Continuous protein purification using simulated moving bed: Taking the chromatography platform to the next level

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Abstract

Most common methods for purifying proteins use one or more liquid chromatographic steps in which single columns are employed in a linear process. Whereas these methods are often sufficient for analytical and lower scale separations, they can become cumbersome and expensive when larger amounts of purified protein are required. The inherent limitations of single column methods, including inefficient use of the solid phase and linear protocol, are addressed by simulated moving bed chromatography (SMBC; 1,2). SMBC elevates the chromatographic platform by conversion of the conventional batch process to a continuous process. SMBC has been successfully applied to small and large-scale binary separations of hydrocarbons, sugars, and enantiomers (1), but has rarely been used for protein purifications. The highly efficient zones of separation created by SMBC enable continuous bind-wash-elute chromatography and peak shaving of high purity target protein. The SMBC process is up to 20-fold more productive than single column elution methods due to the dramatic increase in utilization of the solid phase and continuous operation (1).

We have developed a bench-scale SMBC instrument, the Octave™ Chromatography System, designed for milligram-to-multigram scale purification of chemical and biological compounds. Here we demonstrate the utility of bench top SMBC for continuous SEC-based protein separation and Protein A affinity purification of a monoclonal antibody from cell culture fluid. Comparisons with single column purification revealed that the SMBC process resulted in improved efficiency in chromatography media and buffer utilization.

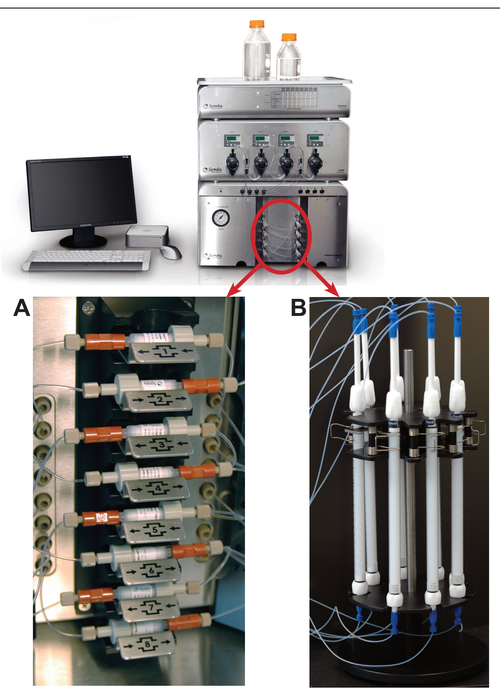


Fig. 1. Column arrangement in the Octave™ Chromatography System

A: Eight 1-ml disposable polypropylene columns placed in the Column Rack of the Octave 10 Chromatography Module.
B: Eight 1-cm ID Omnifit™ glass columns placed in the round Octave Column Stand.

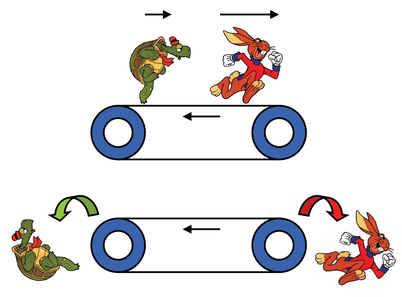


Fig. 2. Principle of countercurrent separation

In countercurrent systems the fluid phase flows in the opposite direction from the solid phase. Two species having different mobilities on the solid phase effectively separate if the transit time of the solid phase is greater than the residence time of the slower (more retained) species and less than the faster (less retained) species. In simulated moving bed systems the solid phase is represented by a series of columns and movement of the fluid and solid phases is achieved by switching the flow streams between consecutive columns at defined intervals.

References

1. Perrin, S.R. and Nicoud, R.M. (2001) *in* "Chiral Separation Techniques: A Practical Approach", Second Edition (ed. by G. Subramanian), pp. 253-285, Wiley-VCH Verlag GmbH.
2. Andersson, J. and Mattiasson, B. (2006) SMB technology with a simplified approach for protein purification. *J Chromatog A* **1107**, 88-95.

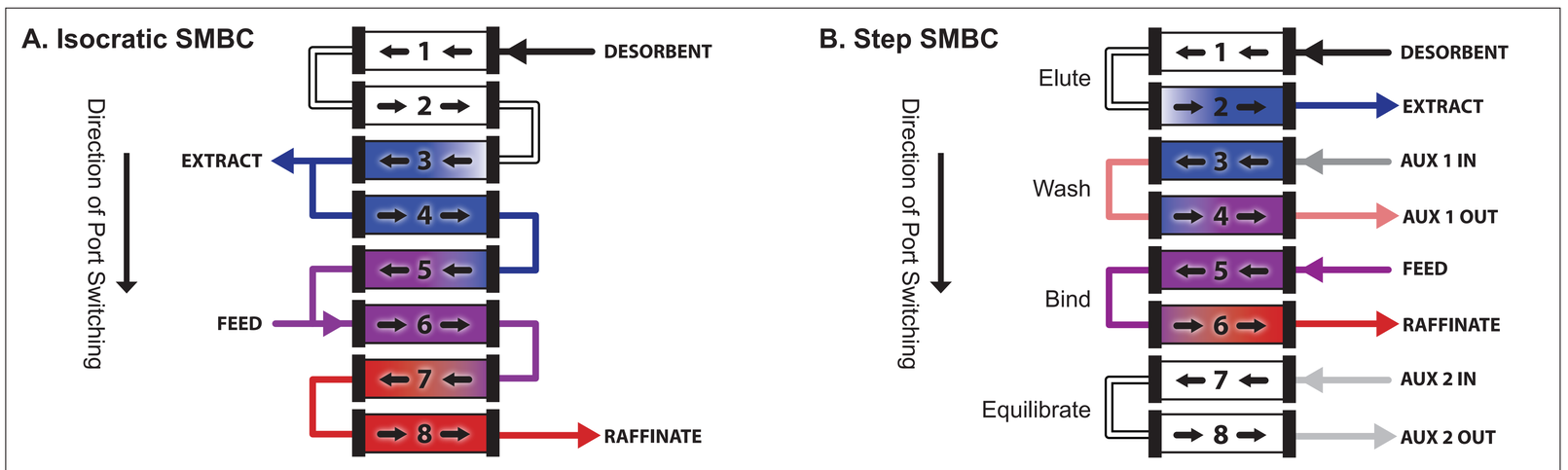
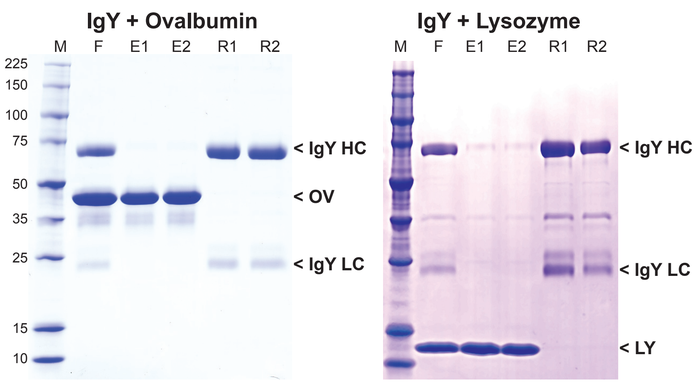


Fig. 3 Isocratic and Step SMBC flow configurations

Panels A and B show the inlet and outlet ports in the Octave System in isocratic and step flow configurations, respectively. In Isocratic SMBC (A), Feed and Desorbent buffers are the same composition, and separation occurs due to the difference in relative mobility of the Extract and Raffinate at a single buffer condition. In Step SMBC (B), four independent zones are established, which enables a protocol using Bind, Wash, Elution, and Equilibration buffers having different compositions. At periodic intervals, all ports are simultaneously switched to the next column in the series to simulate movement of the adsorbent in the opposite direction of fluid flow.



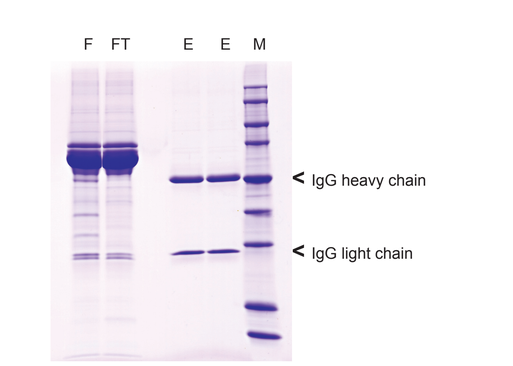
M: markers 10-225 kDa
F: Feed (protein mixture): IgY 180 kDa; OV 43 kDa; LY 14 kDa
E1, E2: Extract fractions (more retained)
R1, R2: Raffinate fractions (less retained)

Feed Mixture	Purity	Productivity SMBC	Productivity SC
IgY + Ovalbumin	IgY > 99% OV > 98%	150 mg/h each	35 mg/h each
IgY + Lysozyme	IgY > 95% LY > 90%	210 mg/h each	35 mg/h each

Columns: SMBC; 8 x 17 ml (1 x 21 cm each)
Single Column (SC); 1 x 136 ml (2 x 43 cm)

Fig. 4. Isocratic SMBC separation of proteins by size exclusion

Chicken IgY was mixed with either chicken ovalbumin or chicken lysozyme, as indicated. The proteins (5 mg/ml each) were separated by isocratic SMBC using eight 1.0 cm x 21.5 cm Omnifit™ glass columns packed with Superdex™ 200 Prep Grade resin. Under these conditions the faster-moving 180-kDa IgY was obtained in the raffinate and slower-moving ovalbumin (43 kDa) or lysozyme (14.3 kDa) was obtained in the extract fractions, as analyzed by SDS-PAGE.



F: Feed (concentrated tissue culture fluid)
FT: flow-through (Raffinate)
E: purified Mab (Extract)
M: markers 10-225 kDa

	Step-SMBC
Purity	> 99%
Recovery	95%
Productivity	200 mg/h

Mode	Resin	Buffer	Time	Productivity
Single Column	100 ml	960 ml	6.2 h	58 mg/h
Step SMBC	8 x 1 ml	320 ml	1.8 h	200 mg/h

Fig. 5. Step SMBC purification of monoclonal antibody with POROS® MabCapture® A resin

Monoclonal antibody was produced in mammalian cell culture. The culture fluid was concentrated, diafiltered, and applied to 8 x 1 ml POROS MabCapture A columns on the Octave System in a Step SMBC configuration. For comparison, a single column packed with 100 ml Protein A Sepharose® Fast Flow was run according to the manufacturer's instructions using the same process buffers and total sample load.

Column	Total DBC* at 30 mg/ml	Flow Rate	Productivity at 95% Yield	Time to Process 5 L	Buffer Volume	Number of Cycles	MabSelect® Sure
8 x 10 ml (SMBC)	2.4 g	16 ml/min	**9.12 g/h**	**5.2 h**	53.4 L	28	**$1,600**
1 x 800 ml (SC)	24 g	163 ml/min	**7.42 g/h**	**6.4 h**	57.6 L	3	**$16,000**

* Linear Velocity: 500 cm/h; Load at 75% dynamic binding capacity for SMBC and SC

Table 1: Comparison of Step-SMBC and Batch (SC) processes used for mAb purification from 5 L, 10 g/L titer culture.

1000 L Bioreactor mAb Titer, g/L	Step SMBC (8 x 750 ml) Liter resin	Cycle #	Process Time	SC or Batch (1 x 25 L) Liter resin	Cycle #	Process Time
3	6	13	10.4 h	25	3	7.5 h
6	**6**	**26**	**10.4 h**	**25**	**6**	**15 h**
9	**6**	**39**	**10.4 h**	**25**	**9**	**22.5 h**

PROSEP® – vA High Capacity SMB column size: 12.6 cm ID x 6 cm SC size: 40 cm ID x 20 cm

Table 2. Effect of mAb titer increases on processing time, cycle number and column volume

Conclusions

Specific affinity interactions simplify the fractionation behavior of complex biological feed streams to binary mixtures for purposes of Step SMBC protein purification and increase the attractiveness of this continuous technique for bioprocessing. Isocratic SMBC enables higher productivity protein SEC, eliminating long columns required for efficient separations at larger scale. The combination of the SMBC process and the Semba Octave Chromatography System allows:

- Unattended multi-gram scale protein purification
- Up to 20-fold savings in chromatography media and buffers
- Higher SEC productivity from smaller columns
- Preparation of concentrated highly purified proteins from dilute samples
- Highly efficient processing of high titer Mab with significant reduction in Protein A media consumption