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Application Note

Multi-Column Chromatography for Efficient Polishing Purification

Summary

Multi-Column Chromatography (MCC) was developed for mAb capture to improve speed and productivity and circumvent bottlenecks associated with bind/elute (b/e) Protein A chromatography. These bottlenecks reduce efficiency and hinder biomanufacturers from implementing a fully continuous process. Adoption of the MCC approach for capture chromatography has resulted in up to 10-fold reductions in resin costs and an increase of up to 40% in productivity.

As MCC is an integral step in achieving a fully continuous capture operations, we investigated the possibility of using the same methodology for polishing chromatography as a component of continuous downstream processing. The studies in this application note focused on Ion Exchange (IEX) chromatography in bind/elute mode using the Mobius® Multi Column Capture system.

Testing involved a model protein, α-protein, with impurities of similar physiochemical characteristics; the difference in isoelectric point (pI) between α-protein product and the β-protein impurity was 0.5, and the difference in conductivity at which the α-protein product and the β-protein impurity elute is approximately 8 mS/cm.

Following multiple rounds of purification on Fractogel® EMD SO3-(M) Cation Exchange (CEX) resin in bind/elute mode using the Mobius® Multi Column Capture system, the α-protein product was separated from the β-protein impurity. The purity of the α-protein product increased from 55.03% to 81.77 %, while impurity levels were reduced from 43.5% to 17.01%, similar to the profile following batch separation. However, the productivity of the MCC purification at 25 g protein/h/L resin was more than double that of batch purification, which achieved 11.5 g protein/h/L resin. This enhanced productivity, while maintaining purity, presents opportunities to integrate MCC with the Mobius® Multi Column Capture system for downstream operations.

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Introduction

Increasing demands for affordable, in-demand biopharmaceuticals have increased efforts from biomanufacturers to move towards intensified and fully continuous manufacturing. Transitioning from batch to continuous processing has been slow and intermittent, and although unit operations have become more integrated, they do not necessarily all operate in continuous mode.

The move towards continuous processing started with the adoption of perfusion bioreactors that enabled continuous production of biological products upstream. Operating continuously increased cell density by up to 10-fold, and titer up to 50-fold. However, to reap the benefits of increased upstream productivity, downstream unit operations need to be modified to efficiently process the harvest material without bottlenecks.

The focus of this application note is to assess the utility of Multi-Column Chromatography (MCC) method using Cation Exchange (CEX) resins in a bind/elute mode to improve productivity without compromising purity. Compared to a flow-through approach, CEX chromatography in bind/elute mode enhances separation of closely related species through selective binding of the target protein.

MCC System Design and Operation

Downstream chromatographic purification steps have been a major impediment to achieving fully continuous processing as one or more steps relies on bind/elute mode of operation. As compared to traditional single column batch chromatography, implementing MCC in CEX purification offers the opportunity to increase productivity while reducing resin volumes, costs and footprint without compromising yield or purity.

The development of automated multi-column capture systems like the Mobius® Multi Column Capture system, take MCC purification to a new level. These systems are typically operated as a 3-column chromatography system with 3 independent pumps using single-use pump heads. Flow paths are fully single-use and include bubble trap, pre-column pH and conductivity meter, post column pH and conductivity meter, and UV meter, **Table 1**.

Parameter	Mobius® Multi Column Capture system Operating Ranges
Flow Range	8 to 120 L/h with inline dilution (ILD)
Column Diameter	From 10 cm to 25 cm*
Process compatible	Continuous 20 days: -1000 L up to 2 vessel volume per day -2000 L up to 1 vessel volume per day -Intensification 48 h (up to 5 g/L) -1000 L/8 h -2000 L in 16–20 h
Maximum Pressure	4 bars
Software	Enables: pH recalibration, dilution control, synchronization of load and non-loading durations column loading switch triggers based on breakthrough (BT), time, column volume (CV), absolute UV, area under the curve
SU Flowmeter accuracy	±3% Measured Value
Dilution Capacity	Use concentrated buffers (based on volume or conductivity) ≈dilution factor 30x
* Can be run with 7 cm columns if necessary	

Table 1. Mobius® Multi Column Capture system parameters

The MCC operation is generally divided into two functional phases: load and non-load, which operate independently. Three columns are connected in series. The first two columns, the primary and secondary columns, are continuously loaded with product via a dedicated pump. The primary column receives the protein feed, while the secondary column captures the flow from the primary column, allowing the primary column to achieve higher breakthroughs and enhancing resin utilization compared to batch processes. The non-load steps (washes, elution, strip, and equilibration) are run independently on a single column by a separate pump, **Figure 1**. When the primary column is fully loaded, it transitions to non-load steps, while the pre-loaded secondary column takes over as the primary column. The third column then becomes the new secondary column. This cycling between load and non-load steps continues until the loading process is complete.

The third pump in the MCC system serves as a dilution pump, allowing for in-line dilution of buffer concentrates with Water for Injection (WFI). This capability further lowers the costs associated with the chromatography step and minimizes the manufacturing footprint.

Although primarily developed for capture chromatography with Protein A resin, the MCC approach can reasonably be applied to any bind/elute chromatographic purification operation to improve productivity and resin utilization.

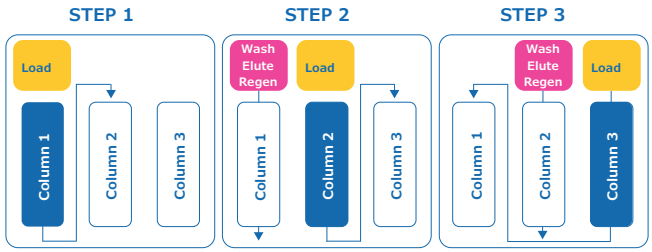

Figure 1. Multi-Column Chromatography workflow. MCC allows the simultaneous loading, wash, elution and regeneration of multiple columns

The increase in productivity and resin utilization during MCC is due to the serial connectivity of the primary and secondary columns which enables the primary column to be loaded beyond the 1% breakthrough (BT) load typical of single (batch) column loading. Any breakthrough from the primary column will be captured in the secondary column. Resin volumes and costs are reduced as a consequence of running many cycles on three small-volume, rather than one single, large-volume column.

Switching between columns can be triggered by UV signal, volume loaded (CV), integrated area under the breakthrough curve, or loading time. The BT detection algorithm monitors the UV signal at the outlet of the primary column and compares it continuously with BT input value. The BT value can be entered as an absolute value (AU) or a value corresponding to 100% BT and a BT percentage to reach.

MCC Application in Polishing Purification

The purpose of this study was to perform process development, optimization and scale-up of MCC to the Mobius® Multi Column Capture system using the polishing chromatography CEX resin Fractogel® EMD SO3-(M), to differentiate and separate closely related molecules.

Specifically, the focus was on purity, dynamic binding capacity (DBC), breakthrough and back pressure profiles in the scaled-up process. In addition, performance of the Mobius® Multi Column Capture system was assessed in terms of accuracy, reproducibility, and how the system responded to variations in temperature, conductivity, and pH that could impact molecule separation. The sensitivity of instruments and software were closely monitored to assess the interactions of software and hardware for controlling the process.

Materials and Methods

Testing involved a feed containing a model protein product, α-protein, with impurities of similar physiochemical characteristics: the difference in pI between α-protein product and the β-protein impurity was 0.5, **Table 2**. The same feed was used in process development and scaled-up process studies. This feed was purified by Protein A chromatography to a concentration of 17.79 g/L, then sterile filtered and diluted with equilibration buffer (40 mM Sodium Acetate, pH 4.3 ±0.2, conductivity 5 ±0.5 mS/cm) to a final concentration of 4.02 g/L, pH 4.3, 5 mS/cm for process development and 4.35 g/L for the scaled-up run using the Mobius® Multi Column Capture system.

Process Development Runs

For process development studies, an aliquot of the model feed was used at a final concentration of 4.02 g/L, pH 4.3, 5 mS/cm. Process development was performed using a single 5 mL (8 mm x 100 mm) pre-packed column containing Fractogel® EMD SO3-(M) CEX resin in an ÄKTA Pure™ 25 chromatography system. Purification steps were adapted from a batch purification protocol. To optimize the MCC method, break through curves were generated on the resin-packed column; the packed bed height was the same in both the single column used for process development and for the process runs in the Mobius® Multi Column Capture system. To establish maximum DBC (equivalent to static binding capacity), the process development column was loaded to 100 % BT at 10 minute residence time (RT). The ÄKTA system data was then exported into a Microsoft Excel-based tool (developed in-house) and analyzed to determine the maximum binding capacity. Following determination of maximum binding capacity at 10 min RT, 3 runs were performed at RT of 1, 2 and 3.25 min RT, **Table 3**.

Based on modelling parameters, this Excel-based tool generated comparative breakthrough curves, and analyzed optimization parameters including resin utilization, DBC, productivity, and protein losses in serially connected columns and identified the optimal conditions for scale up.

The run with 3.25 min RT was identified by the Excel-based tool as the optimal conditions; runs with 1 and 2 minute RT, and linear velocities of 600 cm/h and 300 cm/h, were suboptimal with Fractogel® EMD SO3-(M) resin which has a max linear velocity of 250 cm/h. These shorter, suboptimal RT conditions were predicted to result in protein breakthrough in the secondary column.

Feed Composition	Composition (%)	Molecular weight (kD)	Isoelectric Point (pI)	Description
α-protein	52.27	14,200	4.7–5.2	Product
ß-protein	43.50	18,400	5.2	Impurity 1
Bovine Serum Albumin (BSA)	2.66	65,000	4.9	Impurity 2
IgG, IgA, IgM	1.57	150,000–900,000	5.8–7.3	Impurity 3

Table 2. Feed composition and physiochemical characteristics.

Process Parameters	10 min RT	1 min RT	2 min RT	3.25 min RT
% Resin utilization	100	85	100	80
DBC (g/mL resin)	132.94	111.85	110.33	113.06
Protein loaded to column 2 (g/mL resin)	1.00	12.05	2.21	7.95
% Breakthrough in column 2	1.20	3.20	1.20	0.4
Steady State loading time (min)	124.51	3.20	8.50	15.61
Non load time (min)	50.00	50.0	50.0	50.0
Productivity (g/Lresin/h)	45.7	126.3	285.9	264.8
Buffer consumption (L/protein)	0.94	1.11	1.42	1.11
Continuity/Synchronization Alarm	Synchronized	Not synchronized	Not synchronized	Not synchronized
Load resin max linear velocity alarm	Safe flow rate	Exceeds max flow velocity	Exceeds max flow velocity	Safe flow rate
Non-load resin max linear velocity alarm	Safe flow rate	Exceeds max flow velocity	Exceeds max flow velocity	Safe flow rate

Table 3. Process parameter comparison and selection table from excel based tool dashboard. Values shown for column 2 are predicted from the analysis.

To maintain process continuity during column cycling in multi-column chromatography, the duration of the loading time must be longer than the non-load time (equilibration, rinse, wash, elution, regeneration and sanitation). Once the parameters of the 3.25 min RT run from process development were optimized on the ÄKTA system, they were scaled up for the run on the Mobius® Multi Column Capture system.

Process Scale-up Runs

For scale-up studies in the Mobius® Multi Column Capture system, 1565 L of model feed at a concentration of 4.35 g/L, pH 4.3, 5 mS/cm was loaded on three 10 cm diameter QuikScale® columns, each packed with 0.785 L of Fractogel® EMD SO3-(M) resin with a packing bed height of 10 cm. The feed was chilled at 7-10 °C with gentle stirring throughout loading, **Figure 2**.

Information generated from the process development studies and analyzed by the Excel-based tool was used to size the columns, calculate the buffer requirements for each step, and the expected operation time. **Table 4** shows the close alignment between model predictions and results from midway through the purification, at cycle 15, for each of the three columns in the Mobius® Multi Column Capture system.

Based on input data, the tool predicted 30 cycles with a run time of 110 hours. The model did not account for the recycling of the reserve, which was recirculated back to the feed tank in real-time during the process scale run.

Additional time is included in these calculations for sanitization and installation time for the pre-sterilized Mobius® Flexware components; these were assumed to be 8 hours. If prepacked columns are not used, additional time for packing and qualification of chromatography columns should be considered. Table 5 summarizes qualification information for Quikscale® columns packed with Fractogel® EMD SO3-(M) resin.

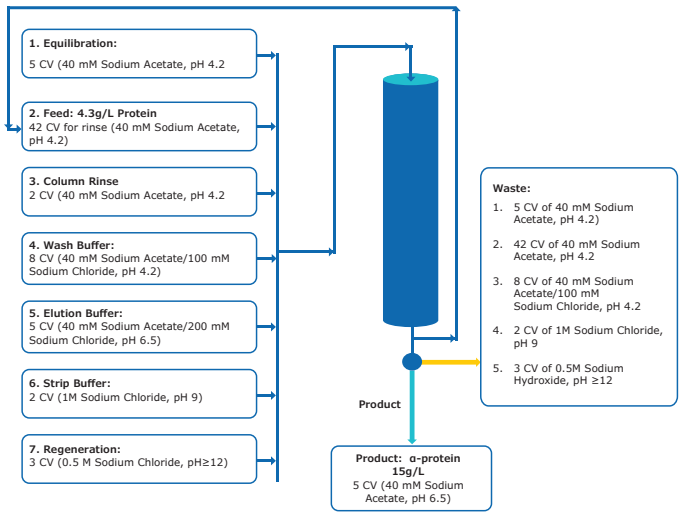

Figure 2. Process scale Mobius® Multi Column Capture system.

Comparison & Decision	Model	Mobius® Multi Column Capture system		
Residence Time (min.)	3.2	Column#1, Cycle#15	Column#2, Cycle#15	Column#3, Cycle#15
% Breakthrough	80%	80%	80%	80%
% Resin Utilization	0.86	0.98	0.93	0.95
Dynamic Binding Capacity (g/mL Resin)	178.47	199.16	194.91	199.48
Protein Loaded to Column 2 (g/mL Resin)	52.50	2.809	7.05	1.94
% Breakthrough in Column 2	1.3%	0.0%	0.0%	0.0%
Steady State Load Time (min)	121.7	151.8	151.76	151.34
Non load time(min)	88.8	50.0	50.00	50.00
Productivity (g protein/L resin/h)	88.0	25.0	25.0	25.0
Buffer consumption(L/g protein)	0.15	0.43	0.43	0.43
Continuity /Zyncronization Alarm	Synchronized	Synchronized	Synchronized	Synchronized
Load Resin Max Linear Velocity Alarm	Safe Flow Rate		Safe Flow Rate	Safe Flow Rate
Non Load Resin Max Linear Velocity Alarm	Safe Flow Rate		Safe Flow Rate	Safe Flow Rate

Table 4. Comparison between model predictions and results from cycle 15 on each column of the Mobius® Multi Column Capture system.

Column	Packed Column Height (cm)	Asymmetry	Height Equivalent to a Theoretical Plate (HETP)
Column 1	10	1.196	0.0654
Column 2	10	1.469	0.0524
Column 3	10	1.353	0.0588

Table 5. Qualification of QuikScale® columns (100mm x 500mm) packed with Fractogel® EMD SO3-(M) resin.

Once the Mobius® Flexware components and columns were installed, the Mobius® Multi Column Capture system editor was used through Human Machine interface (HMI) to manually prime the buffer and product lines with equilibration buffer to remove air from the system with the columns offline. Once completed, the columns were brought online, and each packed column was equilibrated. The optimized run parameters were entered into HMI editor including %BT (80%), impurity profile (0.08AU), elution collection starts and end (0.1 AU). The load linear flow rate was 184.62 cm/h (14.49 L/h), and the non-load linear flow rate was 300 cm/h (23.55L/h).

The method was run continuously without any interruption for 107 hours (4 days) for 29 cycles and included 87 loaded columns. This aligns closely with the predicted run time of 110 hours with 30 cycles of loading.

Purification Results

The process scale chromatography polishing run using the Mobius® Multi Column Capture system successfully separated the target product (α-protein) from product related impurity (β-protein) without any human intervention during the automated cycling purification. Product purity increased from 55% to 82% with an excellent recovery of 92%.

Clarified Feed	% Proportion	Volume(L)	Conc.(g/L)	Amount(g)
α-protein	55.0	1565	4.35	3746.3
β-protein	43.5			2961.4
BSA	2.7			181.1
IgG, IgA, IgM	0.0			0.0

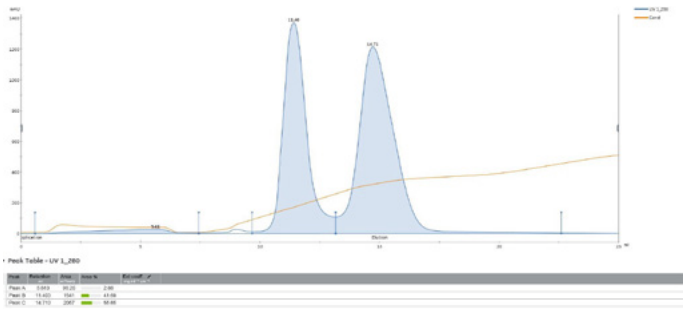

Figure 3. Composition and analytical chromatogram showing peak distribution of feed before purification.

Figure 4 lists the composition of the eluate following purification and the peak distribution on an analytical chromatogram and shows the selectivity of the Fractogel® EMD SO3-(M) resin for separating a protein product from an impurity with similar properties. This effective separation was achieved using the Mobius® Multi Column Capture system and control processing parameters (conductivity, pH and UV) determined from process development studies using an ÄKTA Pure™ 25 chromatography system.

Purified Feed	% Proportion	Volume(L)	Conc. (g/L)	Amount	% Recovery
α-protein	81.8	341.5	12.4	3462.6	92.4
β-protein	17.0				
BSA	0.0				
IgG, IgA, IgM	0.0				

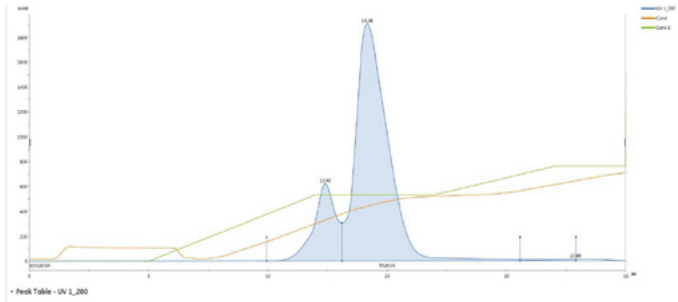

Figure 4. Composition and analytical chromatogram showing peak distribution of elution pool following purification.

During the continuous operation and 87 column loadings, a high degree of inter column reproducibility was observed as demonstrated by the consistency of UV-280 nm profiles, for each of the three columns throughout the 29 cycles of loading for each column, **Figure 5**. The total protein bound to each column was 159 ±3g which translates to dynamic binding capacity at 80% break through (DBC 80%) of 109 g α-protein/L resin.

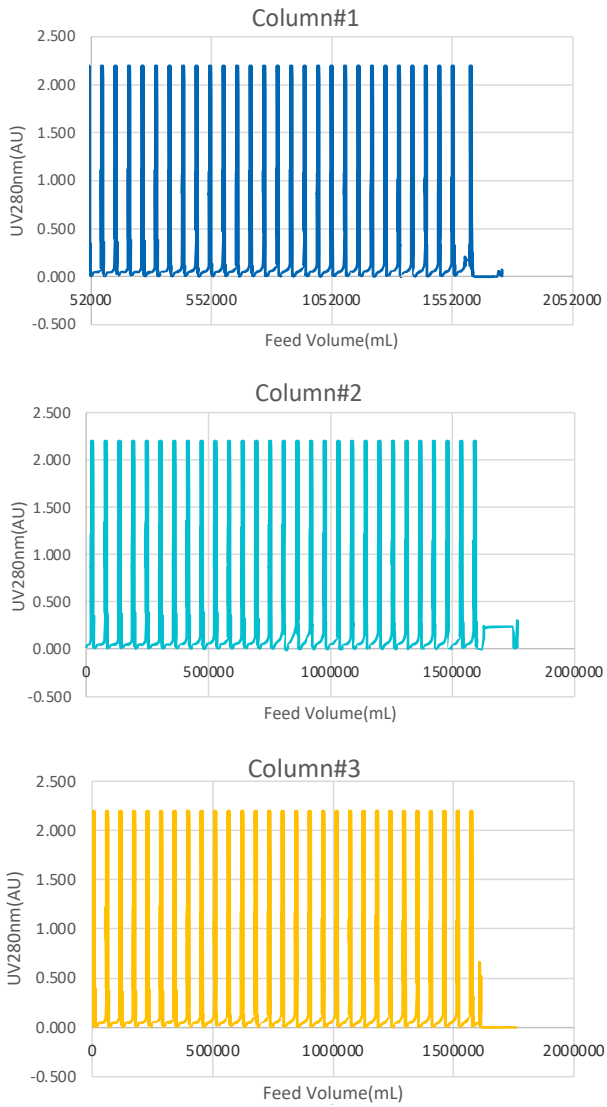

Figure 5. Shows consistency of UV-280 nm profile across the 29 cycles (87 loads) in all the 3 columns containing Fractogel® EMD SO3-(M) resin

Figure 6 compares chromatograms from 3 columns at cycle 15, the midpoint of the purification. During the load process, column #2 shows an early break through as compared to column #1 and column #3. This slight shift was attributed to minor differences in column packing; although all 3 columns passed qualification, minor variations in symmetry and plate numbers remained.

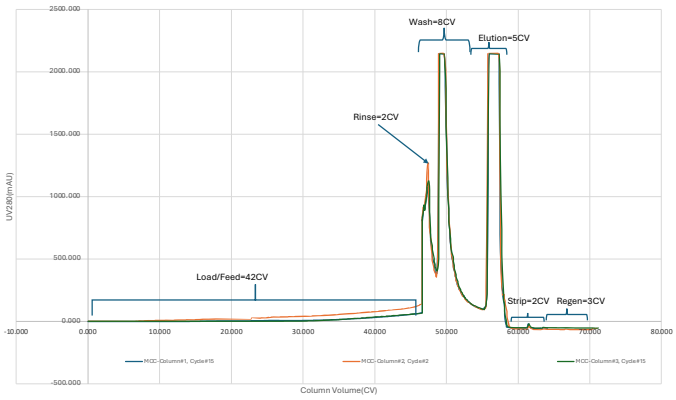

Figure 6. Representative UV-280 nm profile of all 3 columns at cycle #15 of the 29 cycles. Column #2 shows slight early protein breakthrough, most likely attributable to minor variations in column packing.

Discussion and Conclusion

Optimized conditions from process development studies on an ÄKTA Pure™ 25 chromatography system with a single column containing Fractogel® EMD SO3-(M)CEX resin were leveraged and applied to a larger-scale run using a Mobius® Multi Column Capture system.

Even though the target protein exhibited very similar characteristics to the principal impurity, separation using the automated Mobius® Multi Column Capture system with Fractogel® EMD SO3-(M) CEX resin, was highly selective and resulted in protein quality profile that was very similar to that obtained using batch purification methods.

Comparing the multi-column purification to a typical batch purification reveals significant benefits:

- **Productivity:** 25 g protein/h/L of resin with multi column purification as compared to 11.5 g protein/h/L in the single column batch process. This doubling of productivity results in significant cost benefits to the purification steps.
- **Resin volumes and costs:** Multi column purification required 2.4 L of Fractogel® EMD SO3-(M) resin as compared to a predicted volume of 30 L of resin for single column batch purification. These reduced resin requirements result in significant cost benefits for the multi column purification. Buffer consumption, purity and recovery were similar between multi column and batch purification.
- **Yield and recovery:** were similar in multi column and batch purification at 92%. Purity achieved with the multi-column purification was 82%.
- **Automation:** monitoring of chromatographic purification steps using the Mobius® Multi Column Capture system was automated as compared to manual operations throughout the single-column batch process. Automation frees up operators for other tasks while minimizing costs for system monitoring.

These studies demonstrate the effectiveness of the Mobius® Multi Column Capture system for bind/elute downstream purification steps beyond Protein A capture. By adapting this multi column purification approach to cation exchange purification, downstream productivity can be improved and result in significant cost savings. This approach provides another option for process intensification and may help biomanufacturers in the transition to fully continuous manufacturing.

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