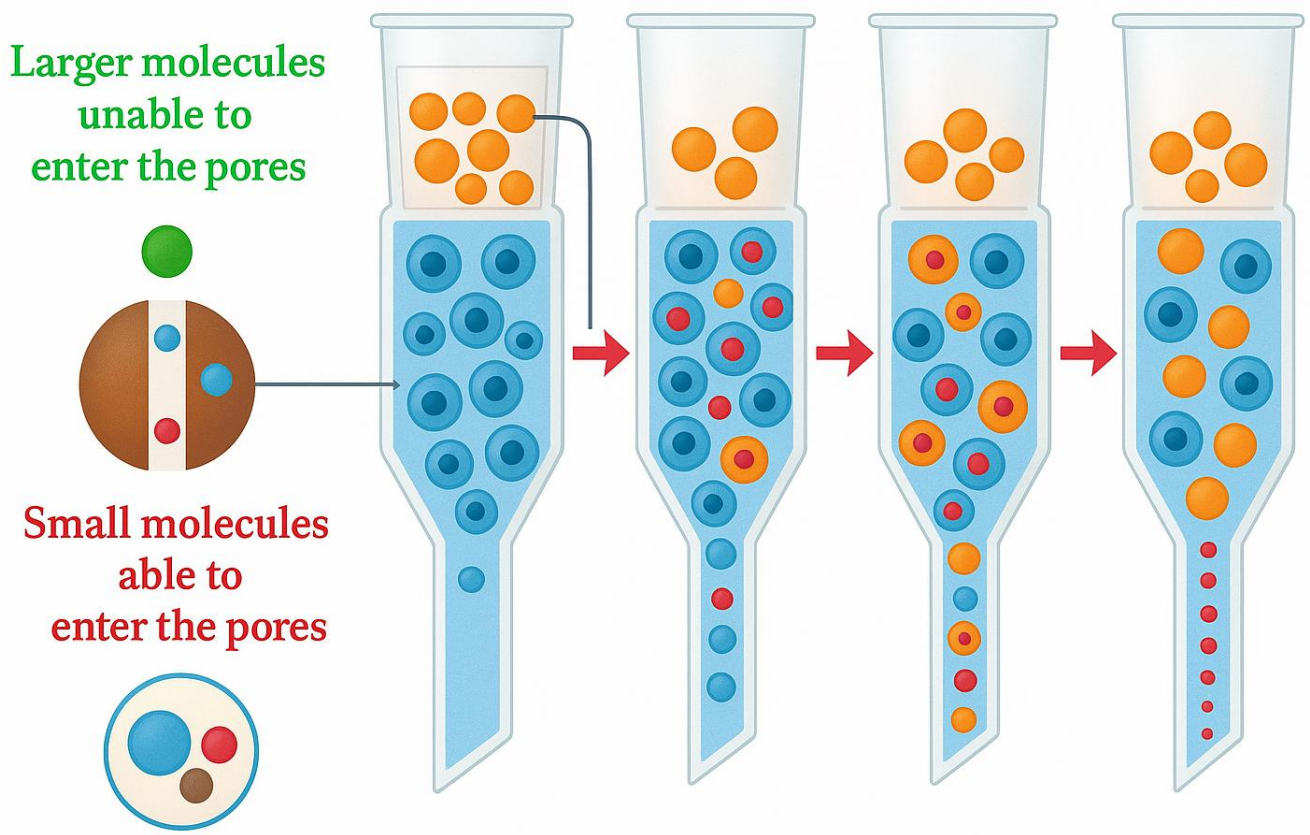
Gel Filtration Chromatography (Size Exclusion Chromatography)

Introduction:

- Gel Filtration Chromatography (GFC), also known as Size Exclusion Chromatography (SEC), is a technique that separates molecules based on their size (molecular weight) as they pass through a porous gel matrix.
    - No interaction occurs between the sample and the stationary phase.
    - Separation depends entirely on molecular size and shape.
- This method is widely used in biopharmaceutical analysis, particularly for the purification and molecular weight determination of proteins, peptides, nucleic acids, and polymer-based drugs.

Technique:



1. Stationary Phase:

- Packed column contains porous beads made of materials like:
    - Dextran (Sephadex)
    - Agarose (Sepharose)
    - Polyacrylamide (Bio-Gel)
    - Silica-based gels (for HPLC SEC)
- These beads have defined pore sizes that allow smaller molecules to enter the pores, while larger ones are excluded.

2. Mobile Phase:

- Typically, aqueous buffer or a suitable solvent that maintains protein/drug stability.
- No need for organic solvents or gradient elution.

3. Separation Mechanism:

- Molecules are larger than the pore size:
  - You cannot enter the beads.
  - Travel between the beads and elute first (shorter retention time).
- Molecules are smaller than the pore size:
  - Diffuse into pores.
  - Take a longer path, so elute later.
- Intermediate-sized molecules elute in between.

4. Elution:

- Isocratic elution (constant mobile phase composition) is used.
- Elution volume correlates with the logarithm of molecular weight.

Applications in Pharmaceutical Analysis:

A. Protein Purification

- Separation of monomers, dimers, aggregates of therapeutic proteins like insulin, monoclonal antibodies, etc.

B. Molecular Weight Determination

- Estimation of molecular weight of peptides, oligonucleotides, polysaccharides.

C. Polysaccharide and Polymer Analysis

- Used to analyze PEGylated drugs or polymeric excipients (e.g., PVP, HPMC).

D. Removal of Salts/Excipients

- Desalting proteins or enzymes by separating low molecular weight salts (which elute later).

E. Quality Control of Biopharmaceuticals

- Detection of aggregates or degradation products in formulations.

F. Liposome and Nanoparticle Analysis

- Separates encapsulated vs free drug, or particles based on size.

Advantages:

- Gentle, non-destructive method (no chemical interaction).

- Suitable for biomolecules and sensitive drugs.
- Predictable and reproducible separation.

Limitations:

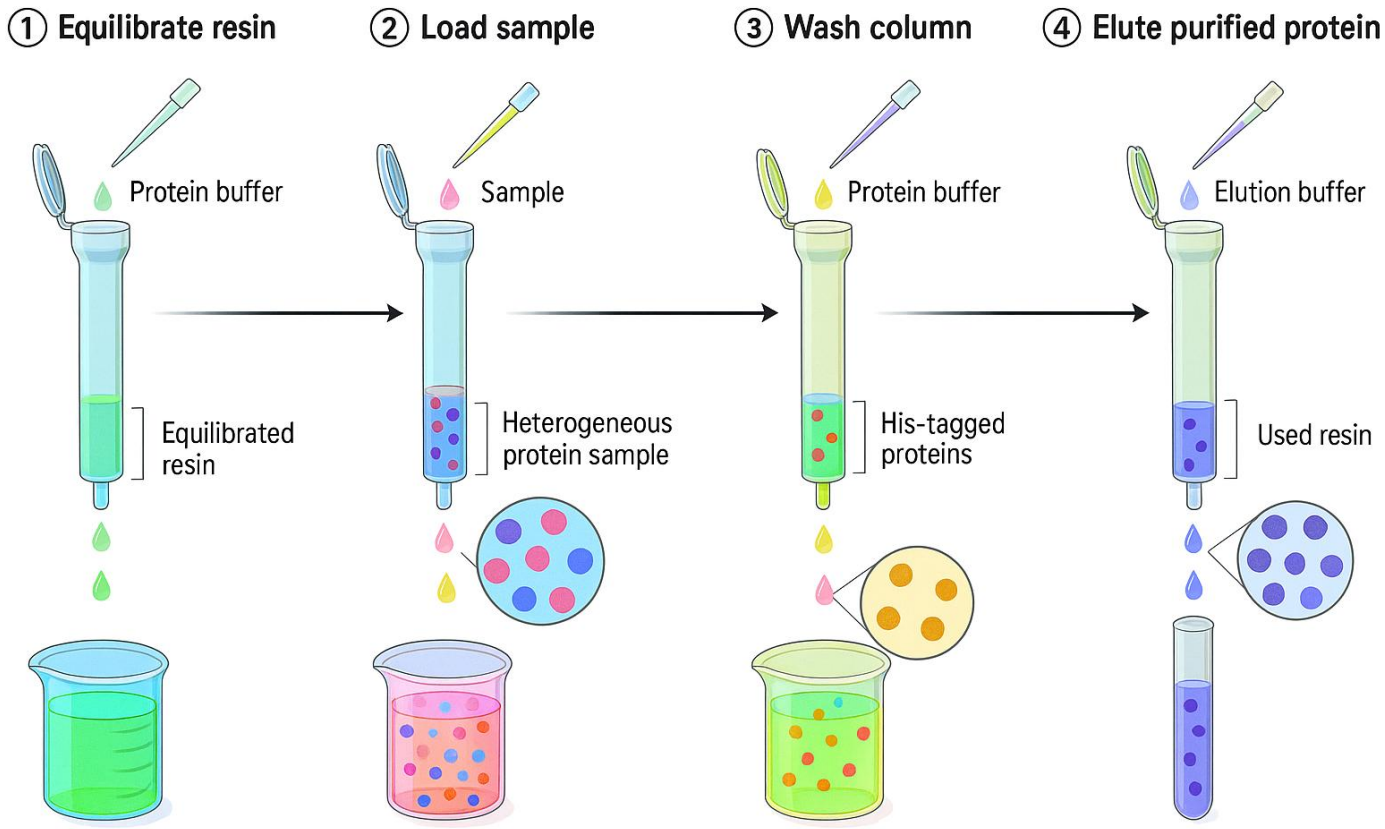
- Low resolution for molecules of similar size.
- Not ideal for complex mixtures with small size differences.
- Requires careful calibration.

Affinity Chromatography

Introduction:

- Affinity Chromatography is a highly selective separation technique that utilizes specific interactions between the target molecule (analyte) and a ligand attached to a stationary phase.
- Based on biochemical affinity between:
  - Enzyme ↔ Substrate
  - Antibody ↔ Antigen
  - Receptor ↔ Ligand
  - Metal ion ↔ His-tagged protein
- It is widely used in pharmaceutical biotechnology for purifying proteins, peptides, enzymes, antibodies, hormones, and other biological drugs.

Technique:



A. Stationary Phase:

- A solid matrix (usually agarose or sepharose) is covalently linked to a specific ligand.
- Ligands can be:
  - Antibodies
  - Enzyme substrates
  - Lectins

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