Increase Antibody Purification Productivity Using Multi-Column Chromatography

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Introduction

- Biotherapeutics, such as monoclonal antibodies (mAbs) and their modifications, have typically relied on multi-step purification processes for optimal removal of impurities such as host cell proteins (HCPs), DNA, adventitious viruses, and antibody aggregates.
- However, additional purification steps increase downstream expenses significantly, including costs of supplementary resin, hardware, buffers, area demand, etc.
- Thus, it is imperative to design and test effective purification procedures for production of high-quality biotherapeutics, but with reasonable production 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 (e.g. Khanal & Lenhoff, 2021; Matte, 2022).
- **To demonstrate the benefits of MCC technology in downstream processing, we here describe a 2-step MCC platform for mAb purification using Tosoh Bioscience's bench-top instrument, Octave™ BIO, using SkillPak™ BIO prepacked columns optimized for MCC applications.**

Octave BIO Multi-column Continuous Chromatography (MCC) Technology

Benefits of MCC:

- Integrated continuous processing with highly consistent purification cycles.
- Smaller equipment and lower solvent consumption.
- Typically, 2 to 5-fold higher productivity vs. batch systems.
- Tosoh Bioscience offers a bench-top Octave BIO (right) for process development and small-scale purifications and a process counterpart Octave PRO, built with the same technology, for industrial applications resulting in scalability from milligrams to kilograms of purified product.

Bench-top Octave BIO MCC instrument.

SkillPak BIO Pre-packed MCC 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
Alkaline stability (mol/L)	0.2 NaOH	0.5 NaOH

Capture Step on TOYOPEARL AF-rProtein-A HC-650F Resin

Experiment overview:

- Sample: mAb (IgG_1)-containing Chinese hamster ovary (CHO) cell culture supernatant (titer 6.5 g/L).
- Three loading columns in series with a total of five columns (Figure 1).
- Once the 1st column was almost fully saturated, it was then moved to the washing state, and the 2nd column that was capturing the breakthrough now moved into the primary capture position.
- The columns were then cleaned and re-equilibrated before re-entering the load zone in a cyclical process for three full cycles (Table 2).
- Protein concentration was measured spectroscopically at AU_{280}. HCPs were measured using Cygnus Technologies F550-1 ELISA kit. TSKgel® UP-SW3000 column (4.6 mm ID × 30 cm) was used for analytical SEC.

Figure 1: Graphic representation of the MCC Protein A method.

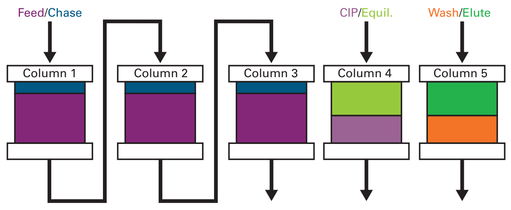
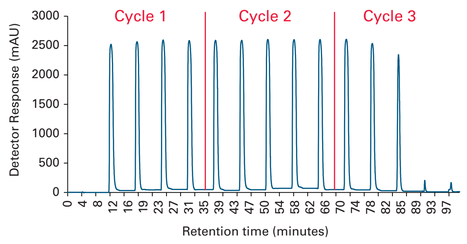


Table 2: Protein A process parameters for MCC.

mAb loading (mg/mL resin)	~65
Max. flowrate (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

Results

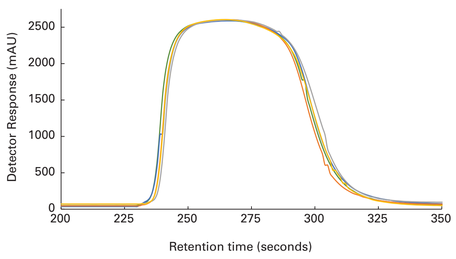
Figure 2: Reproducible elution peaks.



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)

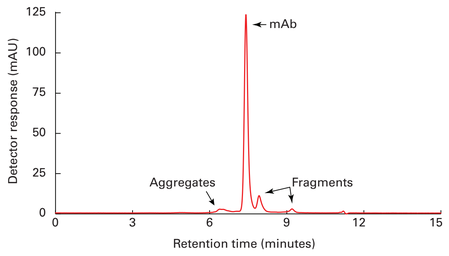
Protein A elution chromatogram where AU_{280} trace is in blue. Cycle 1 represents the start-up mode where the first elution at ~6 min is without protein and cycle 3 the shut-down mode where the two last elution steps are directed to columns with no loaded protein.

Figure 3: Minimal column-to-column variation.



Protein A column-to-column AU_{280} variation during Cycle 2. The different colors represent the five columns used in MCC mode.

Figure 4: Analytical SEC for the eluate.



Column: TSKgel UP-SW3000 column (4.6 mm ID × 30 cm)
Mobile phase: 0.1 mol/L KH_2PO_4/Na_2HPO_4, 0.1 mol/L Na_2SO_4, 0.05% NaN_3, pH 6.7

Analytical SEC for Protein A-purified mAb shows 4.9% aggregated mAb. The amount of mAb fragments (~10%) is typical for this mAb.

- Run chromatogram shows reproducible elution peaks in a "saw-tooth" pattern (Figure 2) with a minimal column-to-column variation during cycle 2 in an overlapped image (Figure 3).
- Figure 4 shows analytical SEC for the eluate. 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).

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%

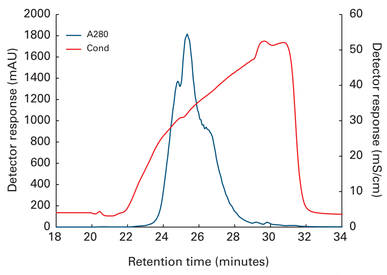
- The steady-state productivity was 106.4 g mAb/L resin/hr. This is ~6-fold higher compared to our similar batch production result (16.8 g mAb/L resin/hr).
- For virus inactivation, the mAb-containing eluate was held at pH 3.8 for 1 hour prior to pH adjustment to 5.0 with 2 mol/L Tris-base.
- As the capture step resulted in 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 host-cell protein (HCP) removal.

Polishing Step on TOYOPEARL Sulfate-650F Resin

Experiment overview:

- TOYOPEARL Sulfate-650F was chosen as the candidate resin for the polishing step due to 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 NaCl gradient experiment at pH 5.0 using Octave BIO system revealed the conductivity (~41 mS/cm) needed for efficient mAb elution (Figure 5).
- Based on this result, 375 mmol/L NaCl in equilibration buffer was selected for the step elution.
- Elution conditions from the single column salt gradient elution were translated into a 4-column TOYOPEARL Sulfate-650F MCC method (Table 4).

Figure 5: Single-column NaCl gradient test.



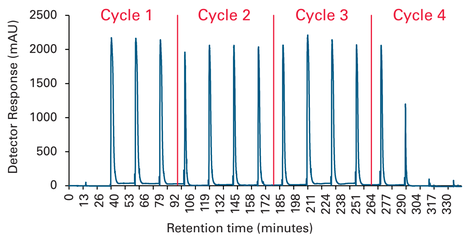
A single-column NaCl gradient test using Octave BIO for determination of NaCl molarity for efficient mAb elution in step-mode on TOYOPEARL Sulfate-650F.

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

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

Results

Figure 6: TOYOPEARL Sulfate-650F elution chromatogram.



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

AU_{280} trace is in blue. Cycle 1 represents the start-up and cycle 4 the shut-down mode.

Table 5: TOYOPEARL Sulfate-650F process results.

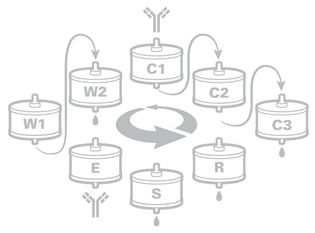
Fraction	Volume (mL)	Protein (mg/mL)	Total (mg)	Yield (%)
Protein A Eluate	165	4.2	689	N/A
Flow-through	251	0.0	4.0	0.6%
Wash	81	0.0	1.9	0.3%
Eluate	195	3.4	655	95.1%
CIP	85	0.3	23	3.3%
Equilibration	159	0.0	0.5	0.1%

Note: 22% of the total protein A eluate (3.15 g) was used for polishing step demonstration.

- The following MCC method in four cycles resulted in adequate elution profiles including very reproducible peaks (Figure 6) and an excellent yield (95.1%) (Table 5).
- The steady-state productivity was 59.9 g mAb/L resin/hr.

Process Summary

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



Conclusions

- Here, we present a two-step MCC platform using an Octave BIO system with TOYOPEARL AF-rProtein-A HC-650F and TOYOPEARL Sulfate-650F resins resulting in a highly satisfactory overall mAb recovery, purity, and process productivity.
- For both MCC steps, the steady-state productivity values were several-fold higher than typically obtained from batch purification processes.
- Using MCC can lead to a reduction in overall protein A resin requirements of up to 90% with a concomitant reduction in buffer consumption of 30-45%.
- The platform is straight-forward to run and easily scalable to an industrial large-scale process using a powerful sister instrument, Octave PRO skid (Tosoh Bioscience LLC), built with the same technology.
- **The Octave BIO chromatography platform presented here strongly supports transformation of biologics purification toward MCC instrumentation due to the MCC technology's beneficial effects on product quality, time savings, and reduction of production costs.**

More About MCC Technology
1. Khanal O. Lenhoff A.M. Developments and opportunities in continuous biopharmaceutical manufacturing. mAbs, 13:1, e1903664, 2021
2. Matte A. Recent advances and future directions in downstream processing of therapeutic antibodies. Int. J. Mol. Sci, 23:15, e8663, 2022

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