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Toward complete continuity in antibody biomanufacture: Multi-column continuous chromatography for Protein A capture and mixed mode hydroxyapatite polishing

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Toward Continuous DSP: Multi-Column Continuous Chromatography for Protein A Capture and Mixed Mode Hydroxyapatite Polishing

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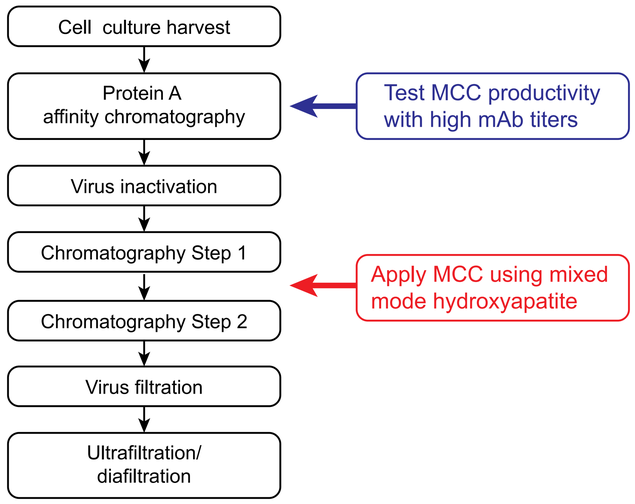
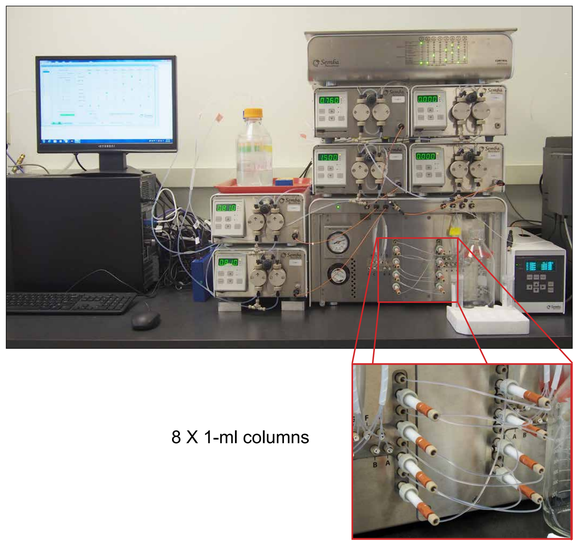
Summary

Multi-column continuous chromatography (MCC) is a scalable technology previously demonstrated to improve productivity and lower the cost of Protein A affinity chromatography versus the standard single column batch process. In this study we employed a lab-scale 8-column MCC process to compare the performance of three different Protein A adsorbents with high-titer human monoclonal IgG_1 culture fluids. Productivities approaching 100 g mAb/L resin/h were achieved with 7.5 g/L titers. We then investigated another MCC process using hydroxyapatite (HA) for aggregate removal and concurrent depletion of impurities from Protein A-purified mAb. Results indicate that MCC-HA was capable of reducing aggregate and other impurity levels to meet FDA standards.



Typical DSP train

Instrumentation

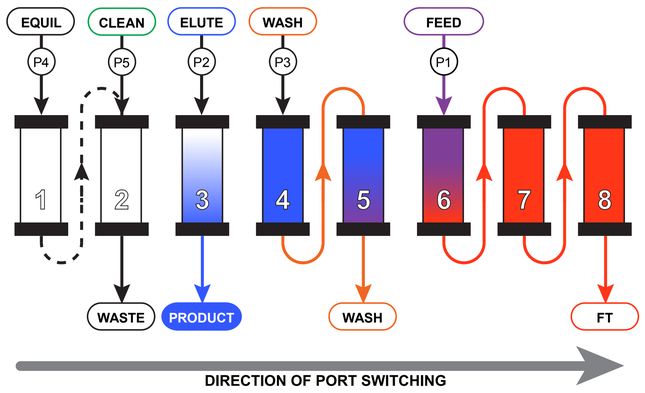


8 X 1-ml columns

Octave™ Chromatography System

- Performs SMBC, MCC and other continuous chromatography protocols
- Runs up to 8 columns, up to 8 pumps
- Patented valve block design
- Scalable from 12 ml/min to 300 ml/min flow rates; grams to kilograms per run
- Large scale GMP-compliant system under development

MCC Process

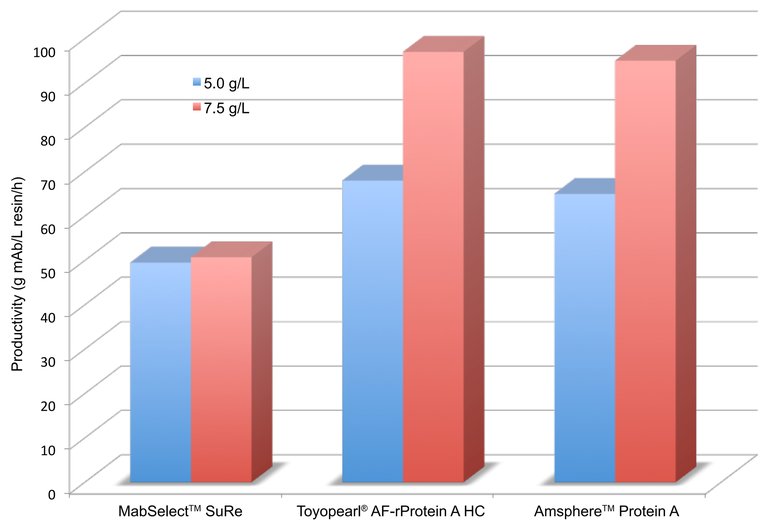


MCC Protein A Capture

Protocol

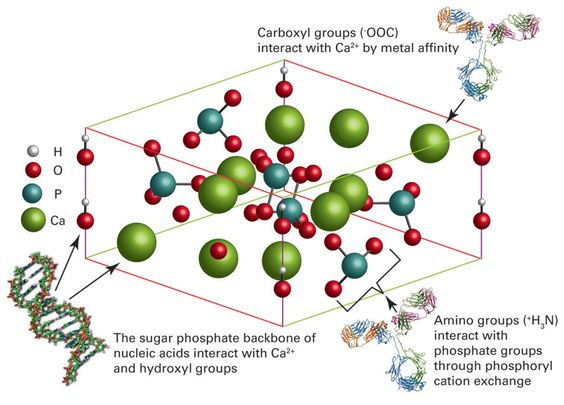
1.	Feed	80% SBC at 0.5 min/column residence time*
2.	Wash	10 CV 25 mM Na phosphate, 1 M NaCl, pH 6.7
3.	Elute	5 CV 100 mM citrate, pH 3.0
4.	Clean	4 CV 100 mM NaOH, 1 M NaCl
5.	Equil	10 CV PBS

* Residence time limited to 0.7 and 1.0 min/col for 5.0 and 7.5 g/L mAb, respectively, for MabSelect SuRe due to limitation of linear flow velocity



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

Mixed Mode Hydroxyapatite



Hydroxyapatite (HA) is a hexagonal crystalline calcium phosphate matrix that interacts with biomolecules via calcium ion affinity, cation exchange, and hydrogen bonding. CaPure™-HA (Tosoh Bioscience) is a spherical form of HA with high capacity and flow properties. HA has been shown to bind IgG aggregates more tightly than monomers (Gagnon 2009). We investigated using this adsorbent in an MCC process for removal of aggregates and other impurities from Protein A-purified mAb.

Gagnon, P. (2009) Monoclonal antibody purification with hydroxyapatite. *New Biotechnol.* **25**, 287.

MCC-HA Method Development

1. Determine [PO_4] and [NaCl] elution conditions by single column gradients
   a. Run Na phosphate (pH 6.8) gradient at 50 mM NaCl: mAb eluted at 60 mM PO_4
   b. Run NaCl gradient at 5, 10, and 20 mM PO_4: at 10 mM PO_4 mAb eluted at 600 mM NaCl and contained 99.5% monomer; aggregate remained on column.
2. Determine binding capacity under loading conditions (10 mM PO_4, 50 mM NaCl, pH 6.8)
   DBC @ 10% breakthrough = 31 mg/ml at 1 min residence time
   = 47 mg/ml at 3 min residence time
3. Run MCC process and optimize [NaCl] elution for monomer yield and purity

Protocol

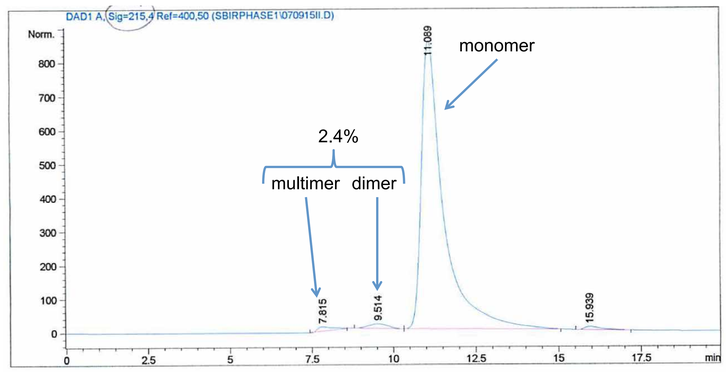
1.	Feed	20 mg/ml PAC mAb at 1 min/column residence time*
2.	Wash	8 CV 10 mM $NaPO_4$, pH 6.8, 50 mM NaCl
3.	Elute	7 CV 10 mM $NaPO_4$, pH 6.8, 500-750 mM NaCl
4.	Clean	5 CV 250 mM $NaPO_4$, 250 mM NaOH
5.	Equil	10 CV 10 mM $NaPO_4$, pH 6.8, 50 mM NaCl

MCC-HA Results

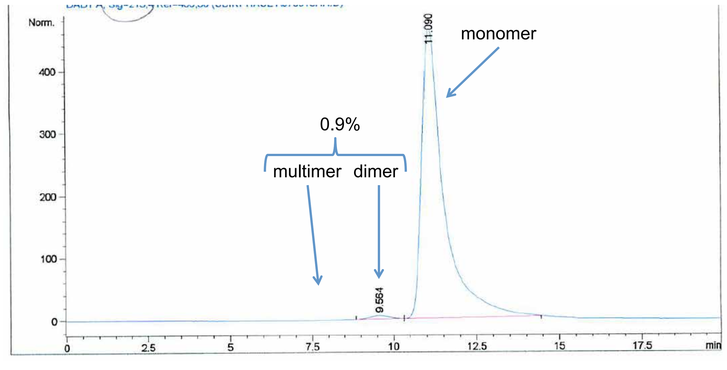
We used two preparations of Protein A-purified mAb containing different amounts of aggregate as samples (Feed) for MCC-HA runs. The High Aggregate (19%) sample was prepared by using glycine pH 2.3 for elution in the MCC-PAC process, and the Low Aggregate (2.4%) sample resulted from using pH 2.6 for elution. In both cases the MCC-PAC sample was incubated for 30 min at low pH, then adjusted to pH 6.8 with 1 M Tris-Cl, pH 9 before use as feed for the MCC-HA runs.

Results show that the continuous HA process reduced the aggregate content 4-5 fold for the High Aggregate sample and 2-3 fold for the Low Aggregate sample, with monomer recoveries ranging from 70-90%. HCP, DNA and Protein A concentrations were reduced to very low levels.

Sample	[NaCl] mM	Monomer Yield (%)	Aggregate %	[HCP] log red.	[DNA] pg/mg	[Protein A] ppm
High Aggregate mAb (19%)	----	N/A	19	1.8	0.4	0.1
	750	91	4.9	2.7	<0.02	0.08
	700	81	4.0	2.7	<0.02	0.06
Low Aggregate mAb (2.4%)	----	N/A	2.4	1.8	0.3	0.1
	700	78	1.2	2.7	<0.02	<0.05
	600	76	1.1	2.8	<0.02	<0.05
	500	70	0.9	2.7	<0.02	<0.05



Low Aggregate Sample (Feed)



Low Aggregate Product