Protein Purification Part III:
Size-exclusion chromatography

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Presentation Outline

- Theory of size-exclusion chromatography
- SEC chromatography media
- R & D vs. Manufacturing
- Size exclusion chromatography operations
- Gel filtration chromatography
- Buffer exchange chromatography

Size exclusion chromatography: Theory

- The separation is based on the size and shape of the molecules applied to the column relative to the media porosity.
- Size exclusion media have a wide variety of separation ranges.
- Gel filtration and buffer exchange are two different applications of size exclusion chromatography.
- 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.

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 contains a chemical or biochemical functional group (ligand).

R & D vs. Manufacturing

- Research scale is relatively independent of column size.
- Research scale columns have lengths of up to 100 cm.
- Manufacturing scale is very dependant on column size.
- Stacks of columns, ranging from 20 - 25 cm in length are used.

Chromatography unit operations

- 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.
- Column inlet equals column effluent with respect to critical parameters.

Column load

Dependant upon two process variables:

- Volume
- Protein mass

Column development

- Follow the column load with the column equilibration.
- Continue to develop the column until one column volume of equilibration buffer has been run through the column.

Column regeneration

- Chemical treatment of the column to return it to “as new” condition.
- Gel must be stable to regeneration conditions (extremes of pH, solvents, chaotropic reagents).

Column storage

- Continued chemical treatment of the column to maintain cleanliness of column following regeneration.
- Gel must be stable to storage conditions (extremes of pH, solvents, chaotropic reagents) for extended periods of time.

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Sections of the sizing column

- Void (exclusion) volume.
- Included volume.
- Salt (total) volume.

Gel-filtration chromatography

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

Buffer exchange chromatography

- Column width (speed) is the most important parameter for buffer exchange.
- Transfer the protein from a less desired buffer or solution into a more desired buffer or solution (preparation for chromatography, SDS-PAGE or formulation).
- Good resolution between large (>5 kD) molecules and small molecules.

Gel filtration vs. buffer exchange

Parameter	Gel filtration	Buffer exchange
Equilibration pH and load conductivity	desired pool pH and cond.	desired pool pH and cond.
Flow rate	very slow	faster
Column length	long (resolution)	short (speed)
Load volume	1 - 5 % of bed	up to 25% of bed
Regeneration	0.5 M NaOH	0.5 M NaOH
Storage	0.1 M NaOH	0.1 M NaOH