

CONTINUOUS ANTIBODY PURIFICATION USING THE OCTAVE™ CHROMATOGRAPHY SYSTEM

Introduction

Protein A affinity chromatography is the predominant method for capture and purification of mAbs produced in mammalian cell culture. While Protein A chromatography is extremely specific and capable of achieving mAb purities greater than 98% in a single step, it is the most expensive component of the downstream process. An excellent reference (Vunnum, 2009) provides general information and method development tips for Protein A affinity chromatography. In recent years, new developments in cell culture technologies have led to significant increases in antibody titers (Birch and Racher 2006). Due to a linear relationship between antibody titer and amount of stationary phase required for capture, increases in antibody titers demand larger Protein A resin columns making the capture step even more costly.

Continuous SMB provides the flexible alternative to the batch process, because smaller columns are recycled toward the useful Protein A lifetime and disposed. The Octave chromatography system manages eight columns connected in series with fluid flow paths controlled through pneumatic valves. Four independent pumps control fluid flow rates. Each column has nine valve positions with inlet access to all four pumps, four outlet lines, and a shutoff valve that controls the flow from one column to the next in the series. By adjusting the various flow rates, the time at which the valves switch the inlet and outlet streams from one column to the next, and the solution compositions, one can create several simultaneously operating zones in which feedstock is continuously loaded, contaminants are washed away, purified product is collected, and columns are equilibrated for the next loading step.

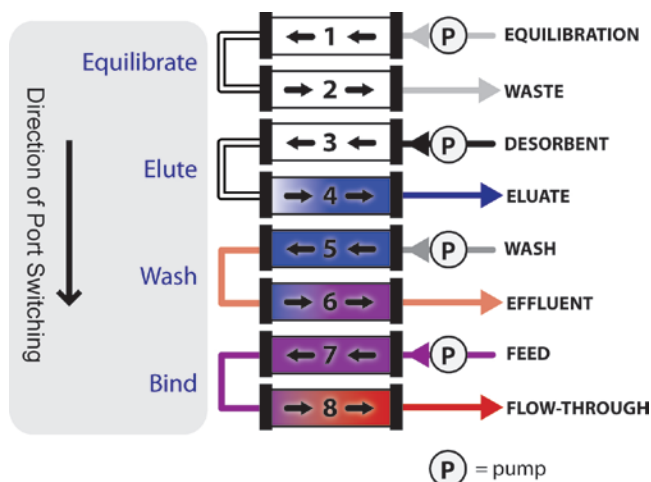


Fig. 1. Four-zone step SMBC configuration

Figure 1 shows a basic step configuration with four zones of two columns each to perform a simple bind, wash, elute, and equilibrate protocol. In the Octave System the columns remain stationary and ports are switched periodically to move the fluid streams among the columns. In this scheme the ports are switched in the direction of fluid flow such that the Bind zone becomes Wash, Wash becomes Elute, Elute becomes Equilibrate, Equilibrate becomes Bind, etc. in a continuous loop.

The step configuration can be modified as needed to accommodate additional solutions and steps in a purification scheme. For example, some bioprocessing protocols such as Protein A-mediated mAb capture might require up to 8 independent zones and include clean-in-place and column regeneration zones. The scheme in Figure 2 employs two additional pumps (for a total of six pumps) to add zones for strip and clean-in-place steps to the process.

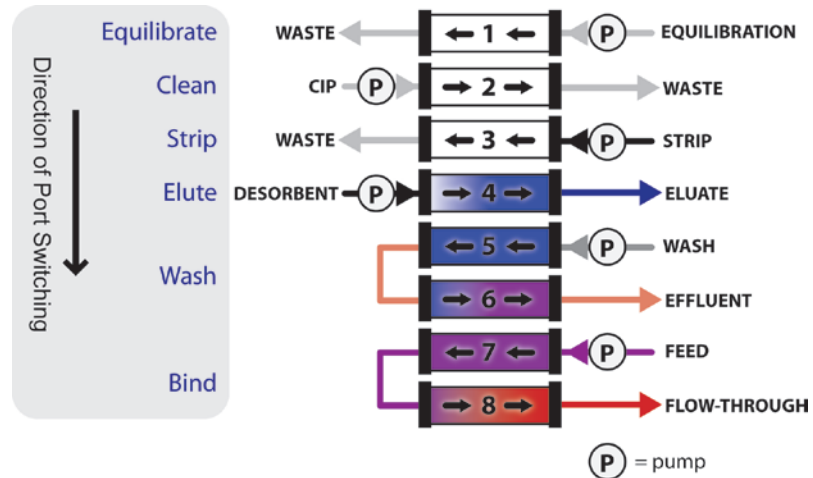


Fig. 2. Six-zone step SMBC configuration

The Octave System SembaPro™ software can control up to eight pumps and enables independent valve control with an unlimited number of steps. Intermediate steps can be created in which specific pumps are temporarily turned off or on in one or more zones to allow specific columns to be washed, soaked, cleaned, or otherwise processed. Figure 3 shows a scheme designed to incorporate a fifth zone for column cleaning into the basic four zone configuration that still uses four pumps. Each “major” step is comprised of two substeps A and B; in substep A, a CIP solution is used in one zone, and that zone is washed/equilibrated in substep B by turning off the CIP solution pump and opening/closing appropriate valves to divert flow from the wash zone into the former CIP zone.

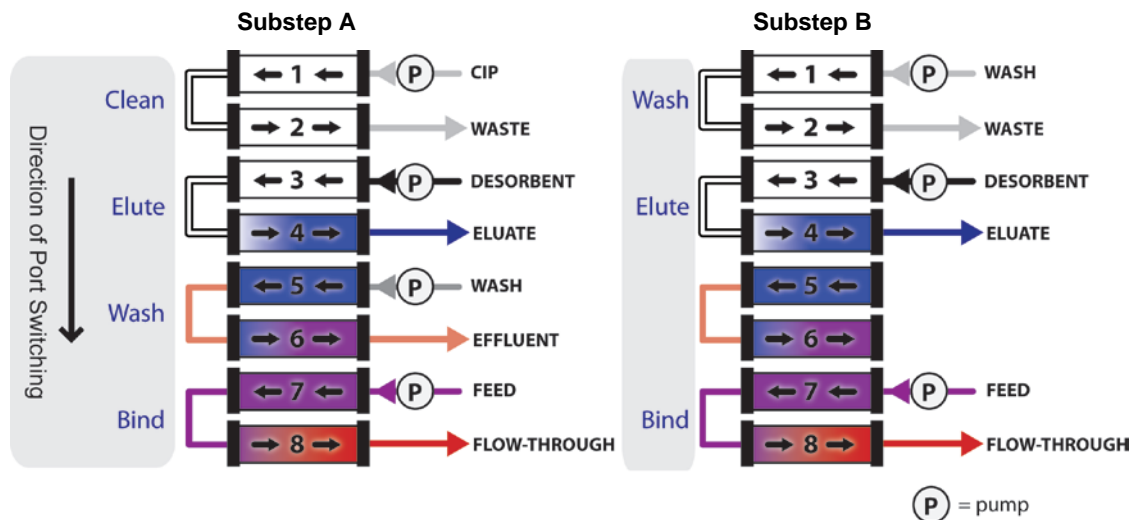


Fig. 3. Five-zone step SMBC configuration using four pumps

These examples illustrate only a few possible process configurations using the Octave System. The flexibility of valve programming and use of up to eight pumps enables the creation of an almost unlimited variety of schemes for antibody affinity purification.

Step SMBC Protocol for Antibody Purification

1.1 Determining Parameters for the Continuous Method

In most cases the starting conditions for a continuous method are derived from those established for a sequential batch protocol, including number of steps (e.g. bind, wash, elute, clean, equilibrate, etc.) and composition of the various solutions and buffers. Fig. 1 shows a 4-step protocol. The Octave™ System employs 8 columns and up to 8 pumps, so it can accommodate a variety of methods. Once the desired column configuration and buffer compositions are established, the main parameters to determine for Step SMBC are the flow rates of each pump, switch time, and number of cycles. These can be determined for a specific purification by knowing:

1. Capacity of a single column for the target antibody (varies with species, subclass, or individual antibody)
2. Total column volume in ml ($\pi r^2 h$, where r and h are in cm)
3. Approximate concentration of the target molecule in the sample (e.g. cell culture medium)
4. Volume of sample to be processed

The amount of sample (Feed) loaded on each column is determined by programming the duration (seconds) of the step or 'switch time' using the SembaPro™ software such that approximately 75% of the manufacturer's recommended column binding capacity for the target protein would be achieved at the flow rate and target protein concentration estimated in the sample. The Feed flow rate can be increased to accommodate dilute samples; however, in practice the flow rate should be kept slow enough to allow sufficient contact time between the target protein and the resin to allow complete adsorption. The flow rates for the wash, desorbent, and regeneration buffers are each set independently to achieve the desired flow volume in their respective zones.

1.2 Example Parameter Calculations

The following example describes the calculation of Step SMBC parameters for the 4-zone column configuration shown in Figure 1 using Semba's 1-ml Octave MabCapture® A Columns. The resin is comprised of a macroporous backbone that retains linear adsorption capacity at very high flow rates, and can tolerate much higher back pressures than conventional agarose-based resins.

1. Calculate the appropriate switch time (T).

$$T = \text{switch time} = [S / (Q_F \times C_{TP})] \times 0.75$$

Where:

S = Target protein capacity per column	45 mg
C _{TP} = Estimated concentration of target protein in feed	1 mg/ml
Q _F = Feed flow rate	15 ml/min

In this example, $T = [45 \text{ mg} / (15 \text{ ml/min} \times 1 \text{ mg/ml})] \times 0.75 = 2.25 \text{ min (135 sec)}$.

The Feed flow rate was selected based on the recommended linear velocity* (cm/h) to achieve 45 mg/ml resin binding capacity, and 1-ml column with cross-sectional area of 0.35 cm².

Thus in 135 seconds the first column in the binding zone should receive about 33.8 mg antibody or 75% of the column capacity. Antibody that is not captured by the first column in the binding zone would be captured by the second column in that zone. Depending on the antibody concentration and adsorption kinetics, the feed flow rate, switch time, and target protein load can all be adjusted to maximize capture efficiency and throughput.

2. Calculate the flow rates of the other zones based on the switch time, the number of columns in each zone, and the amount of buffer (in column volumes) that will be put through each column.

$$Q_j = \text{flow rate in Zone J} = (X_j \times V) / (T \times N_j)$$

Where:

X_j = column volumes of buffer used for each column in Zone J

V = total volume of 1 column

N_j = number of columns in Zone J

In this example the Wash, elution, and Regeneration zones will use 10, 6, and 10 column volumes, respectively:

$$Q_W = \text{Wash zone flow rate} = (10 \times 1 \text{ ml}) / (1.35 \text{ min} \times 2) = 3.7 \text{ ml/min}$$

$$Q_E = \text{Elution zone flow rate} = (6 \times 1 \text{ ml}) / (1.35 \text{ min} \times 2) = 2.22 \text{ ml/min}$$

$$Q_R = \text{Regeneration zone flow rate} = (10 \times 1 \text{ ml}) / (1.35 \text{ min} \times 2) = 3.7 \text{ ml/min}$$

3. Enter the parameter values (switch time, flow rates) into the appropriate script in SembaPro. The valves in all of Step scripts included with the Octave System are pre-programmed in a 4 x 2 column configuration with 8 steps. Open any of these scripts (e.g. Step-1ml-45-1) and modify accordingly. Refer to the Octave System User Manual for using SembaPro software.

1.3 Running the Method on the Octave Instrument

1. Set up pump connections for Step SMBC mode as described in the Octave System User Manual, where Pumps 1–4 are connected to inlets A–D, respectively.
2. Connect tubing to outlets E–H. Outlet E will be the purified product, so it can be run through an appropriate detector. All outlets should have a minimum 40 psi backpressure (maximum 270 psi); when running 1-ml or 5-ml polymeric columns (particle diameter > 30 microns), use the 40 psi backpressure regulators supplied with the system. *Note: If an outlet stream is connected to a UV/Vis detector, the backpressure regulator should be placed on the detector outlet to prevent air bubble formation in the flow cell.* Set up appropriate collection vessels. Unbound material (raffinate) will come from outlet F, G will contain wash effluent, and H will contain equilibration buffer used following elution.
3. Purge the system using jumpers as described in the Octave System User Manual.
4. Connect columns.
5. Prepare sufficient amounts of all solutions: Feed, Wash, Elute, Regenerate (Equilibrate). Filter all solutions through 0.45-micron filters before running through the Octave System.
6. Power up the Octave System and open the appropriate script in SembaPro.
7. Connect all inlet lines to appropriate reservoirs except use Bind buffer for Pump 1 (Feed). Prime the pumps with a syringe.
8. Run the script with Bind buffer instead of Feed for one cycle to equilibrate the system.
9. Transfer the Pump 1 inlet to the Feed reservoir and run the script for the desired length of time.
10. During the second cycle, analyze samples of Extract, Raffinate, Wash effluent, and (if desired) Regeneration effluent for the target protein.

* Linear velocity (cm/h) = [volumetric flow rate (ml/min) x 60 min/h]/column cross-sectional area (cm²)

Results and Troubleshooting

Figure 4 illustrates results of the continuous SMB purification of a mAb from cell culture fluid using eight 1-ml columns with the Octave 10 System. This SMB process produced 200 mg/h of antibody; purity was greater than 99% and recovery was 95%.

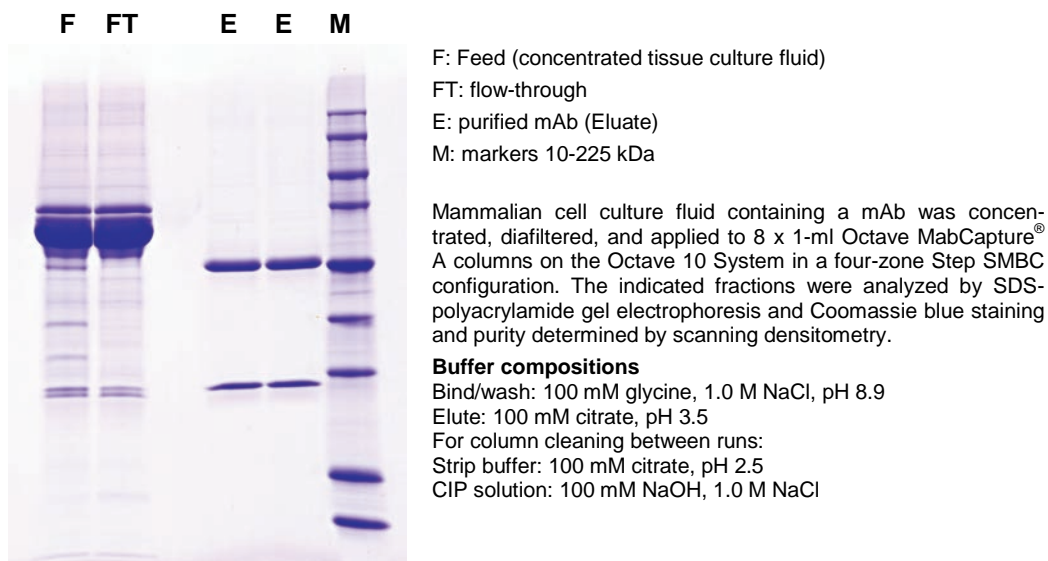


Figure 4. Continuous purification of a monoclonal antibody with Octave™ MabCapture® A Columns

Running parameters (flow rates, switch time) can be adjusted based on the results. Guidelines for adjustments are given in the table below.

Symptom	Possible reason	Possible solutions/steps
Target protein in Raffinate (flow-through); no other problem	Inefficient binding or target protein concentration in Feed too high for script conditions.	Decrease Feed flow rate; ensure column is properly equilibrated in Bind buffer; ensure target protein concentration is in appropriate range.
Target protein in Raffinate (flow-through); poor yield in Extract	Loss of column binding capacity	Ensure columns are not exhausted or dirty; ensure correct buffer formulations; ensure that columns are fully equilibrated in Bind buffer in Regeneration zone (increase flow rate of Pump 4); test an individual column for ability to bind target
Insufficient purity of target protein	Stringency/duration of Wash zone too low	Increase flow rate of Pump 2 (Wash); change composition of Wash buffer (e.g. decrease pH, increase salt, add detergent, organic solvents, glycols, etc.); increase switch time
Target protein in Regeneration effluent	Incomplete elution in Elute zone	Increase flow rate of Pump 3 (Elute)

References

- Birch, J.R and Racher, A.J. 2006. Antibody production. *Adv Drug Deliver Rev* **58**, 671–685.
- Vunnum, S., Vedantham, G. and Hubbard, B. 2009. "Protein A-based affinity chromatography." *In: Process Scale Purification of Antibodies*, Gottschalk, U. (ed.), John Wiley & Sons, Hoboken, New Jersey, 79–102.

Protein A Affinity Columns Available From Semba Biosciences

Product	Size	Cat. No.
Octave MabCapture® A Column	8 x 1 ml	C1007S
	2 x 1 ml	C1009S
	8 x 5 ml	C1008S
	1 x 5 ml	C1008E