

Chromatography

Generic Protocol

1. Prepare Column (\pm)
2. Apply Sample
3. Wash
4. Elute
5. Analyze Fractions

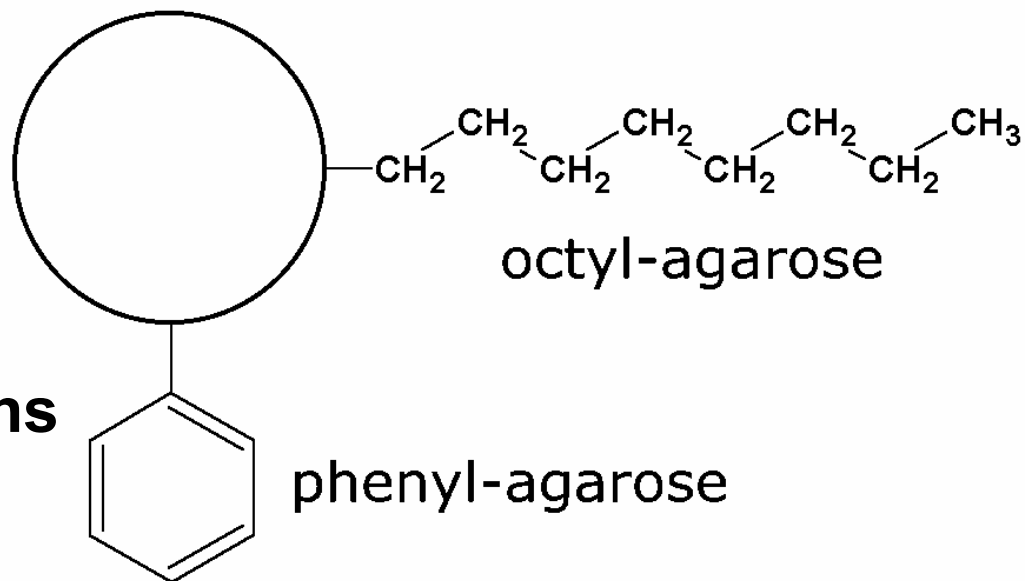
Types

- adsorption
- ion exchange
- hydrophobic
- gel filtration
- affinity

Hydrophobic Interaction Chromatography (HIC)

- separates proteins based on differences in hydrophobicity

- absorb proteins to hydrophobic matrix
- high salt promotes hydrophobic interactions
 - eg, 1 M $(\text{NH}_4)_2\text{SO}_4$



← increasing salting out effect
anions: PO_4 , SO_4 , Cl , Br , NO_3 , ClO_4 , I , SCN
cations: NH_4 , Rb , K , Na , Li , Mg , Ca , Ba
increasing chaotropic effect→

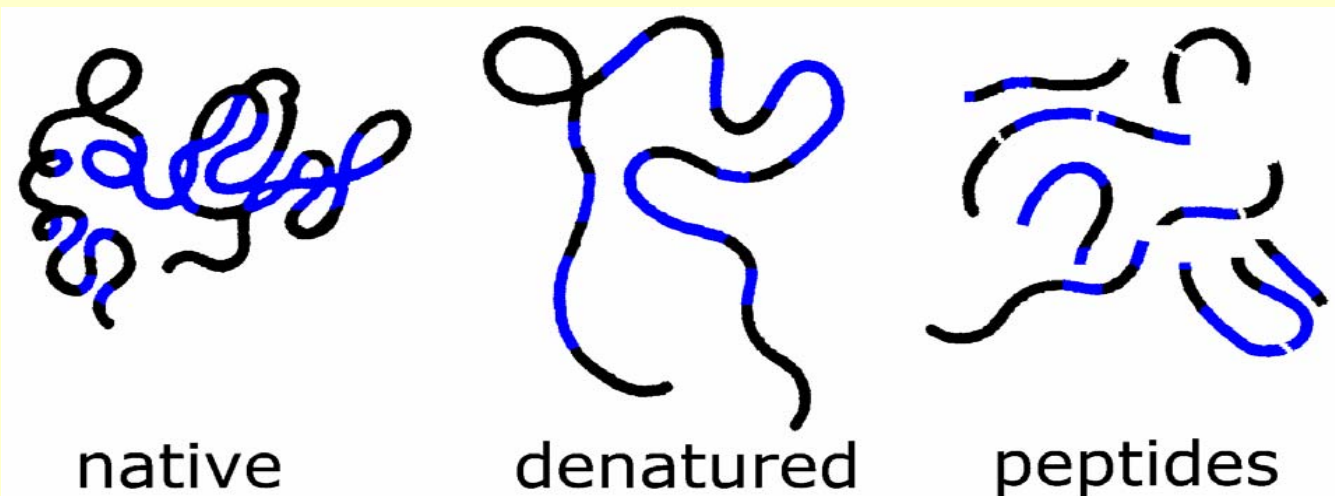
Elution from Hydrophobic Columns

- **decrease ionic strength**
 - eg, 1 → 0 M $(\text{NH}_4)_2\text{SO}_4$ gradient
- **decrease solvent polarity**
 - eg, ethylene glycol
- **chaotropic agents**
 - eg, salts, urea
- **detergents**

Reverse Phase Chromatography

	HIC	vs	RPC
Mobile Phase	Polar Solvent		Nonpolar Solvent
Conditions	Native		Denatured
Solute Discrimination	Surface Residues		Total Residues

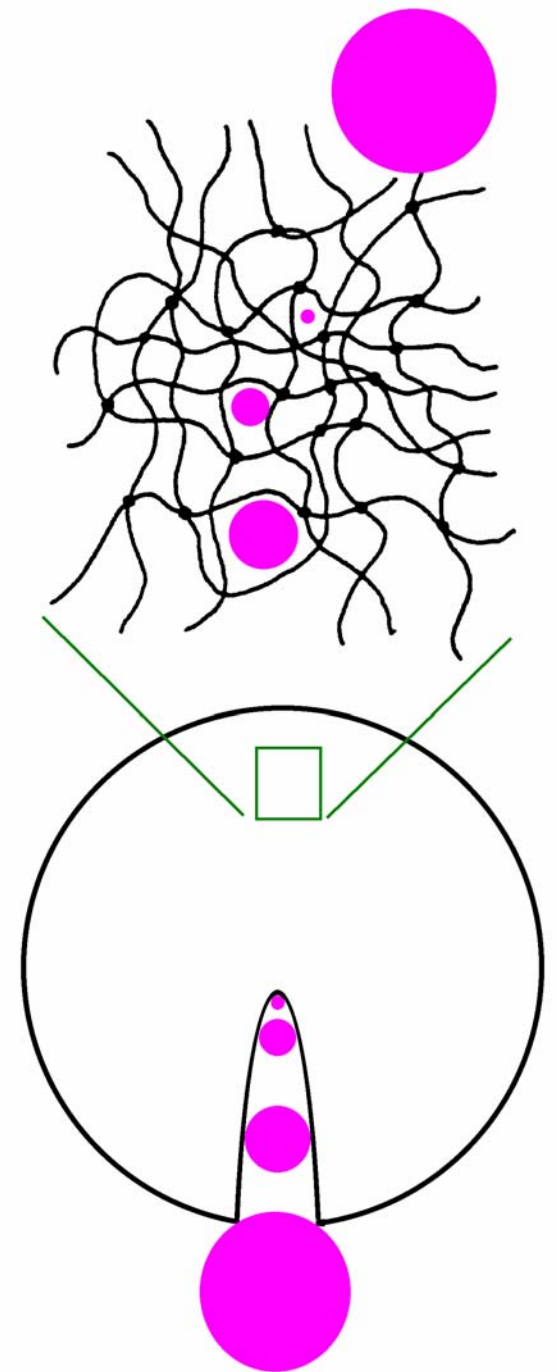
- separation based on total hydrophobicity
- generally used to separate small peptides

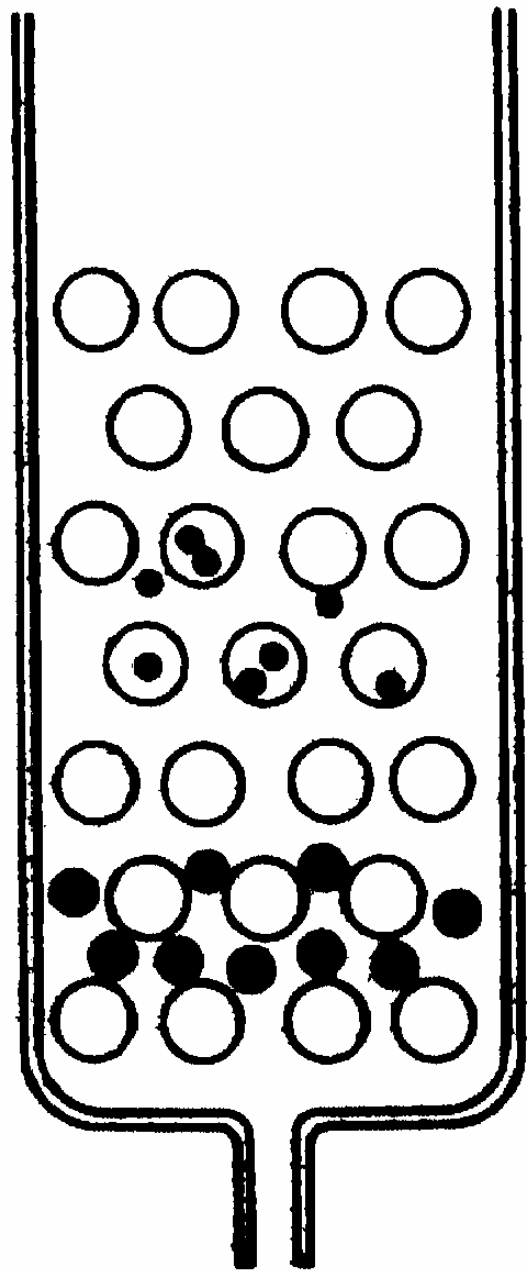
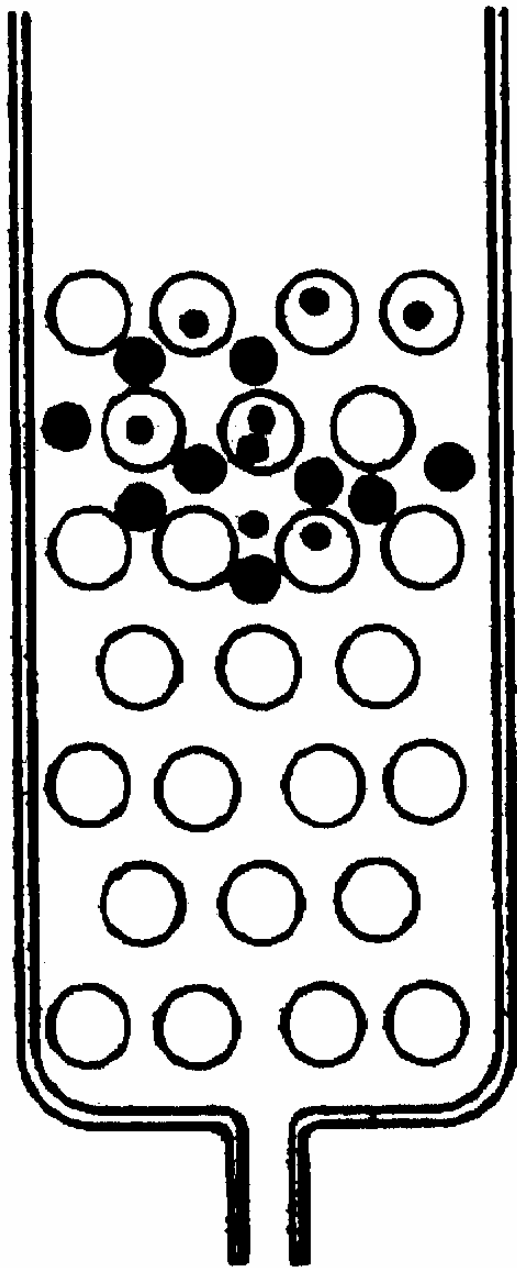
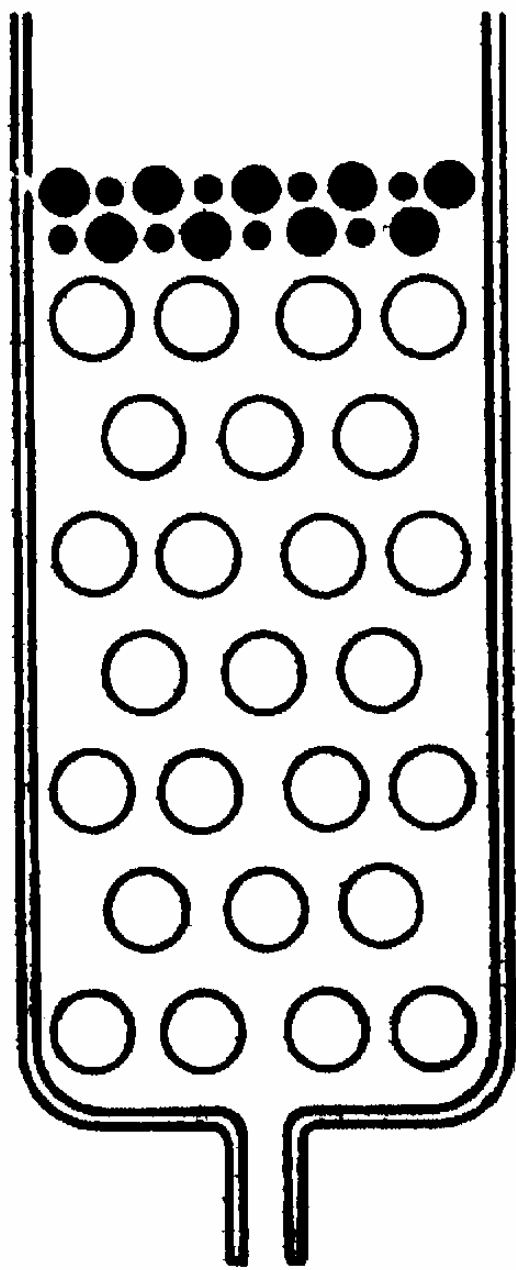


Gel Filtration

- separation based on size, aka
 - molecular sieve chromatography
 - size exclusion chromatography
- media composed of cross-linked polymers
- 'pore' size of matrix determines degree of interaction
 - larger molecules are excluded and migrate faster
 - smaller molecules are included and are retained longer

- Dextran (=Sephadex[®])
- Agarose (=Sepharose[®])
- Polyacrylamide





Practical Considerations

- choose matrix with desired characteristics
 - size range
 - does not interact with solute
- load sample in smallest possible volume
 - include 0.15-1 M NaCl
- elute in one column volume

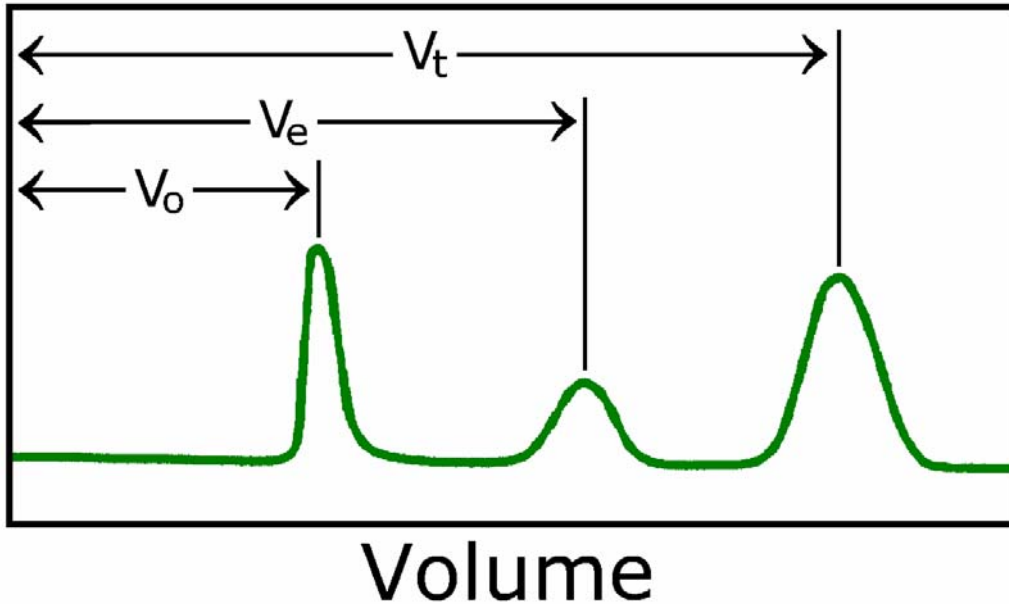
Sephadex

Code	Range (kDa)
G-25	1-5
G-50	2-30
G-100	4-150
G-150	5-300
G-200	5-600

Applications:

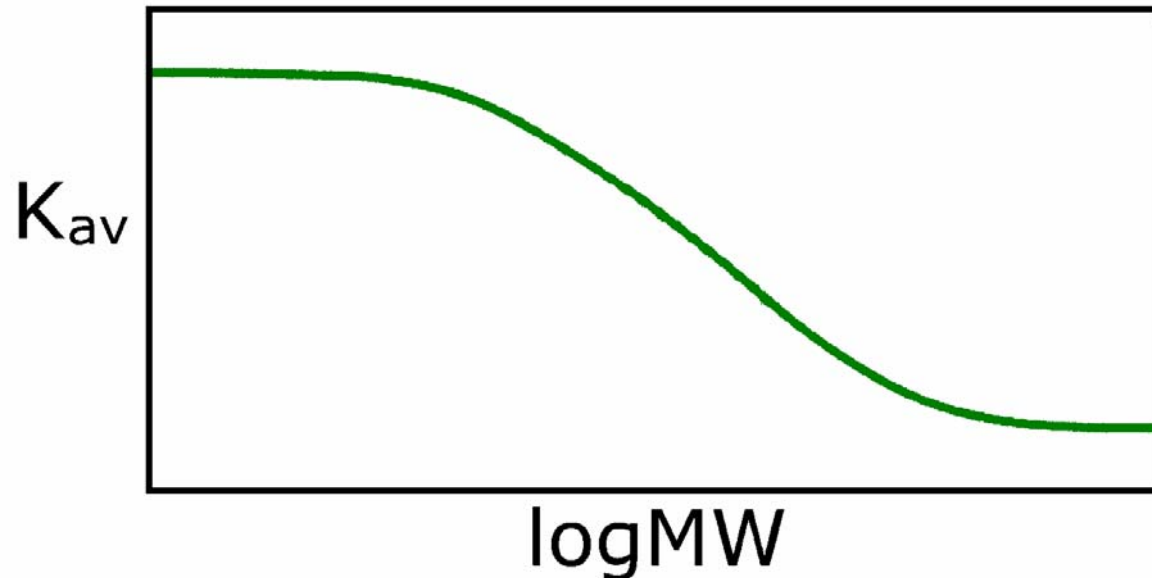
- purification
- desalting
- size determination

Calculating Size



V_o = void volume
 V_t = total volume
 V_e = elution volume

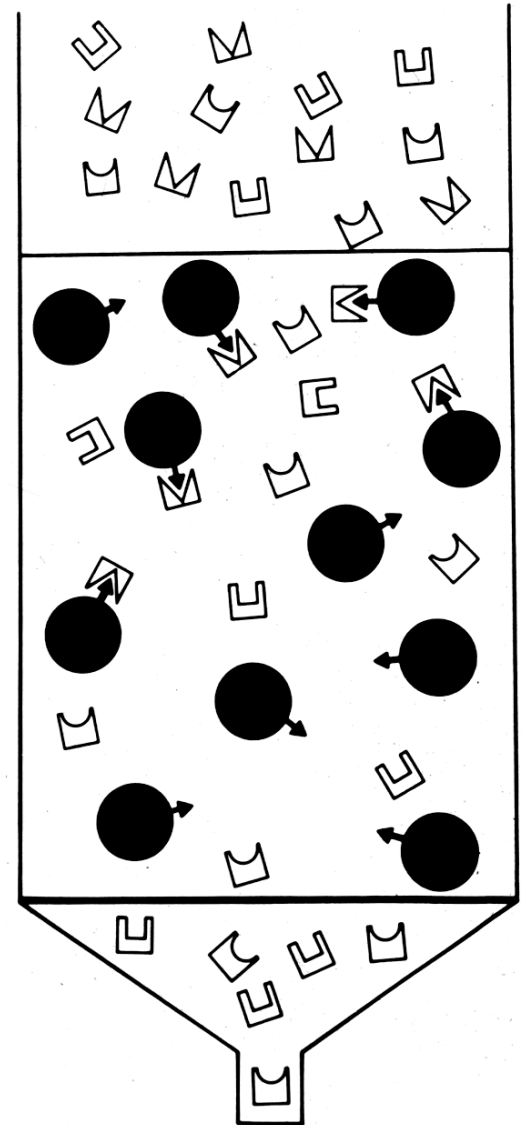
$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$



- use size standards
- (relative MW)
- migration also affected by shape

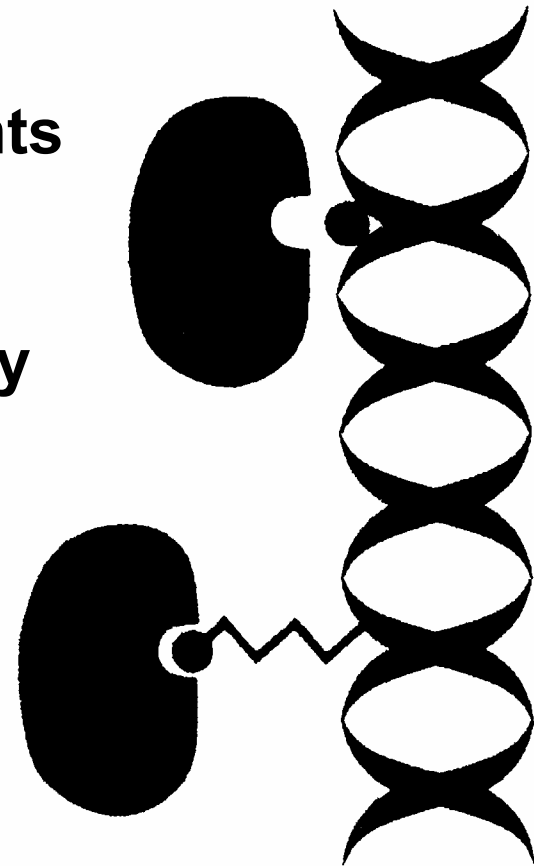
Affinity Chromatography

- based on specific binding of protein to “ligand”
- ligands can include:
 - substrate analogs
 - inhibitors
 - natural ligands
 - co-factors
 - metals
 - binding proteins
 - antibodies
 - etc



Affinity Chromatography

- **Prepare column**
 - ligand attached to matrix (eg, CNBr-activated sepharose)
 - linker arms
 - matrix should not absorb contaminants
 - covalent attachment of ligand should not alter binding properties
 - binding should be specific, but affinity not so high as to prevent elution
- **Elution: destabilize binding**
 - compete with free ligand
 - change pH, ionic strength
 - chaotropic or denaturing agents



Chromatography Summary

Chromatography	Discrimination
Ion Exchange	Charge
Gel Filtration	Size and Shape
Hydrophobic	Surface Hydrophobicity
Reverse Phase	Total Hydrophobicity
Affinity	Specific Amino Acids
Adsorption	Amino Groups?