The Effect of Surface Area on the Protein Binding Capacity of Membrane-based Cation-exchange Adsorbers

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Introduction

- An increased demand for protein therapeutics motivate the need for more efficient downstream processing.
- Protein capture remains one of the slowest and most expensive steps of this process
- The current method of resin-based chromatography has a low mass throughput.
- A need exists for a high throughput technology that can accommodate larger batch sizes.

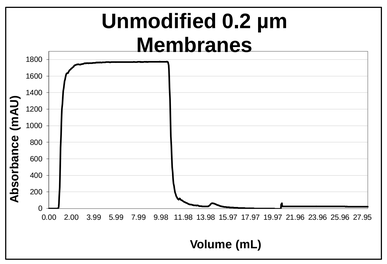
Objectives

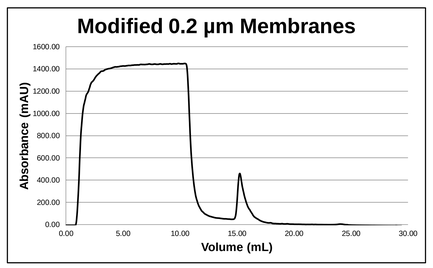
- Surface modify membranes to create cation-exchange adsorbers with high protein binding capacity and high throughput
- Investigate the effect of membrane surface area (pore size) on protein binding capacity of surface-modified membranes
- Compare column performance across a range of flow rates

Results

- The 0.20 µm pore-size membranes were tested at three different flow rates: 3.85 , 7.70, and 12.83 mL/min

Typical Chromatograms



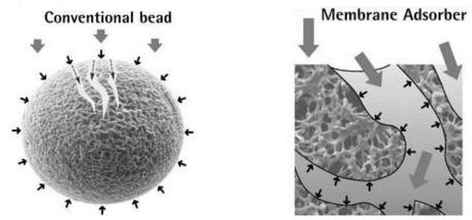


Lysozyme Binding and Elution

Flow Rate (mL/min)	Approximate Dynamic Binding Capacity (mg/mL)	Recovery (%)
3.85	5 ±2	16.57
7.7	15± 1	4.89
12.83	20 ±3	3.91

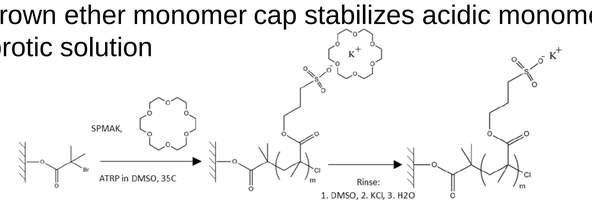
Discussion

- Membrane chromatography offers lower pressure drop and higher throughput than conventional bead method



Sartorius-Stedim Biotech SA

- Crown ether monomer cap stabilizes acidic monomer in aprotic solution



- Addition of ascorbic acid in 1:1 molar ratio reverses oxidation of Cu (I) catalyst in ATRP solution
- A dry layer thickness of 10 nm was targeted to accommodate 0.20 µm pores. Elipsometry measurements of silicon wafers revealed actual thickness of 5 nm.
- Approximate Binding Capacity = $\frac{C_0(V_{Br} - V_d)}{V_c}$

C_0 = initial concentration *V_{Br} = breakthrough volume (modified)*
V_d = dead volume (unmodified) *V_c = column volume*

- Percent Recovery = $\frac{protein_{eluted}}{protein_{bound}} \times 100\%$

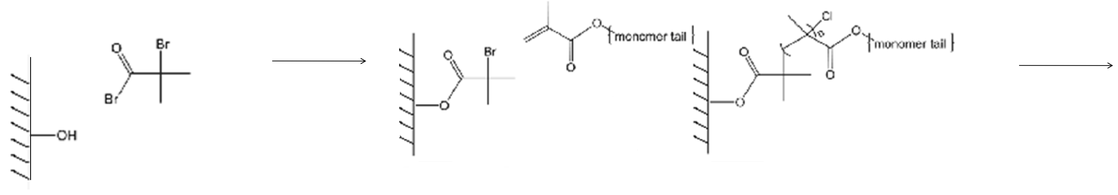
Conclusions

- The data show how ATRP can be used to create cation-exchange adsorbers and demonstrate the relationship between surface area and dynamic binding capacity

Future Work

- Investigate the effect of polymer chain grafting density on protein binding capacity of surface-modified membranes
- Determine the optimal polymerization time (chain length) to maximize binding capacity

Experimental Methods



GE Life Sciences

Surface Functionalization
Initator: 2-BIB (2-Bromoisobutyryl bromide)

Atom Transfer Radical Polymerization (ATRP)
Monomer: SPMAK (3-sulfopropyl methacrylate, potassium salt)
Substrate: Membranes of 1.0, 0.45, and 0.20 µm pore sizes
Solvent: DMSO (Dimethyl Sulfoxide)
Catalyst: Cu (I)/HMTETA complex
Monomer Cap: 18C6

Dynamic Binding Capacity Measurements
Binding Buffer: 50 mM Tris
Elution Buffer: 50 mM Tris + 1 M KCl
Regeneration Buffer: 0.3 M KOH
Protein: lysozyme in binding buffer

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