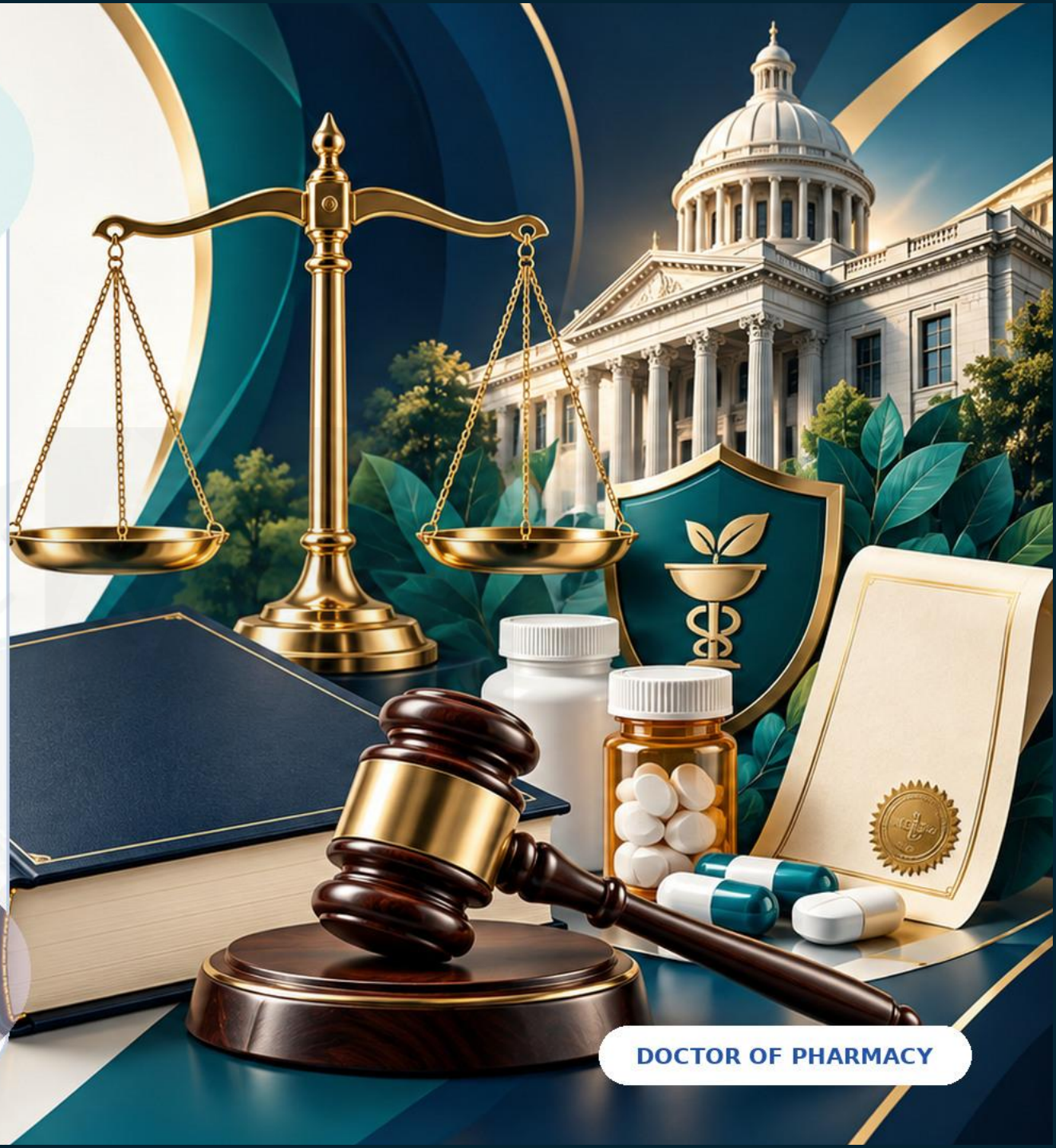
PHARM.D • YEAR 3

Pharmaceutical Analysis

CHAPTER 2.9

Gel Filtration and Affinity Chromatography

PPT CHAPTER COVER



DOCTOR OF PHARMACY

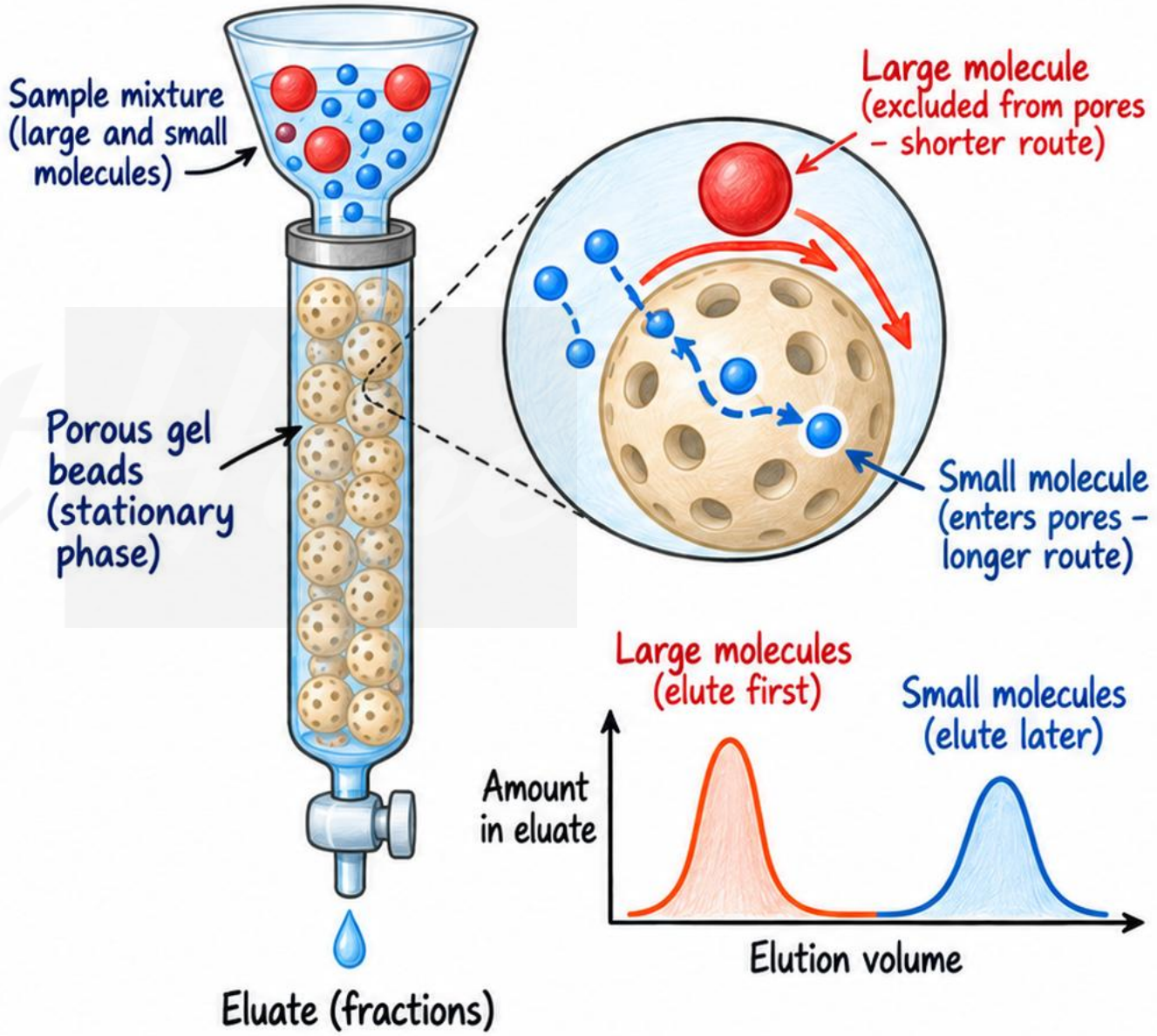
GEL FILTRATION: SIZE-EXCLUSION PRINCIPLE

Principle

- Gel filtration separates molecules mainly by hydrodynamic size using porous beads.
- Large molecules excluded from the pores follow a shorter route and elute first.
- Smaller molecules enter more pores and elute later.

Important considerations

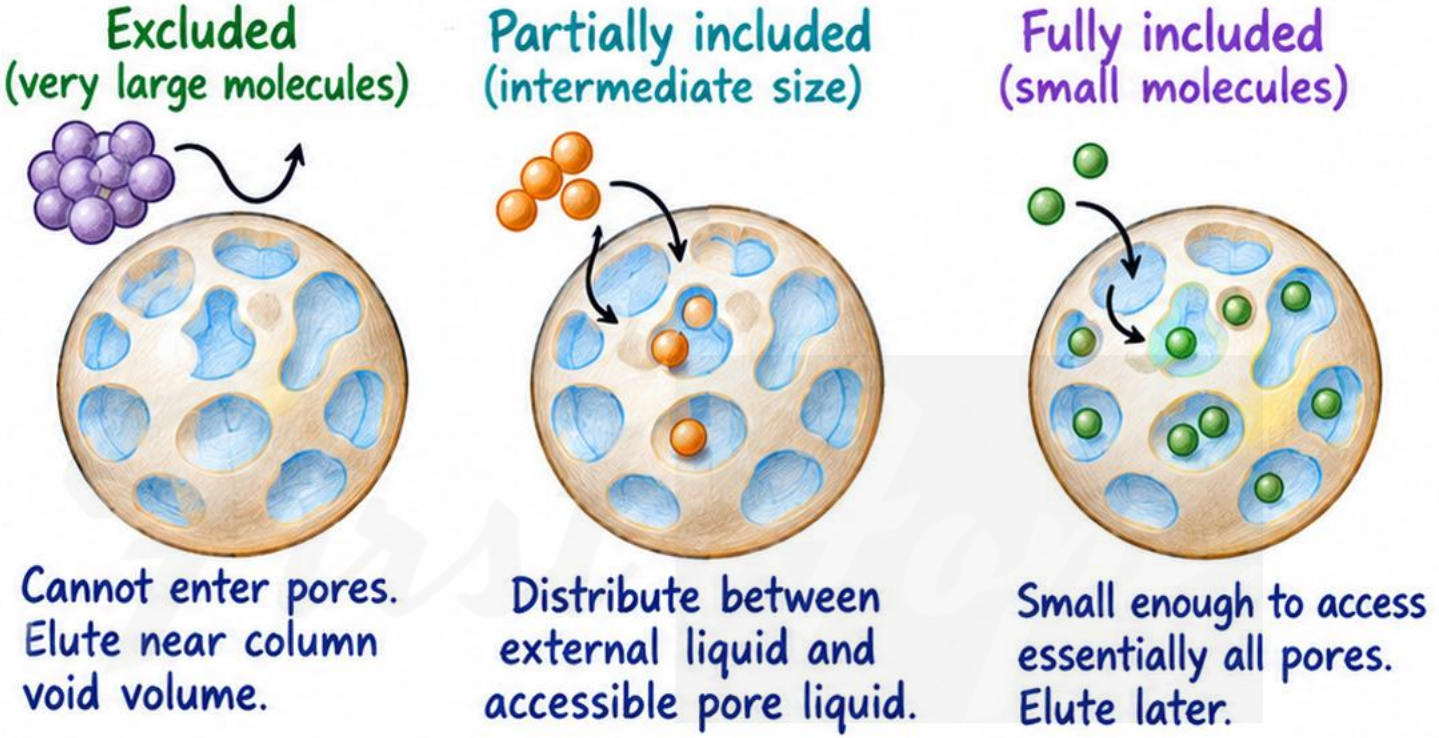
- Ideally there is little adsorption to the stationary phase.
- Shape and nonideal interactions can affect retention, so elution order is not determined by molecular mass alone.



PORE ACCESS AND ELUTION VOLUME

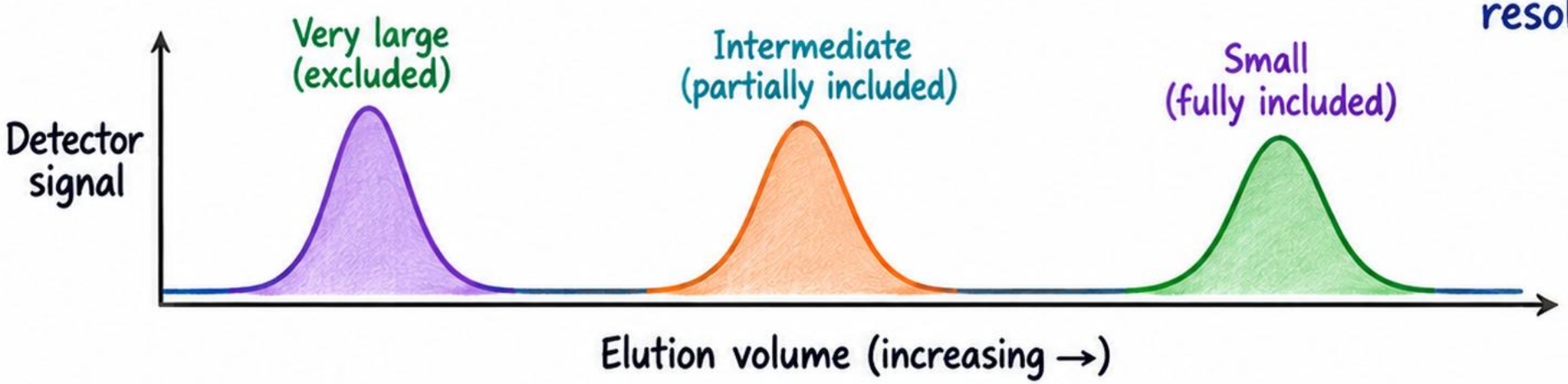
Very large molecules elute near the column void volume because they cannot enter the pores.

Smaller molecules distribute between the external liquid and accessible pore liquid.

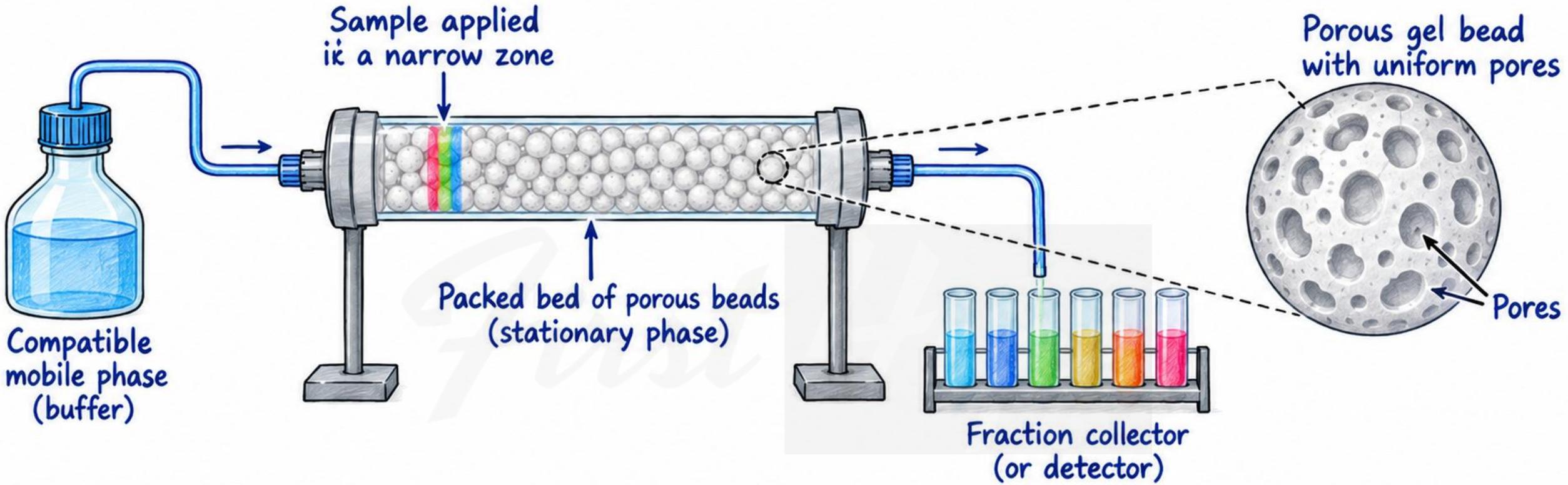


Useful separation occurs within the resin fractionation range.

Outside that range, differently sized molecules may elute together and are not resolved.



GEL-FILTRATION MATERIALS AND APPARATUS



Stationary Phases

- Include cross-linked dextran, agarose, polyacrylamide and suitable porous silica.
- The packed bed must have appropriate pore size and chemical compatibility.

Operation and Apparatus

- Sample is applied in a narrow zone and eluted with a compatible mobile phase, commonly isocratically.
- A detector or collected fractions follows the separated components.

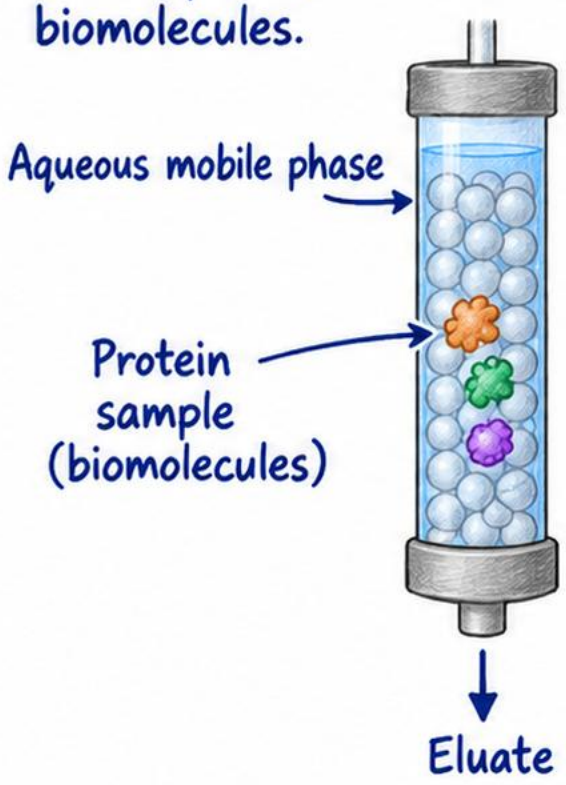
Practical Considerations

- Avoid unwanted adsorption, bead compression and unstable sample conditions.

GEL FILTRATION VERSUS GEL PERMEATION

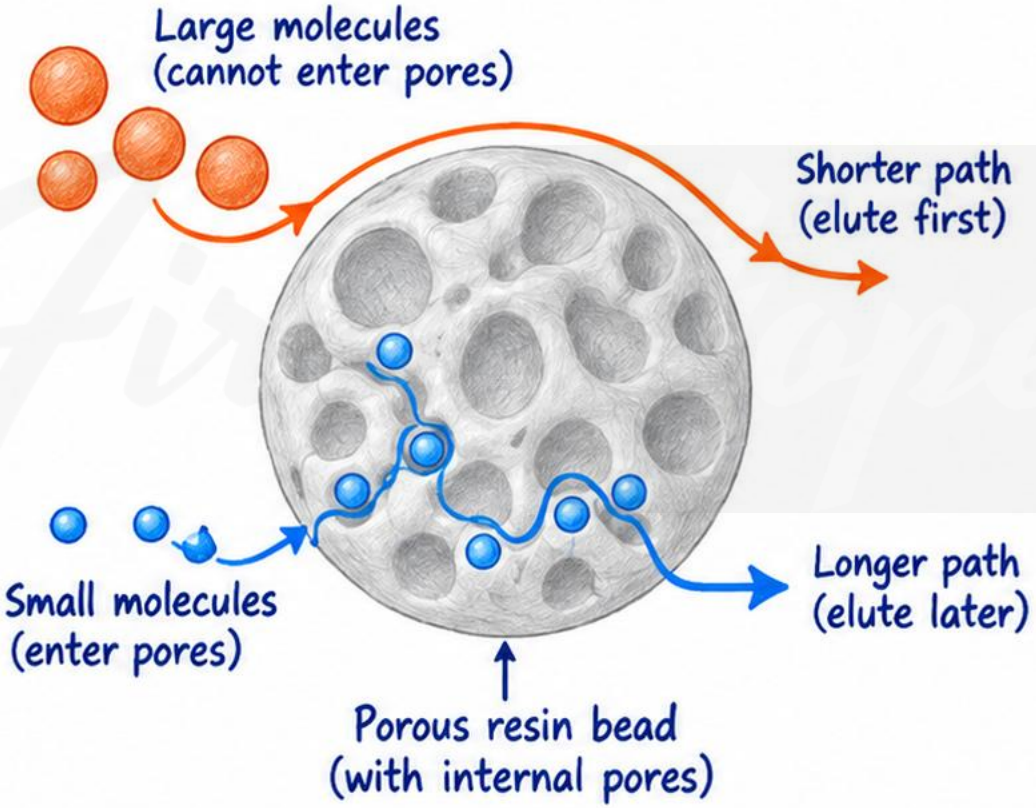
Gel filtration

- Usually refers to size-exclusion separation in an aqueous mobile phase, often for biomolecules.



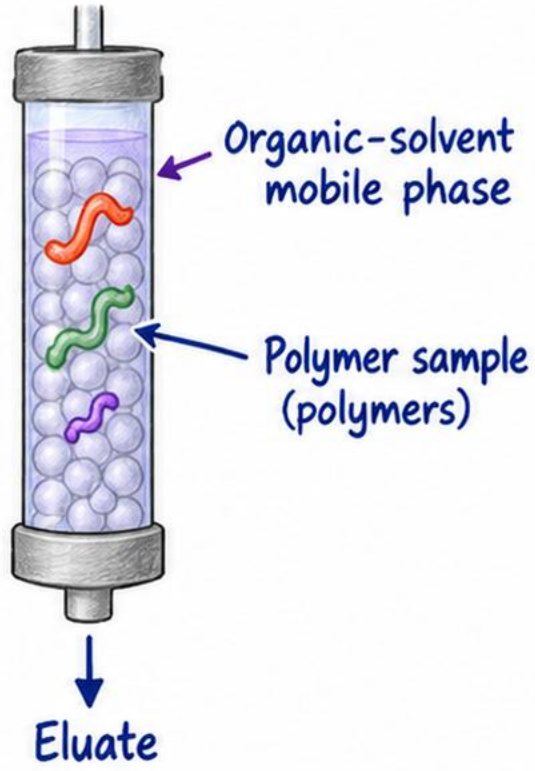
Common mechanism (size-exclusion)

Both use pore access as the main separation basis.



Gel permeation chromatography

- Commonly refers to organic-solvent size-exclusion methods, often for polymers.



Solvent, resin and analyte must remain compatible; aggregation, adsorption or changes in molecular shape can invalidate a simple size interpretation.

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