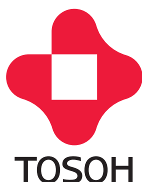


Application Note

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

Monoclonal antibody (mAb) drug biomanufacturers typically have relied on multi-step processes using batch-mode for optimal 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, but with reasonable process costs, time, and manufacturing space requirements.

Innovations in downstream mAb processing technology, such as the use of multi-column continuous chromatography (MCC) instrumentation, have recently been shown to significantly reduce operational costs, footprint, and time investment by increasing process productivity. To demonstrate the benefits of MCC technology in downstream processing, here we describe a two-step MCC platform for mAb purification using Tosoh Bioscience's bench-top instrument, Octave® BIO, using SkillPak™ BIO pre-packed columns optimized for MCC applications.

Experimental Conditions

MCC Instrumentation

The Octave BIO bench-top MCC system (*Figure 1*) is based on a rugged, reliable, and patented Octave technology made for bioseparation. The system can run methods using up to eight columns using six pumps. It contains a low dead volume replaceable manifold block (valve block), which is hydraulically triggered to regulate flow within each column. Three exchangeable valve block sizes account for four different sizes of pumps with flow rates ranging from 12 to 300 mL/min. For complete data recording, the system has integrated detectors (four of each) for UV, conductivity, and pH. The system can also run gradients in a single-column batch mode with a sample injector. That is 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. Protein A exhibits a competitive binding profile, especially at fast flow rates, which is a highly sought attribute for MCC processes. 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 instrument with SkillPak BIO pre-packed columns.

Table 1. Specifications of SkillPak BIO prepacked 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 stability (mol/L)	0.2	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 proprietary 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. 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

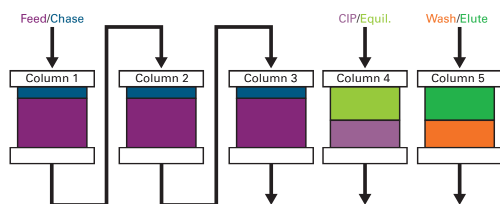
MCC Capture of mAb – TOYOPEARL AF-rProtein A HC-650F

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 MCC system. 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 column capacity. 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 where the AU280 nm analysis measuring >200 mAU redirected through a 3-way peak-collect valve and pooled separately from the rest of the process elution stream.

Table 2. Protein A process parameters for MCC.

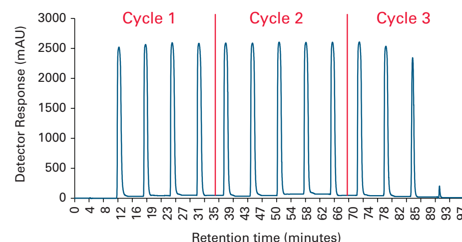
Parameter	Value
mAb loading (mg/mL resin)	~65
Max. flow rate (cm/hr)	300
Residence time for load (min)	0.5
Switch time (min)	6.7
Cycle time (min)	33.6
Number of columns	5
Number of cycles	3

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 the start-up mode where the first elution at ~6 min is without protein and cycle 3 the shut-down mode where the two last elution steps are directed to columns with no loaded protein.

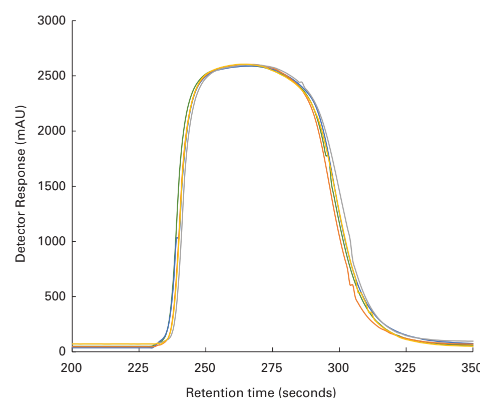


Equil. buffer: phosphate-buffered saline (PBS), pH 7.4 (6 CV)
Chase: equilibration buffer (3 CV)
Wash: 10 mmol/L Na-phosphate, 0.5 mol/L NaCl, pH 6.7 (7 CV)
Elution: 100 mmol/L acetate (NaOH), pH 3.0 (6.3 CV)
CIP: 100 mmol/L NaOH, 1.0 mol/L NaCl (5 CV)
Temperature: ambient (room temperature)

Table 3. Protein A process results.

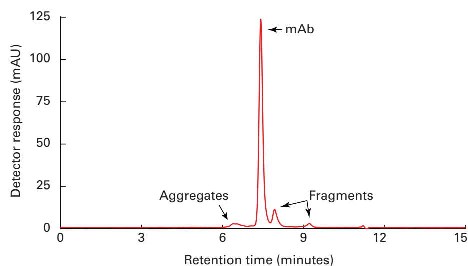
Fraction	Volume (mL)	Protein (mg/mL)	Total (g)	Yield (%)
Feed	525	6.5	3.41	N/A
Flow-through	1028	0.0	0	0.0%
Wash	551	0.2	0.12	3.4%
Eluate	163	19.3	3.15	92.1%
CIP	398	0.0	0	0.0%

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.



For virus inactivation, the mAb-containing eluate was held at pH 3.8 for one hour before 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, ideally benefiting from bind-and-elute chromatography mode, to enhance aggregate and HCP removal.

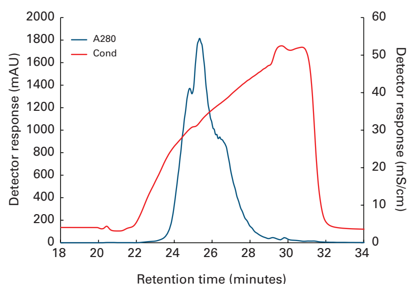
Figure 5. Analysis of Protein A-purified mAb using analytical SEC.



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 system 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.

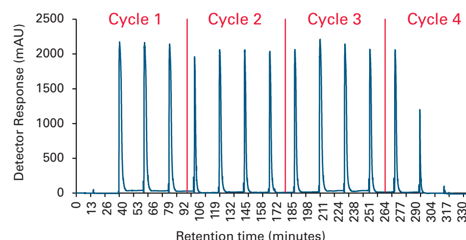


Elution conditions from the single column salt gradient elution were translated into a four-column TOYOPEARL Sulfate-650F MCC method (*Table 4*). mAb loading was optimized over two columns in series to prevent any significant mAb loss in the flowthrough fraction while still maintaining a high productivity. Two additional columns were required to complete the remaining 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.

Parameter	Value
mAb loading (mg/mL resin)	~90
Max. flowrate (cm/hr)	204
Residence time for load (min)	1
Switch time (min)	21.4
Cycle time (min)	85.7
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: protein A eluate, diluted 1:5 into equilibration buffer

Table 5. TOYOPEARL Sulfate-650F process results.

Fraction	Volume (mL)	Protein (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%

Process Summary

- The final 2-step process yield:
  - 87% (based on a 92.1% recovery from Protein A and 95.1% recovery from Sulfate-650F)
- The steady-state productivity:
  - Protein A step: 106.4 g mAb/L resin/hr
  - Sulfate-650F step: 59.9 g mAb/L resin/hr
- HCP clearance:
  - Feed: 20,389 ng/mg mAb protein
  - Protein A eluate: 302 ng/mg mAb protein
  - Sulfate-650F eluate: 29 ng/mg mAb protein
- Aggregate reduction:
  - Protein A eluate: 4.9%
  - Sulfate-650F eluate: 1.2%

Conclusions

A two-step MCC platform using the Octave BIO MCC system with TOYOPEARL AF-rProtein-A HC-650F and TOYOPEARL Sulfate-650F resins result in highly satisfactory overall mAb recovery, purity, and process productivity. The platform is straightforward to run and easily scalable to an industrial large-scale process using an Octave PRO MCC System (coming soon), which is built with the same technology. The Octave chromatography platform supports transformation of biologics purification toward MCC instrumentation because of the technology's beneficial effects on product quality, time savings, and reduction of production costs.

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