Introduction to Size-Exclusion Chromatography "SEC"

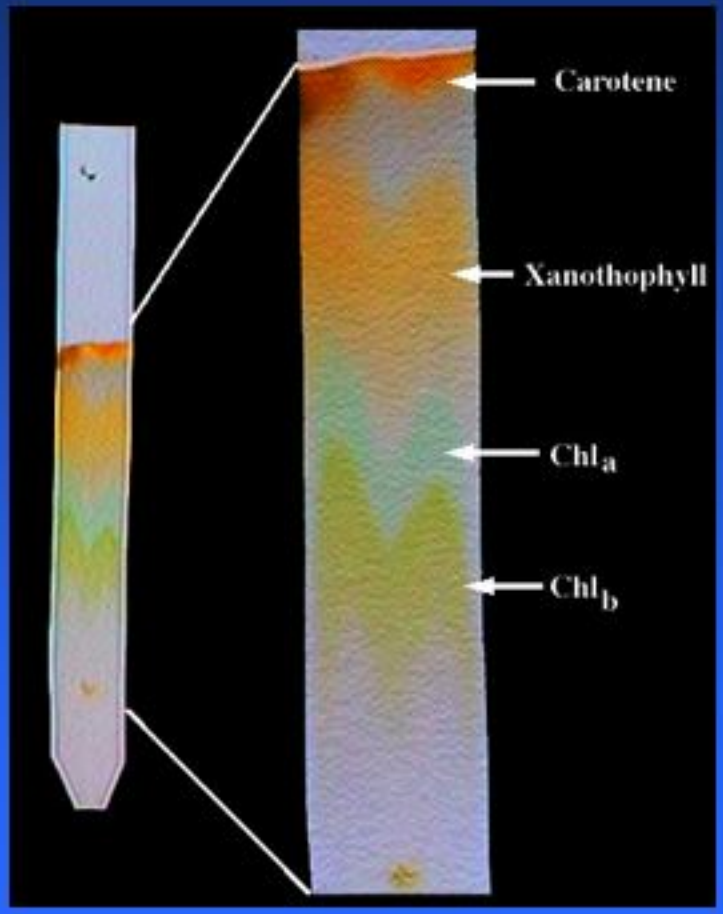
Outline of Topics

- Chromatography Overview
- Theory of Size-Exclusion Chromatography
- SEC chromatography media
- Size Exclusion Chromatography operations
- Gel filtration chromatography

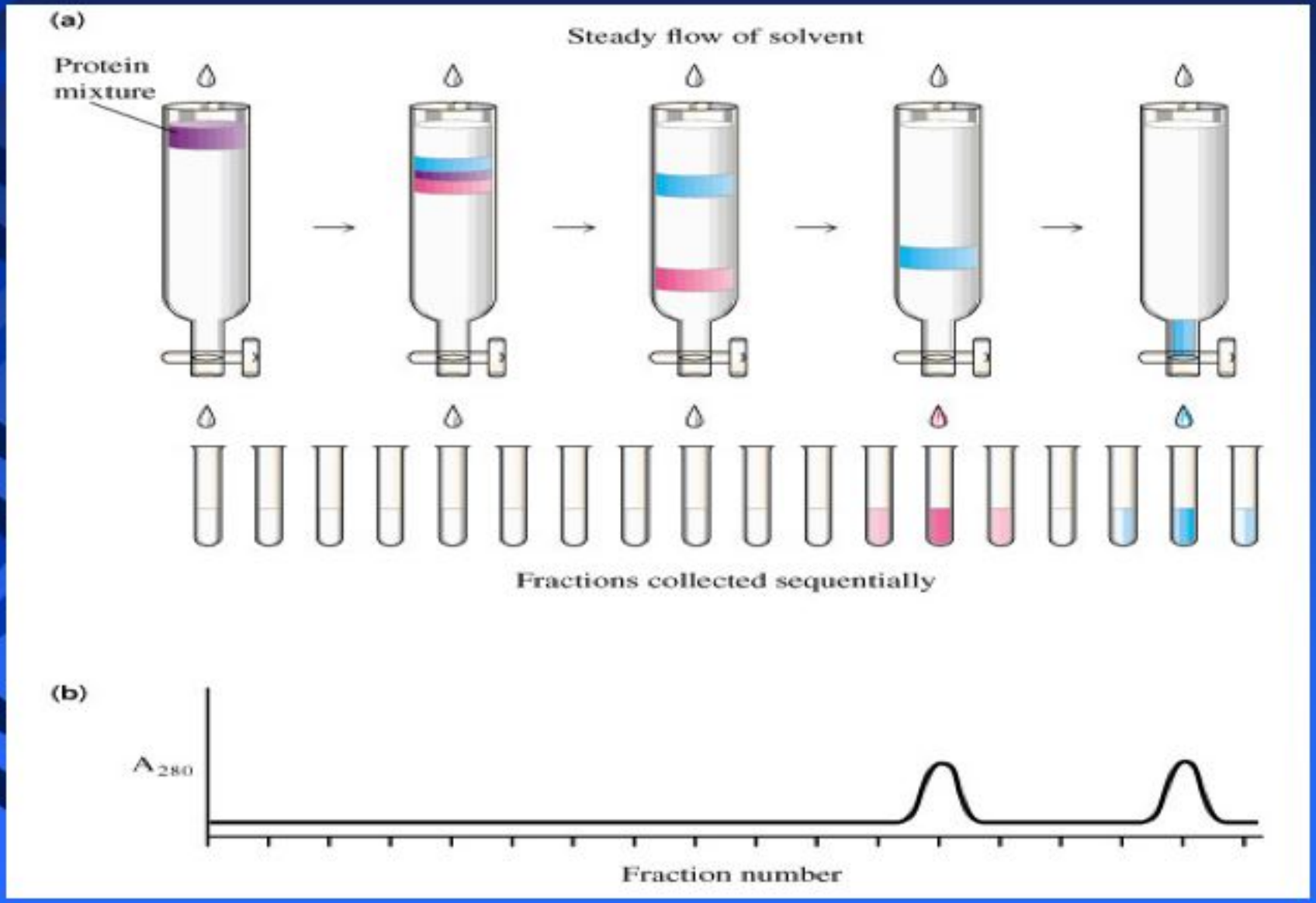
Production Steps

1. **Fermentation** in Host cell: Yeast , mammalian cells, bacteria, or fungi
2. **Recovery**: collecting cells or cell culture supernatant
3. **Purification** : chromatography or precipitations
4. **Formulation / filling**

Paper Chromatography



Chromatograms



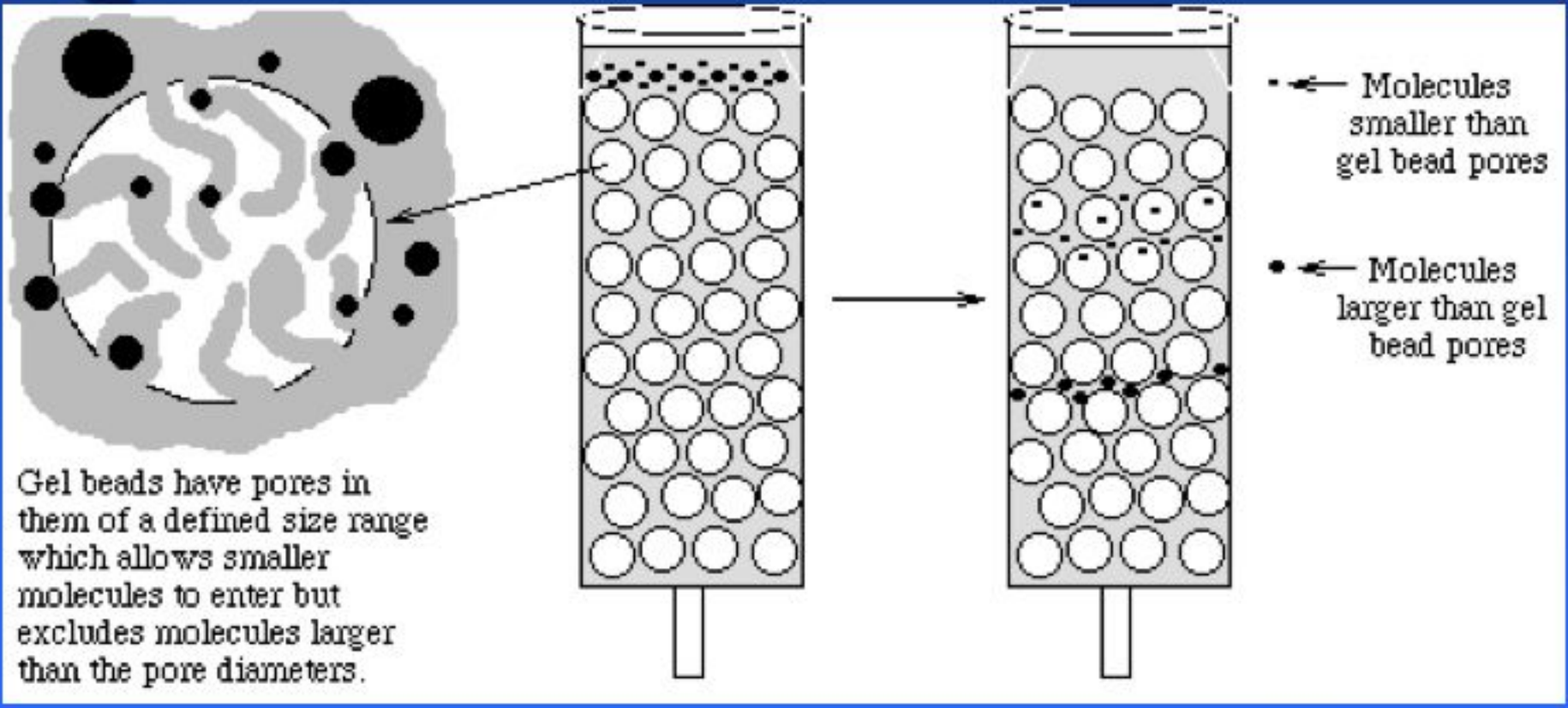
Size Exclusion Chromatography: Theory

- The separation is based on the SIZE & SHAPE of the molecules applied to the column relative to the porosity of the media packed into the column.
- Size exclusion media have a wide variety of separation ranges.
- **Must** run at slow enough speed to allow travel through pores to allow sieving action. A size exclusion chromatography.

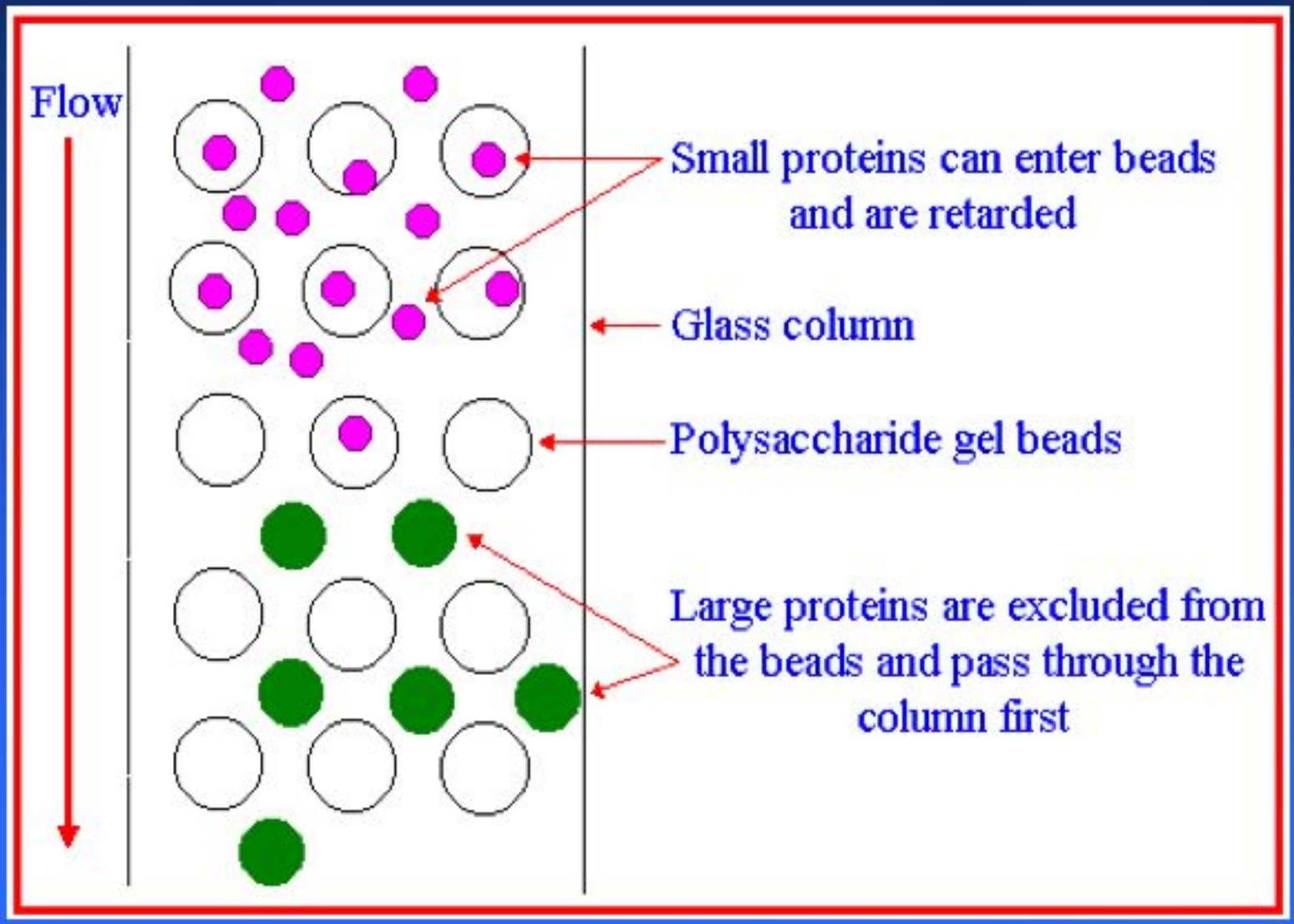
SEC Theory, cont.

- Gel filtration is used to separate classes of molecules that may be relatively close in apparent molecular weights.
- (Buffer exchange is used to separate large molecules (≥ 5,000 MW) from smaller ones.)

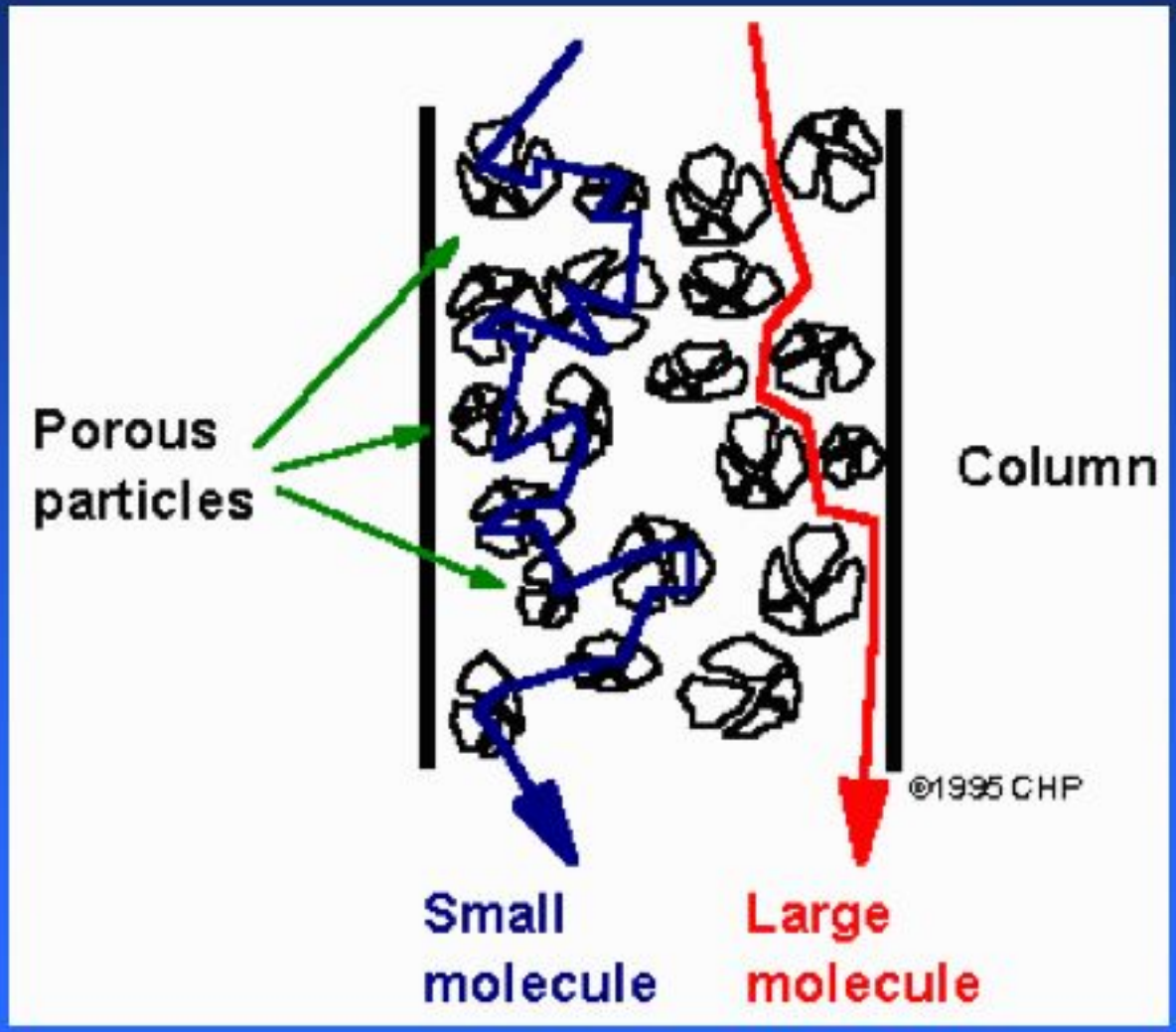
A View of Size Exclusion



Size exclusion SEC



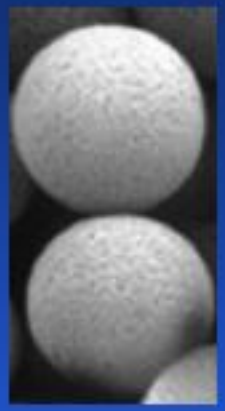
Another View of SEC



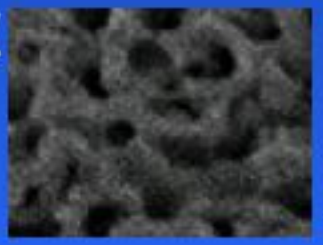
Which exits column first, small or large molecules?

SEC Chromatography Media

- The base bead is made from carbohydrate, or a synthetic substance. The bead should be hydrophilic to promote favorable interactions with water.
- The bead will have a defined porosity range for separation of molecules.
- The media does not contain a chemical or biochemical functional group (no ligand).

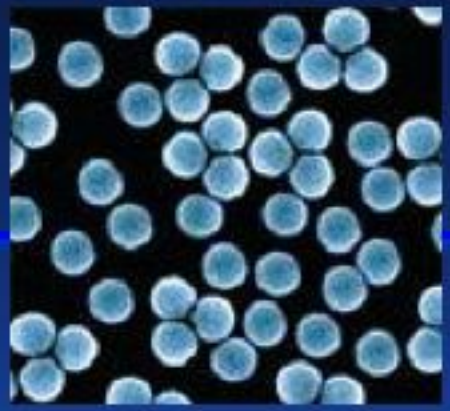


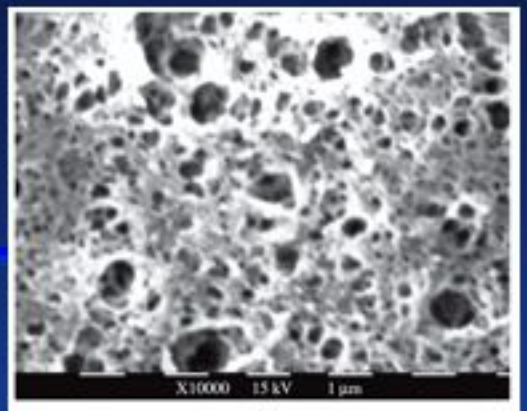
Beads

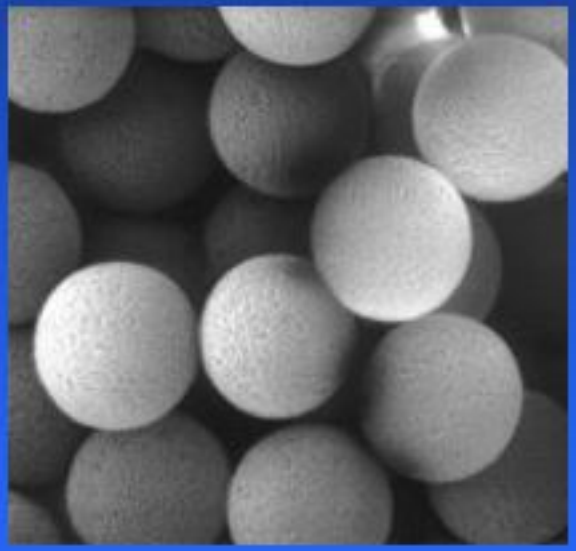


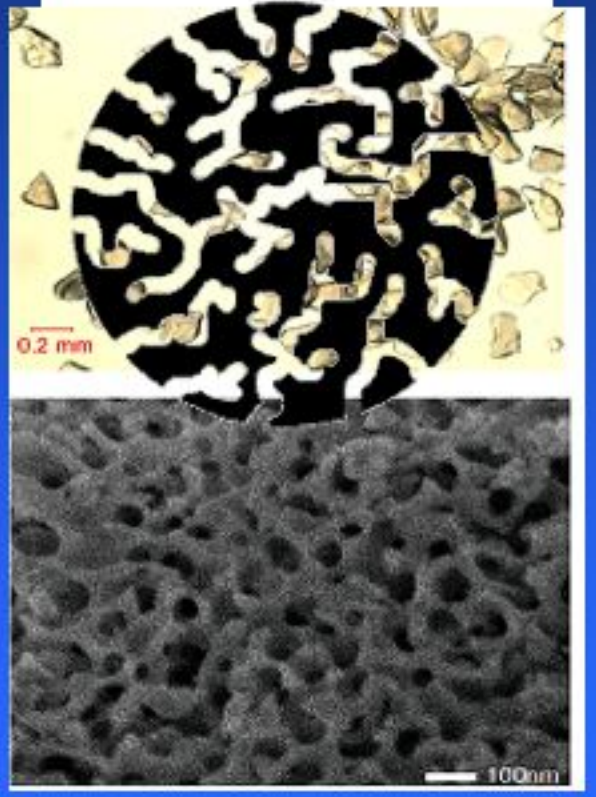
Pores


HiTrap™ 1 ml
Heparin HP
HiTrap™ 5 ml
Heparin HP




X10000 15 kV 1 µm




0.2 mm
100nm

Chromatography Unit Operations, or Steps

- Equilibration
- Load
- Column development
- Regeneration
- Storage

Column Equilibration

- Equilibrate the column with the buffer that the protein is to be exchanged into.
- Include a small amount of salt to provide a minimum of ionic strength.
- Equilibration buffer displaces whatever storage solution the column is stored in.
- Equilibration = Column inlet equals column effluent with respect to critical parameters. The pH, conductivity etc. of the buffer entering the column equals that of the buffer exiting the column.

Column Load

Dependant upon two process variables:

- Volume
- Protein mass

Column Development

- Follow the column load with column equilibration buffer.
- Continue to “develop” the column until one column volume (1 CV) of equilibration buffer has been run through the column.

Column Regeneration

- Means chemical treatment of the column to return it to "as new" condition, because the columns can be reused.

- Column gel must be stable to regeneration conditions [extremes of pH, solvents, chaotropic reagents (which **cause molecular structure to be disrupted**)].

Gel-filtration Chromatography

- Column **length** (impacting resolution) is the most important parameter in **gel-filtration** chromatography.
- Used for separation of molecules that may be closely related in size (at least two fold different).
- And removal of aggregated materials or lower molecular weight forms from the desired protein.

Large Scale Purification Results – Characterization By SEC

Suggests that majority species is monomeric (~40 kD).
Shoulder indicates possible dimers (~80 kD).