Multi-Column Continuous Chromatography for Protein A Capture and Orthogonal Polishing of Monoclonal Antibodies

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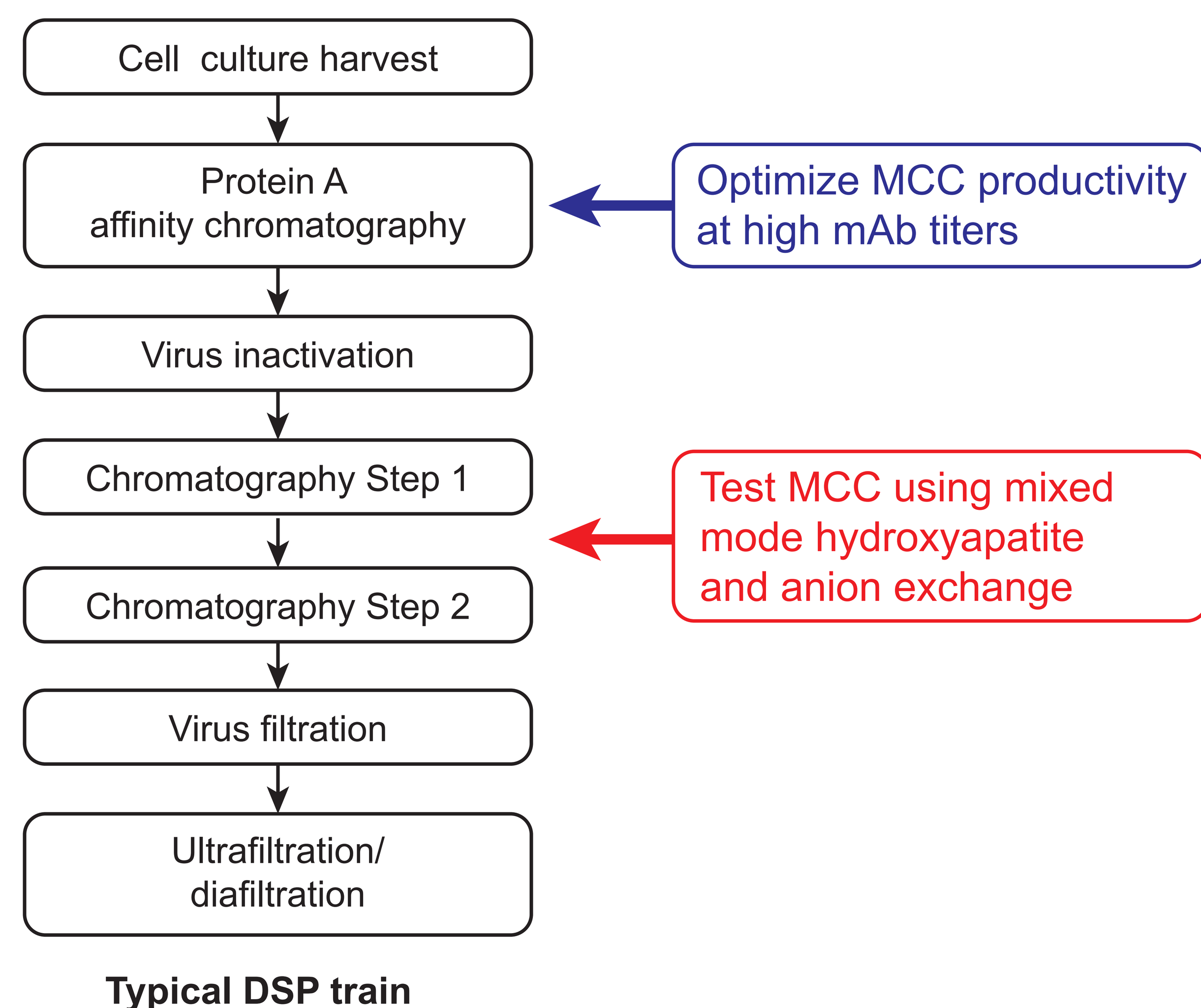
Introduction

The benefits of simulated moving bed (SMB) or multi-column continuous chromatography (MCC) for increasing the productivity of chemical separations have been known for over 60 years. MCC also offers significant economic advantages over traditional batch methods for purification of mAbs, including increased resin capacity utilization, reduced buffer consumption, and decreased column volume. The Protein A capture step is a primary target to apply MCC due to its high cost, which is driven even higher as improvements in upstream processing have produced a steady increase in mAb titers. Drawbacks of increased titers include higher levels of aggregates, fragments, variants, and process impurities.

We have used a lab-scale SMB/MCC instrument (Semba Octave™ 10 System) to develop and optimize a continuous Protein A capture process. Here we present productivity data with five commercial Protein A adsorbents using feedstocks at 5 and 7.5 g/L mAb titers. With an 8-column process and appropriate capture resins, productivities approaching 100 g mAb/L resin/day were achieved with 7.5 g/L feedstock. In addition, we have examined hydroxyapatite and anion exchange as orthogonal MCC polishing steps for aggregate removal and concurrent depletion of impurities following Protein A capture. Results indicate that a completely continuous downstream process, including only two chromatographic steps, may be possible to further increase efficiency and reduce cost in mAb biomanufacture.

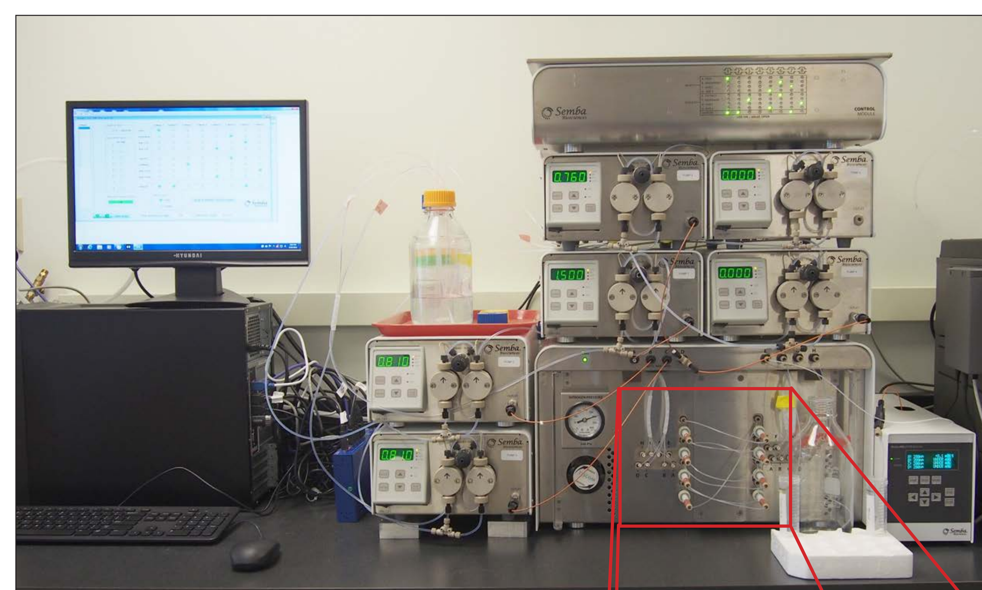


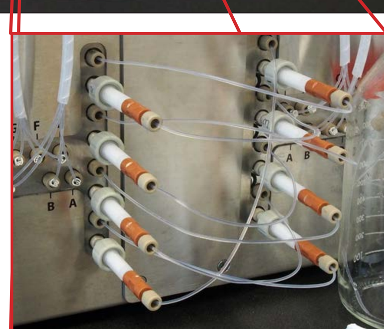
Typical DSP train

Methods

We have taken an experimental approach to optimize productivity using single column binding data, a simple Excel-based modeling tool, and small-scale MCC.

1. For bind-elute processes, determine dynamic and static binding capacities of the adsorbent; single column breakthrough analysis for DBC, saturation binding for SBC.
2. For flow-through processes, determine single column breakthrough of aggregate/impurities vs. monomer at increasing salt concentrations.
3. Model MCC process at various configurations (e.g. number of columns, zone flow pattern).
4. Test process models and optimize parameters using bench top MCC instrument (Octave 10 System).





Octave™ 10 System

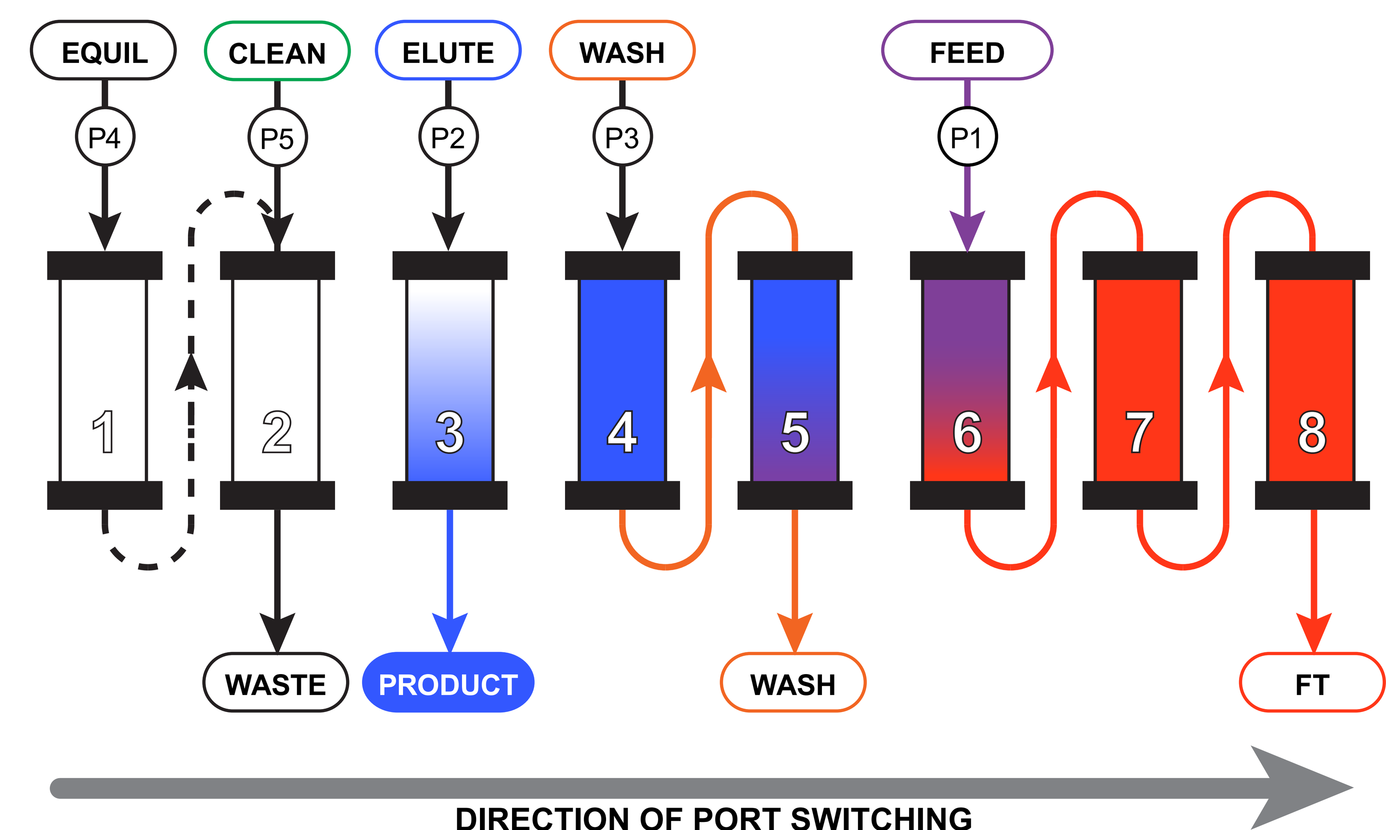
Up to 8 columns
2 valve blocks
4 inlets/outlets
Up to 8 pumps

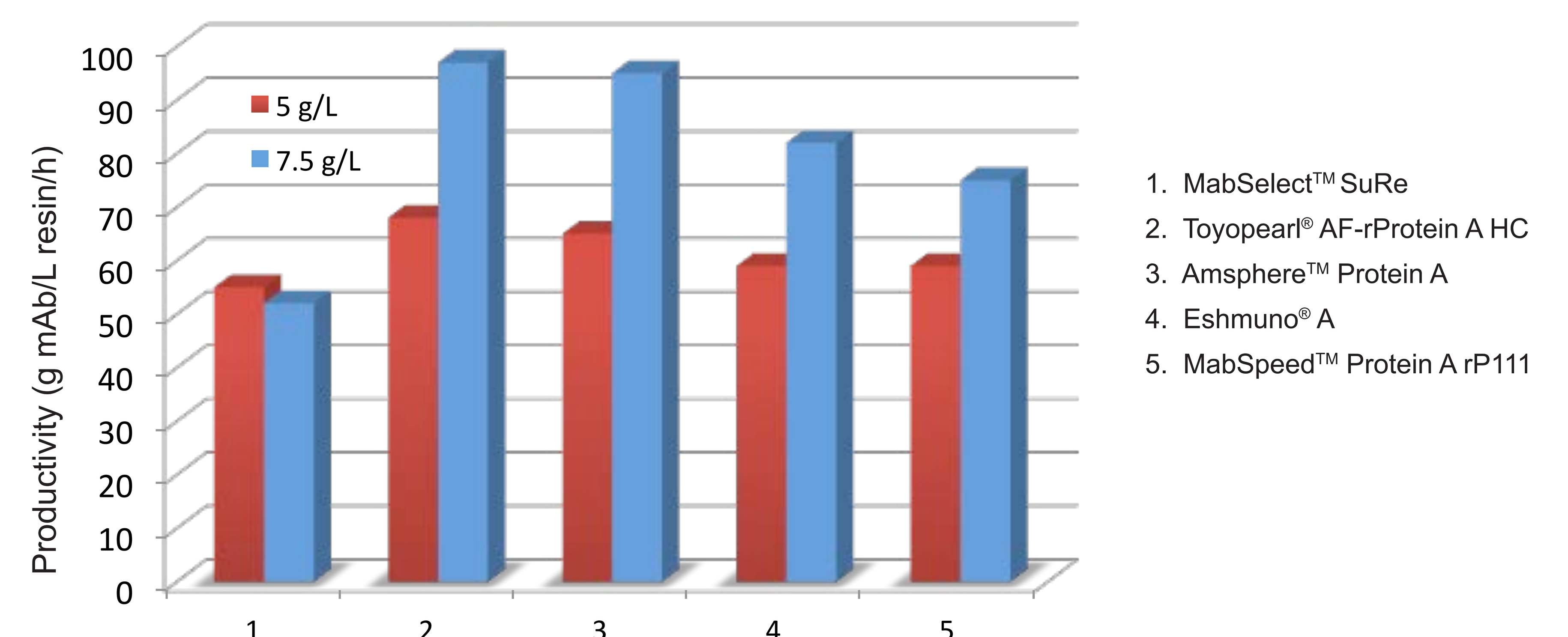
(future)
Semba ProPD™ System
Semba ProGMP™ System

Up to 8 columns
1 or 2 valve blocks
6 inlets/outlets
6 pumps

Semba Biosciences

MCC Protein A Capture





MCC productivity of five different Protein A resins with mAb feed concentrations of 5.0 and 7.5 g/L

MCC Polishing

We used two preparations of MCC Protein A-purified mAb containing different amounts of aggregate as samples (Feed) for MCC polishing runs by anion exchange (AEX) or Hydroxyapatite (HA). In both cases the MCC-Protein A sample was eluted with 100 mM glycine, incubated at low pH for >30 minutes to inactivate virus, and adjusted to pH 6.8-7.0 with Tris-Cl prior to AEX or HA polishing.

Results show that AEX and HA continuous polishing processes were capable of aggregate reduction to 1% or less with monomer recoveries of 88% and 70%, respectively. HCP, DNA, and Protein A impurities were effectively reduced to very low levels by either method.

MCC Process	Condition	Monomer Yield (%)	Aggregate (%)[1]	[HCP] log red.	[DNA] pg/mg	[Protein A] ppm
Anion Exchange Toyopearl NH_2-750F (flow-through)	Start[2]	N/A	3.1	1.7	0.3	<0.05
	A	95	2.6	2.3	not detected[3]	<0.05
	B	88	1.0	2.5	not detected[3]	<0.05
Hydroxyapatite CaPure™-HA (bind-elute)	Start[2]	N/A	2.4	1.8	0.4	0.1
	700 mM NaCl	78	1.2	2.7	<0.02	0.06
	600 mM NaCl	76	1.1	2.8	<0.02	<0.05
	500 mM NaCl	70	0.9	2.7	<0.02	<0.05

1. Aggregate determined according to Bond et al. (1)
2. Starting material; Protein A capture eluate
3. Less than the lower limit of quantification using resDNASEQ Quantitative CHO DNA Kit (Life Tech)

Conclusions

1. Optimal productivities with high-titer mAb feedstocks were obtained at 0.5 min residence time with 3 columns in the capture zone. For example, 97 g/L resin/h was achieved using Toyopearl AF rProtein A HC resin. This value represents 86% of the modeled process (112.5 g/L/h), which assumes 100% yield.
2. MCC-Protein A purified mAb can be treated by low pH inactivation followed by either continuous HA bind-elute or AEX flow-through polishing without dilution or buffer exchange.
3. Both HA and AEX orthogonal polishing steps reduced mAb aggregate, DNA, and Protein A impurities to acceptable levels.
4. Bind-elute HA was more effective for HCP reduction than flow-through AEX.
5. Future MCC downstream polishing studies will examine mixed-mode and membrane devices to improve quality, process efficiency, and economics in mAb biomanufacture.

References

1. Bond et al. (2009) *J. Pharm. Sci.* **99**, 2582–2597.
2. Gagnon, P. (2009) *New Biotechnol.* **25**, 287–293.