Bench top continuous chromatography: an enabling platform for highly productive mAb purification

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*This work was supported in part by an SBIR grant from the US National Cancer Institute of the NIH.

Abstract

Simulated moving bed (SMB) chromatography and its variant multicolumn continuous chromatography (MCC) have the potential to elevate the industrial chromatographic platform by conversion of conventional batch processes to more efficient continuous processes. The high productivity, recovery and purity achieved by SMB chromatography on an industrial scale for small molecules hold promise for biomolecule manufacture.

We used a lab-scale SMB device to perform continuous Protein A Capture (PAC) for the purification of mAbs. Rather than using one large adsorbent column and a sequential batch protocol, the SMB device uses eight small columns, multiple input and output streams, and a continuous protocol ("**Step-SMB**") in which several protocol steps occur simultaneously in different columns, analogous to the "row, row, row your boat" song sung in rounds. Flexibility in programming column and flow configurations enable optimization of purity, yield, and adsorbent utilization.

Unlike batch, Step-SMB is primarily a volume-driven process in which a given volume of feed material can be processed with the same amount of adsorbent, irrespective of titer. As mAb titers increase, flow rates and productivity increase. To take full advantage of the potential productivity gains afforded by Step-SMB for processing high titer mAb, per column residence times must be decreased while maintaining efficient capture.

For this study we purified a humanized IgG_{1a} mAb from CHO culture fluid using the Octave System in a Step-SMB process and an AKTA System in a standard batch process. We compared the performance of an agarose-based Protein A adsorbent with two polymethacrylate-based Protein A adsorbents in both processes.

Step-SMB Chromatography

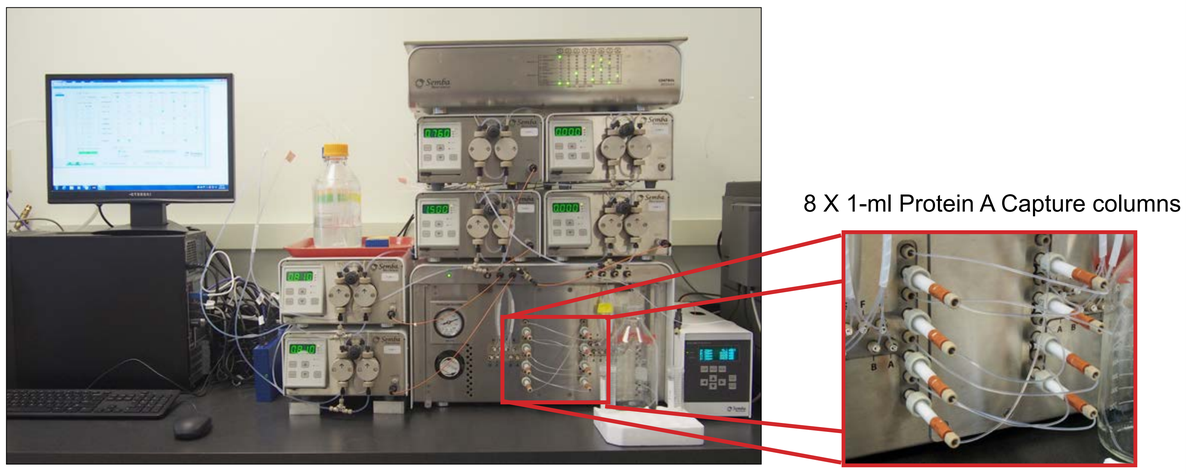


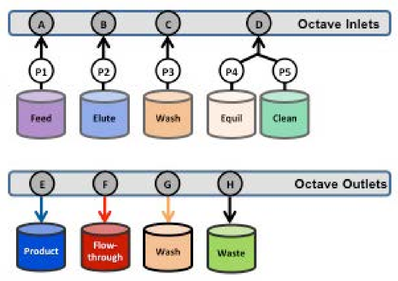
Figure 1. Octave™ Chromatography System

- Capable of performing SMBC/MCC and other continuous chromatography protocols
- Runs up to 8 columns, up to 8 pumps
- Proprietary valve block design; 72 two-way valves; low dead volume; non-metallic flow path
- Scalable from 12 ml/min to 300 ml/min flow rates; grams to kilograms per run

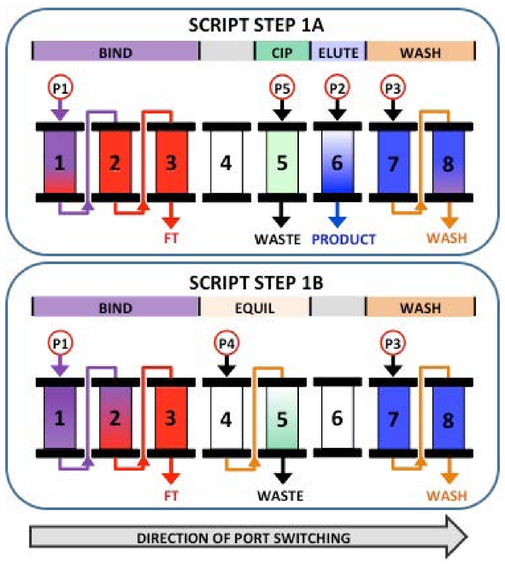
Protocol

1. Equilibrate 10 CV PBS
2. Bind (Feed) 80-100% static capacity at various res times
3. Wash 10 CV 25 mM Na phosphate, 1 M NaCl, pH 6.7
4. Elute 5 CV 100 mM citrate, pH 2.5 or 3.5
5. Clean 4 CV 100 mM NaOH, 1 M NaCl

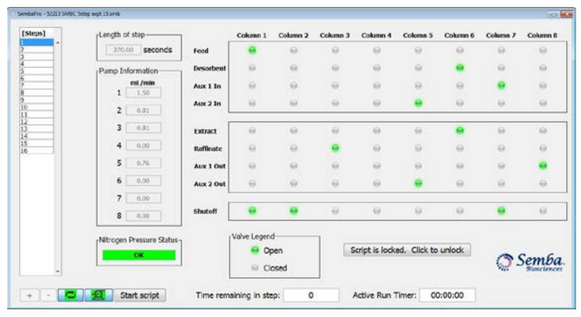
Octave inlet and outlet assignments



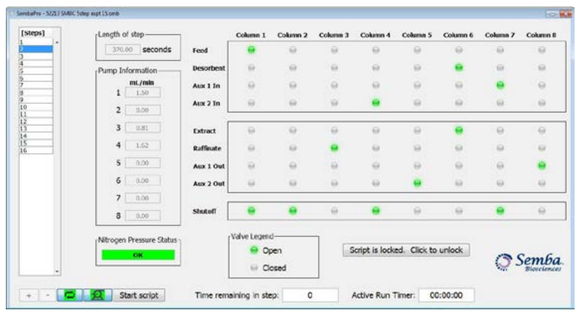
Column configuration



Octave program



Script Step 1A



Script Step 1B

Figure 2. Step-SMB process design

Results

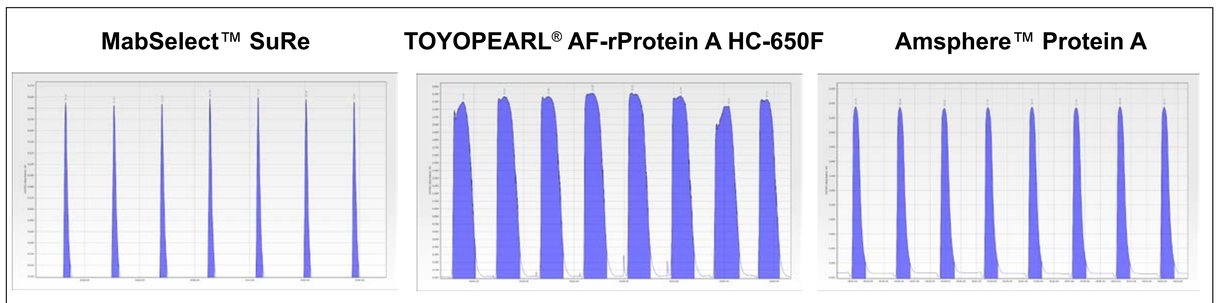
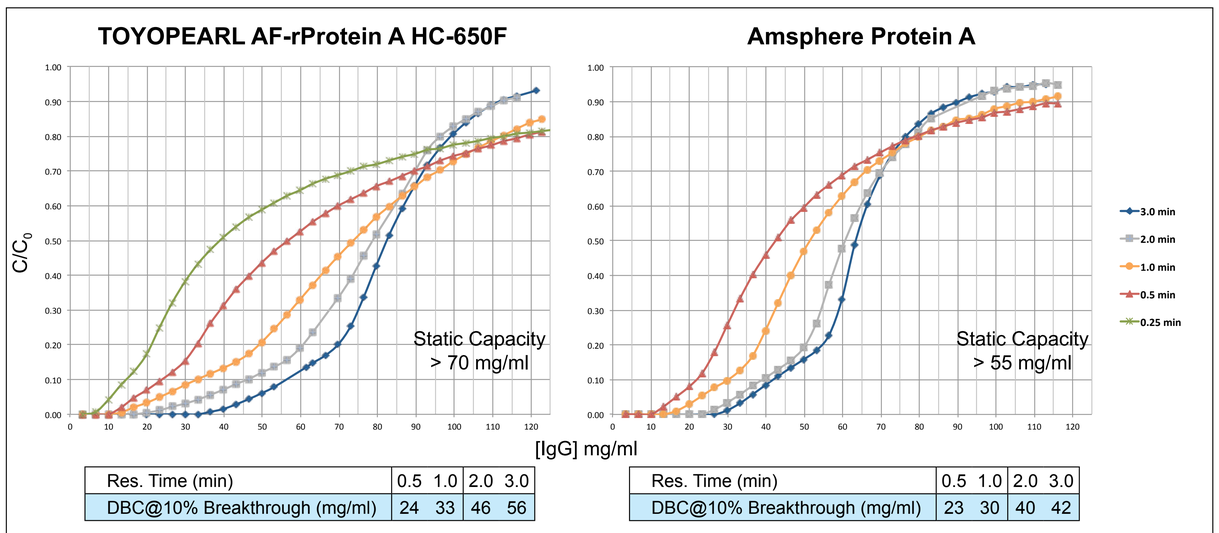


Figure 3. Representative chromatograms from Step-SMB PAC runs

Peaks represent A_{280} of elutions from successive columns. Each run was performed for at least 4 complete cycles (32 elutions). Purity analysis was performed on samples taken after 3 cycles.

	MabSelect SuRe			TOYOPEARL AF-rProtein A-650F		TOYOPEARL AF-rProtein A HC-650F	
	SC	SMB	SMB	SC	SMB	SMB	SMB
mAb titer (g/L)	2.37	2.37	5.0	2.37	2.37	5.0	7.5
DNA (pg/mg protein)	58.6	42.2	ND	524	70.8	ND	ND
HCP (ppm)	2.8 log	2.8 log	ND	2.8 log	3.3 log	2.0 log	2.0 log
Protein A (ppm)	1.8	2.3	ND	7.7	1.9	< 0.5	< 0.5
Monomer (%)	98.1	98.6	98.5	98.5	99.3	99.3	99.0
Res. Time (min/column)	2.0	0.5	0.7	2.0	0.5	0.5	0.5
mAb Loaded (mg/ml resin)	49	48	50	40	42	57	57

Table 1. mAb purity after single column (SC) and Step-SMB PAC



Res. Time (min)	0.5	1.0	2.0	3.0
DBC@10% Breakthrough (mg/ml)	24	33	46	56

Res. Time (min)	0.5	1.0	2.0	3.0
DBC@10% Breakthrough (mg/ml)	23	30	40	42

Figure 4. Binding capacities of polymethacrylate Protein A adsorbents

Dynamic binding capacity (DBC) was measured by running CHO culture fluid through single columns at the indicated residence times and measuring mAb concentration in the flow through. Static binding capacities were determined by batch binding for 2 h with 23 ml mAb at 5.9 mg/ml (136 mg) per ml resin.

	MabSelect SuRe		TOYOPEARL AF-rProtein A HC-650F		Amsphere Protein A	
mAb titer (g/L)	5.0	7.5	5.0	7.5	5.0	7.5
Productivity (g mAb/L resin/h)	49.5	50.7	**68**	**97**	**65**	**95**
Yield (%)	90	89	95	97	86	83
Res. Time (min/column)	0.7	1.0	0.5	0.5	0.5	0.5
Loading Flow Rate (cm/h)	250	169*	343	343	343	343
mAb Loaded (mg/ml resin)	50	50	57	57	42	42

* Maximum linear velocity possible due to flow rate maximum of 500 cm/h for Equilibration step

Table 2. Step-SMB PAC productivity with three capture resins at high mAb titers

Conclusions

1. The mAb produced from a Step-SMB PAC process performed on the Octave platform was equivalent or superior in quality to that produced by the analogous batch process. This finding was consistent between three resins: MabSelect SuRe (GE Life Sciences), TOYOPEARL AF-rProtein A-650F, and TOYOPEARL AF-rProtein A HC-650F (Tosoh Bioscience). **See Table 1.**
2. Two polymethacrylate Protein A capture resins exhibited high DBC at low residence times. The Step-SMB PAC process with 3-column capture zone configuration allowed a per column residence time of only 0.5 min while loading to capacities only possible with 3 min residence time using single-column capture. Combined with alkaline resistance and rigid structure enabling high flow rates, these properties make the resins ideal for processing > 5 g/L mAb titer feedstocks using multicolumn continuous chromatography. **See Figure 4.**
3. TOYOPEARL AF-rProtein A HC-650F demonstrated the best performance in Step-SMB at a mAb titer of 7.5 g/L: productivity was 97 g mAb/L resin/h with 97% yield. **See Table 2.**