TOSOH

2-step mAb purification with multi-column chromatography

Your Challenge

- You deal with increased demand and throughput concerns for antibody therapies.
- You struggle in designing intensified purification for both capture and polishing steps on the same platform.

Our Solution

Octave BIO and SkillPak BIO™ with best-in-class resins

- Perform capture and polish purification with Multi-column Chromatography.

What was done?

- A 2-step purification process was developed, tested, and intensified at the 1 and 5 mL scale.

What was the result?

- Increased process productivity while exhibiting robust impurity clearance and high yield.

The Octave BIO can both design and intensify a 2-step purification process, while TOYOPEARL resins packed in SkillPak columns show robust purification performance in MCC settings.

Your Benefit

Simplify the development of intensified purification with integrated solutions.

Intensified purification of antibody therapies

TOSOH BIOSCIENCE

SEPARATION & PURIFICATION

CONNECTING MINDS.
TOUCHING LIVES.

https://www.separations.eu.tosohbioscience.com/solutions/multi-column-chromatography/octave-bio-system

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Application Note

Two Chromatography Step Monoclonal Antibody Purification Using a Multi-Column Continuous Chromatography Platform

Monoclonal antibody (mAb) drug biomanufacturers typically rely on three batch chromatography steps in their downstream processes for removal of impurities such as host-cell proteins (HCPs), DNA, adventitious viruses, and aggregates. However, additional purification steps increase downstream expenses significantly, including costs of supplementary resin, hardware, buffers, and area demand. Thus, it is imperative to design and test effective purification procedures for high-quality biotherapeutics, that have reasonable process costs, time, and manufacturing space requirements.

Reducing the number of chromatography steps necessitates a reduction in operating and materials costs. Further, the use of multi-column continuous chromatography (MCC) significantly reduces operational costs, footprint, and operating time by increasing process productivity. Here we combine both these cost-savings measures and demonstrate the effectiveness of a two-chromatography step process, operated in MCC mode, for mAb purification using Tosoh Bioscience's Octave™ BIO, bench-top MCC system, and SkillPak™ BIO, pre-packed columns optimized for MCC applications.

Experimental Conditions

MCC System

The Octave BIO bench-top MCC system (*Figure 1*) is based on a reliable, and patented Octave technology made for bioseparation. The system can run methods using up to eight columns and six pumps. It contains a low dead volume replaceable manifold block (valve block), which pneumatically controls the flow path. Three exchangeable valve block sizes account for four different sizes of pumps with flow rates ranging from 0.1 to 300 mL/min. The system has integrated detectors, 4 each of UV, conductivity, and pH, for data collection. The system can also employ gradients in a single-column batch mode with a sample injector particularly useful for initial resin and method development. Furthermore, a three-way peak-collect valve enables collection of highly concentrated product.

Chromatography Resins

TOYOPEARL® AF-rProtein A HC-650F high-capacity affinity resin can be used for mAb capture, where it exhibits competitive binding performance. This is especially true at fast flow rates, which is advantageous for MCC processes. An operating binding capacity of up to 70 g mAb/L resin is observed for this resin in MCC operations. The maximal binding capacity of ~70 g mAb/L resin typically is observed for this resin in MCC operations.

Figure 1. Octave BIO bench-top MCC system with SkillPak BIO pre-packed columns.

Table 1. Specifications of SkillPak BIO pre-packed MCC columns for this study.

Parameter	TOYOPEARL AF-rProtein A HC-650F	TOYOPEARL Sulfate-650F
Column dimensions (cm)	1.6 ID × 2.5 BH	0.8 ID × 2.0 BH
Volume (mL)	5.0	1.0
Particle size (µm)	45	45
Pore size (nm)	100	100
Max. flow rate (cm/hr)	600	600
Max. Binding Capacity (g/L)	~70	>120
Max. operating pressure (MPa)	0.3	0.3
NaOH concentration suitable for cleaning (mol/L)	0.1	0.5

TOYOPEARL Sulfate-650F resin is a strong cation exchange resin that provides separation of mAb aggregates. It has a high salt-tolerance, a wide working pH range, and a dynamic binding capacity of >120 g mAb/L resin.

These resins are packed into Tosoh Bioscience's SkillPak BIO pre-packed columns, which are specifically suited for the Octave BIO system. (*Table 1*)

Analytical Techniques

Protein concentration was measured spectroscopically at AU280 nm. Host cell proteins (HCPs) were measured using Cygnus Technologies F550-1 ELISA kit. TSKgel UP-SW3000 column (4.6 mm I.D. × 30 cm) was used for analytical size exclusion chromatography (SEC) with a mobile phase of 0.1 mol/L KH_2PO_4/Na_2HPO_4, 0.1 mol/L Na_2SO_4, 0.05% NaN_3, pH 6.7

Results and Discussion

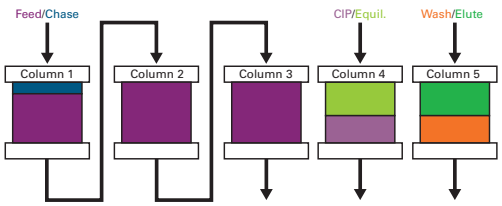
MCC Capture of mAb – TOYOPEARL AF-rProtein A HC-650F

The Protein A-based capture step is well established in batch mode, in which mAb binding and post-loading wash are carried out at neutral pH followed by low-pH mAb elution. In the study herein, a similar purification process was tested in MCC mode (*Table 2*) using the Octave BIO. Protein A columns (5 mL) were equilibrated and loaded with mAb-containing clarified Chinese hamster ovary (CHO) cell culture supernatant (titer 6.5 g/L) to ~90% of the static binding capacity (SBC). This load level is notably higher than the 80% of dynamic binding capacity (DBC) load levels typically seen in batch chromatography. The benefits of MCC in terms of resin capacity utilization are evident. Loading was optimized onto three columns in series with an additional two columns performing the remaining wash, elution, and CIP steps (*Figure 2*). Elution was carried out at pH 3.0. The eluate was collected based on a >200 mAU UV criteria, and isolated using the system's automated three way peak-collect valve.

Table 2. Protein A process parameters for MCC.

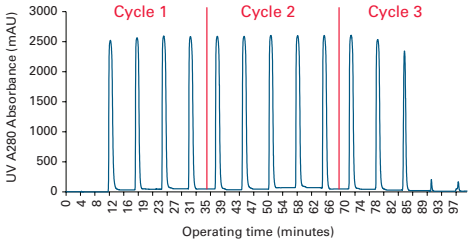
mAb loading (mg/mL resin)	~65
Max. flow rate (cm/hr)	300
Residence time for load (min)	0.5
Switch time (min)	6.7
Cycle time (min)	33.6
Number of columns	5
Number of cycles	3

Figure 2. Schematic representation of the MCC Protein A method.



The MCC method was tested in three purification cycles using five-column mode. The resulting elution chromatogram (*Figure 3*) exhibited reproducible elution peaks in a saw-tooth pattern with a negligible column-to-column variation during cycle two in an overlapped image (*Figure 4*). The yield (92.1%), concentration of the main elution fraction (19.3 mg/mL), and mass balance in different fractions were as expected (*Table 3*). Analytical SEC (*Figure 5*) for Protein A-purified mAb shows 4.9% aggregated mAb. The amount of mAb fragments (<10%) is typical for this mAb.

Figure 3. MCC Protein A elution chromatogram. AU280 nm trace of the elution outlet during run is in blue. Cycle changes are indicated with red line. Cycle 1 represents start-up, where the first elution at ~6 min is without protein, cycle 2 is equilibrium, where each column is loaded equally, and cycle 3 is shut-down, where the two last elution steps are directed to columns with no loaded protein.

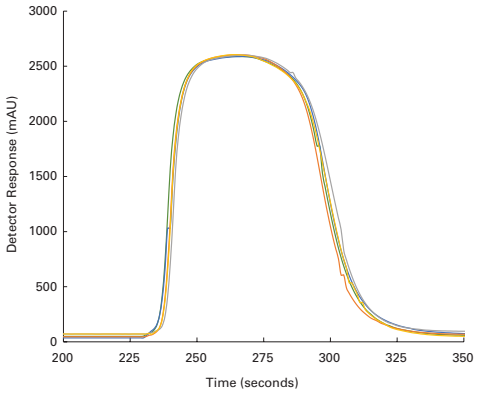


Equil. buffer:	phosphate-buffered saline (PBS), pH 7.4 (6 CV)
Chase:	equilibration buffer (3 CV)
Wash:	10 mmol/L Na-phosphate, 0.5 mol/L NaCl, pH 6.7 (7 CV)
Elution:	100 mmol/L Na-acetate, pH 3.0 (6.3 CV)
CIP:	100 mmol/L NaOH, 1.0 mol/L NaCl (5 CV)
Temperature:	ambient (room temperature)

Table 3. Protein A process results.

Fraction	Volume (mL)	mAb Conc. (mg/mL)	Total (g)	Yield (%)
Feed	525	6.5	3.4	N/A
Flow-through	1028	0.0	0.0	0.0%
Wash	551	0.2	0.1	3.4%
Eluate	163	19.3	3.1	92.1%
CIP	398	0.0	0.0	0.0%

Figure 4. Protein A column-to-column variation analysis during cycle 2. The different colors represent the five columns used in MCC mode. An overlapped image of the AU280 nm elution peaks from each column is shown.

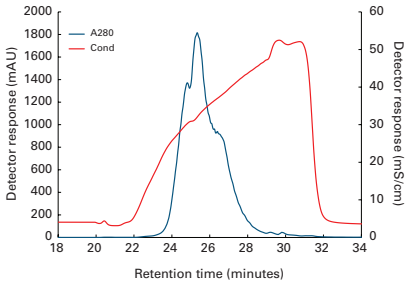


The mAb-containing eluate was then subjected to a low pH hold for an hour before a pH adjustment to 5.0 with 2 mol/L tris base. Because the capture step provided satisfactory purification and recovery of mAb, the next step was to find a suitable MCC polishing step. A bind-and-elute chromatography mode is ideal to take advantage of the added capacity and productivity benefits of MCC due to the overloading nature and increased capacity utilization of the process.

MCC Polishing for removal of impurities – TOYOPEARL Sulfate-650F

TOYOPEARL Sulfate-650F was chosen for the polishing step because of its favorable pressure-flow characteristics and excellent impurity clearance in mAb processes. First, optimal NaCl concentration was tested for efficient elution of mAb from sulfate resin. A single-column linear salt gradient experiment at pH 5.0 using Octave BIO revealed the approximate conductivity (~41 mS/cm) needed for mAb elution (*Figure 6*). Based on those results, 375 mmol/L NaCl in equilibration buffer was selected for the step elution.

Figure 6. A single-column NaCl gradient test using Octave BIO for determination of NaCl molarity for efficient mAb elution in step-mode on TOYOPEARL Sulfate-650F.

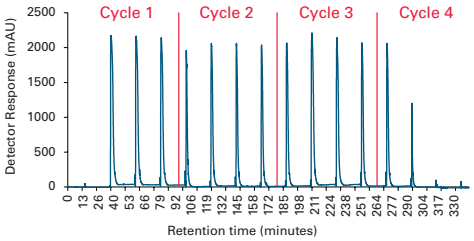


Elution conditions from the single column salt gradient were translated into a four-column TOYOPEARL Sulfate-650F MCC method (*Table 4*). mAb loading was optimized over two columns in series to prevent any significant mAb loss in the flowthrough fraction while still maintaining a high productivity. Two additional columns were required to complete the wash, elution, CIP, and reequilibration steps. For eluate collection, the AU280 nm peaks measuring >200 mAU were pooled. The following MCC method in four cycles resulted in adequate elution profiles, including highly reproducible peaks (*Figure 7*) and an excellent yield (95.1%) (*Table 5*).

Table 4. TOYOPEARL Sulfate-650F process parameters for MCC.

mAb loading resin	~90 mg/mL
Max. flowrate	204 cm/hr
Residence time for load	1 min
Switch time	21.4 min
Cycle time	85.7 min
Number of columns	4
Number of cycles	4

Figure 7. MCC Sulfate-650F elution chromatogram. AU280 nm trace of the elution outlet during run is in blue. Cycle changes are indicated with red line. Cycle 1 represents the start-up and cycle 4 the shut-down mode.



Equilibration: 50 mmol/L Na-acetate, pH 5.0 (10 CV)
Wash: equilibration buffer (5 CV)
Elution: 50 mmol/L Na-acetate, 375 mmol/L NaCl, pH 5.0 (12 CV)
CIP: 0.1 mol/L NaOH, 1.0 mol/L NaCl (5 CV)
Temperature: ambient (room temperature)
Sample: adjusted protein A eluate, diluted 1:5 into equilibration buffer

Table 5. TOYOPEARL Sulfate-650F process results.

Fraction	Volume (mL)	mAb Conc. (mg/mL)	Total (g)	Yield (%)
Protein A Eluate	165	4.2	689	N/A
Flow-through	251	0.0	4.0	0.6%
Wash	81	0.0	1.9	0.3%
Eluate	195	3.4	655	95.1%
CIP	85	0.3	23	3.3%
Equilibration	159	0.0	0.5	0.1%

Results Summary

	Protein A Capture	Sulfate-650F Polishing	Overall Process
Step Yield	92.1%	95.1%	87.6%
Steady-State Productivity (g mAb/L resin/hr)	106.4	59.9	NA
HCP Levels* (ng/mg mAb)	302	290	29 ng/mg mAb
% Aggregate	4.9%	1.2%	1.2%

*from an initial HCP level of 20,389 ng/mg mAb

Conclusions

A two-step MCC platform using the Octave BIO with TOYOPEARL AF-rProtein-A HC-650F and TOYOPEARL Sulfate-650F resins achieved an overall process recovery of 88%, aggregate levels of 1.2%, and HCP levels of 29 ng/mg. These results are comparable to what could be expected from a three chromatography step batch process, but with a significant reduction in cost and increase in productivity. The platform is straightforward to run and easily scalable to an industrial large-scale process using an Octave PRO MCC System, which is built with the same system architecture. A two-step MCC approach to downstream processing, using the Octave and SkillPak platforms, can achieve suitable product quality and recovery, while greatly reducing production costs.