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Application Note

Efficient Protein A Capture in Intensified Processes: Process Characterization and Scalability

Maximizing productivity of monoclonal antibody (mAbs) and biosimilars manufacturing relies heavily on the successful integration of intensified processing into the workflow. As the first step in downstream processing, the protein A capture step is highly selective in terms of delivering product quality and purity, but it can be a bottleneck in terms of processing efficiency and is costly, due to the large volumes of resin needed. Low productivity can be improved by shifting the protein A capture step from a batch operation to continuous capture. This transition improves resin utilization through increased loading, effectively decreases the cost of intermediate resin storage, and reduces buffer consumption.

The Mobius® Multi Column Capture system was developed to facilitate the shift from batch to continuous capture. This system is fully automated, and incorporates single-use, closed technologies enabling continuous, closed, capture chromatography. The study described in this application note was performed to evaluate the performance of the system in a representative biomanufacturing environment. The objectives were to assess the efficiency of continuous mAb capture from a clarified and filtered harvest cell culture fluid (HCCF), using the Mobius® Multi Column Capture system and to evaluate process scalability.

The study was executed in three phases:

Step I: Determination of operating parameters for a continuous capture process.

Step II: Process characterisation and evaluation of feasibility at lab-scale.

Step III: 400 L capture process using Mobius® Multi Column Capture system and previously determined operating parameters.

Figure 1. Mobius® Multi Column Capture system

Experimental Methods

Cell culture harvest

HCCF containing the target mAb was clarified using Millistak+® HC C0SP filters followed by filtration using Multilayer Durapore® 0.45/0.22 µm filters. The mAb concentration (titer) was 2.76 g_{mAb}/L as determined using Protein A HPLC method that uses MAbPac™ Protein A, 12 µm, 4 × 38 mm, column (ThermoFisher Scientific) and an Alliance 2498 UV/Vis from Waters™ system. The equilibration buffer was 50 mM NaH_2PO_4 pH 7,2 (Sigma-Aldrich) and the elution buffer was 50 mM NaH_2PO_4 pH 2.5.

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Step I – Determination of operating process parameters – constant volume approach

The dynamic binding capacity (DBC) study used Eshmuno® A resin to determine the appropriate product loading in column volumes (CVs).

For those tests, a Vantage® L11 column was packed with Eshmuno® A resin and qualified using an ÄKTA Avant™ system (BH: 11.6 cm, CV = 11 mL, As: 1.39, 2440 plates/m). The basic test procedure with residence times (RT) of 1, 2, and 4 min is summarized in **Table 1.**

Table 1. Process sequence for DBC study.

Solution	Column Volume
Equilibration	>3 until baseline
HCCF	Until 100% BT (UV_{280nm})
Wash	1.1 - 1.5
High Salt Wash	2
Wash	>3 until baseline
Elution	5
Equilibration	3
Cleaning	3

Step II – Scale down model assessment

This step simulated a multi-column capture process using an ÄKTA Avant™ system with external modifications. Addition of single-use assemblies enabled the system to be operated in continuous mode using three columns to reproduce a key feature of the Mobius® Multi Column Capture system. The lab-scale setup also included a reserve collection feature to recover unbound mAb from the first column's wash volume, allowing for reprocessing to improve yield and productivity (**Figure 2**).

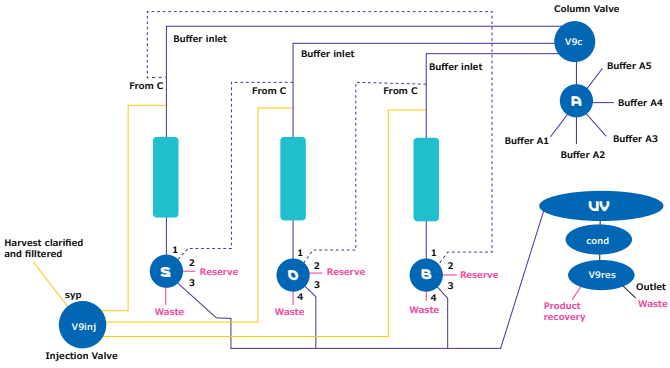


Figure 2. Lab-scale multi-column capture set-up

To operate the ÄKTA Avant™ system continuously, buffers were managed using Valve A and diverted to a selected column with the column valve (**Figure 2**). The sample was applied using the sample pump and diverted to the selected column using the injection valve.[1]

Each column was connected to an inlet valve (S, O and B) allowing diversion of the flow through to the next column (1), to waste (2), to the reserve (3) or to collection (4, elution). **Figure 3** shows a schematic of the Mobius® Multi Column Capture system illustrating loading and non-loading methodology between the various columns.

Only one set of sensors was available so pH, conductivity and absorbance (UV 280 nm) were monitored for the column in the non-loading phase only. The multi-column capture process was automated using Unicorn® 7.1 software.

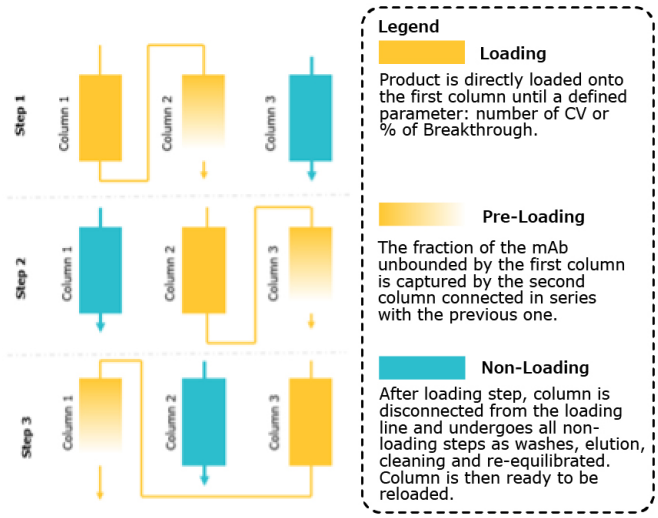


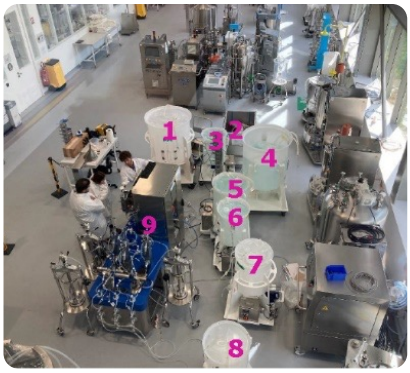
Figure 3. Mobius® Multi Column Capture system methodology.

Step III – Large scale process

After determination of parameters and process simulation at lab-scale using the ÄKTA Avant™ system, the at-scale continuous process was performed on the Mobius® Multi Column Capture system using a Common Control Platform® recipe, **Figure 4**. For this run, three QuikScale® 200 columns packed with Eshmuno® A resin were used (Bed Height: 6 cm, CV: 1.85 ±0.05 L, Assymetry: 1.77 ±0.04, NETP: 3275 ±1070 plates/m).

Buffer composition, physico-chemical properties, and CV triggers determined during the lab-scale run were used for the large-scale trial.

[1]For additional details on set-up and operation of the ÄKTA Avant™ system for continuous capture operations, please contact your local representative.



1. Filtered feed tank
2. Unfiltered feed tank
3. High salt buffer
4. Equilibration buffer
5. Elution buffer
6. Sanitization buffer
7. Eluate tank
8. Reserve tank
9. Mobius® Multi Column Capture system

Figure 4. Mobius® Multi Column Capture System during the bioprocessing workflow.

Results

Step I

Resin capacity was assessed with clarified and filtered HCCF at 2.76 g_{mAb}/L and residence times of 1, 2, and 4 min, following typical DBC procedures (**Figure 5**).

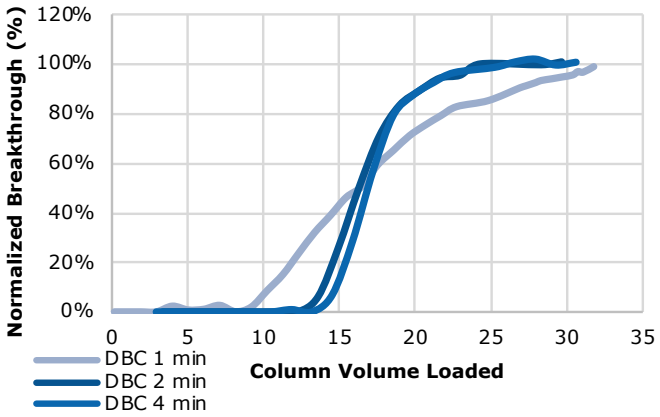


Figure 5. Breakthrough curves obtained at different residence times, for DBC calculation.

Figure 6 shows the DBC at 10% of the breakthrough (BT) was determined to be 28.6 g_{mAb}/L_{resin} at 1 min residence time, 39 g_{mAb}/L_{resin} at 2 min residence time, and 41 g_{mAb}/L_{resin} at 4 min residence time. The difference in capacity between 2 min and 4 min of residence time was unexpectedly low (2 g_{mAb}/L_{resin}). Given this productivity difference, 2 min was used for the capture process condition. The protein A resin used for the study had been used numerous times, and it was expected that binding capacity would be below the typical value (<50 g_{mAb}/L_{resin} at 4 min RT, 90% DBC at 10% BT).

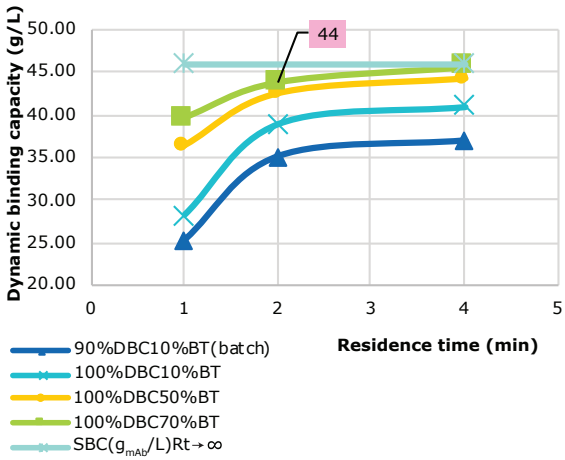


Figure 6. Breakthrough (%) selection influence on DBC as function of residence time (min).

Breakthrough selection is also critical factor for continuous operation, especially as the goal is to maximize resin use.

A 70% breakthrough at 2 min of residence time enabled a loading level of 44 g_{mAb}/L_{resin} (**Table 2**). The resin utilization was evaluated at 95% against 80% for classical batch operation with 37 g_{mAb}/L_{resin} under 4 min residence time (90% DBC, 10% BT vs. 100% DBC, 70% BT), static binding capacity (SBC) being assessed at 46 g_{mAb}/L_{resin} (calculated based on the average of the integrals at the tested residence times)

Resin utilization is given by the following formula:

$$\text{Resin Utilization (\%)} = \frac{\text{Quantity of mAb retained (g)} * \text{Column Volume (L)}}{\text{Static binding capacity (g/L)}}$$

Table 2. Impact of residence times on protein breakthrough.

Parameters	Conditions			Units
Residence time	1	2	4	(min)
100% DBC, 10% BT	28	39	41	(g_{mAb}/L)
Resin saturation	61%	85%	89%	(%)
% of mAb loaded (g) flowing throughs next column	1.6%	<1%	<1%	(%)
90% DBC, 10% BT (typical for batch)	25	35	37	(g_{mAb}/L)
Resin saturation	55%	76%	80%	(%)
% of mAb loaded (g) flowing throughs next column	N/A*	N/A*	N/A*	(%)
100% DBC, 50% BT	36	43	44	(g_{mAb}/L)
Resin saturation	79%	93%	96%	(%)
% of mAb loaded (g) flowing throughs next column	12.6%	5.9%	4.4%	(%)
100% DBC, 70% BT	40	44	46	(g_{mAb}/L)
Resin saturation	86%	95%	99%	(%)
% of mAb loaded (g) flowing through next column	20.5%	10.8%	9.0%	(%)
SBC Rt →∞	46* ±1%			(g_{mAb}/L)

*N/A – No mAbs are flowing through when a safety factor is applied on the DBC selected (here, 90% of the DBC)

The number of CVs corresponding to 70% breakthrough was 16.5 with a 2 min residence time; residence utilization was 95% (**Table 3**). These parameters were used in the continuous process.

Table 3. Selected process parameters.

Residence time (min)	Breakthrough (%) – batch	CV loading (N/A)	Capacity (g_{mAb}/ L_{resim})	Resin utilization (%)
2	70	16.5	44	95

Details of one capture cycle is shown in **Table 4**.

Table 4. CV details for one capture cycle

		Small scale		
Step	Details	CV	Flow rate (mL/min)	Residence time
Equilibration	90% DBC. 10 % BT	5	6.7	1.65
Loading	HCCF filtered through 0.22 µm	16.5	5.5	2
Wash	50 mM $NaHPO_4$ pH 7.1	1.1	6.7	1.65
Wash 1	50 mM $NaHPO_4$ 1 M NaCl pH 7.1	4	6.7	1.65
Wash 2	50 mM $NaHPO_4$ pH 7.1	3	6.7	1.65
Elution	50 mM Sodium Acetate pH 3.5	5	6.7	1.65
Regeneration	0.1 M NaOH	3	6.7	1.65
Storage	20% (v/V) Ethanol or 0.1 M NaOH	3	6.7	1.65

The capture step can be split into the loading step (16.5 CV) and the non-loading step (21.1 CV). To maintain continuity during the running of multiple columns, the residence time of the non-loading step was adjusted to align with the duration of the loading step.

Step II

Using the parameters previously determined (70% BT at 16.5 CV loading, 2 min residence time), the capture process was performed on the modified ÄKTA Avant™ system. Due to the limited number of sensors on the system, only the column in the non-loading step was monitored (**Figure 7**).

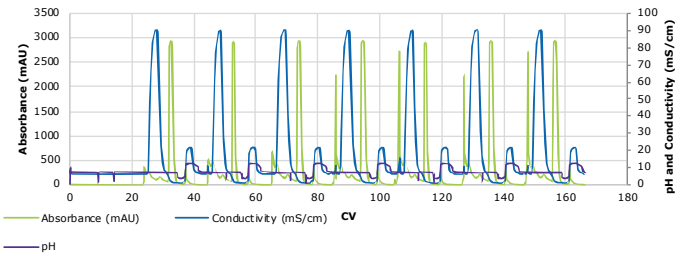


Figure 7. Chromatogram of lab-scale multi-column capture set-up – run 1 (7 captures)

In total, 1270 mL of HCCF at 2.76 g/L were processed on the ÄKTA Avant™ system to simulate a multi-column capture operation.

The amount of mAb contained in the reserve (first 1.1 CV of each wash step) was assessed at 0.27 g_{mAb}. The amount of product directed to the reserve was 8% of the initial amount injected (3.51 g_{mAb}).

The volume of the elution pool was 467 mL with a concentration of 6.41 g_{mAb}/L (5 CV of collection); as no fractionation was performed, concentration was relatively low. Aggregate levels in the final eluate pool were 2.32%. Process yield was estimated at 95.0% (reserve and eluate recovered).

Figure 8 shows a chromatogram of replicate run 2. There is close agreement between run 1 (**Figure 7**) and run 2 (**Figure 8**) the ÄKTA Avant™ system.

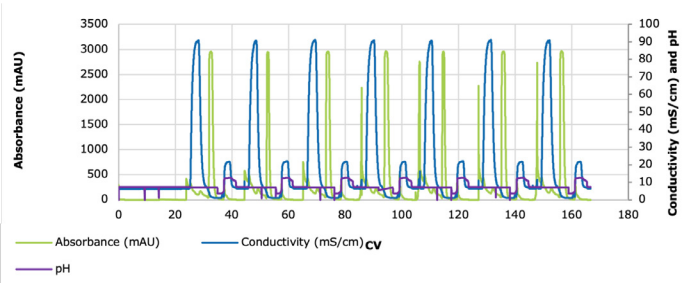


Figure 8. Chromatogram of lab scale multi-column capture set-up – run 2 (replicate) (7 cycles)

The volume of the elution pool for the second run, was 471 mL with a concentration of 6.571 g_{mAb}/L (5 CV of collection); concentration was relatively low. Aggregate level in the final eluate pool was assessed at 2.32%. Process yield was estimated at 97.7% (reserve and eluate recovered).

Step III

After parameters were determined in the process simulation at lab-scale, the capture step was performed on the Mobius® Multi Column Capture system (**Table 5**). The overall, automated process required 8 hours during which time 12 complete and 2 partial loadings were performed (corresponding to the last combination of loaded and preloaded columns). A chromatogram of the run is shown in **Figure 9**.[2]

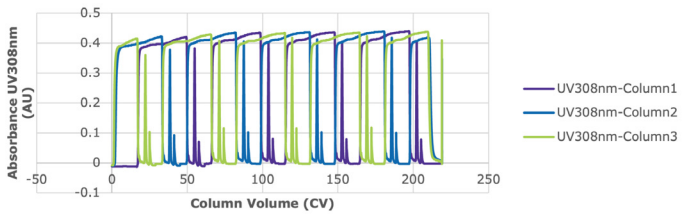


Figure 9. Chromatogram of the Mobius® Multi Column Capture system run showing absorbance at UV 308 nm after each column.

[2]The Optical path length of the spectrophotometer used was 2.5 mm instead of 1 mm causing the UV 280 nm to saturate. This necessitated the use of a second wavelength (UV 308 nm) vailable on the Mobius® Multi Column Capture system to enable full monitoring.

Based on the system feed flow sensor totalizer function, the total feed volume processed was estimated at 395 L at a concentration of 2.8 $g_{mAb}/L_{,resin}$, representing 1.1 kg of mAb. The recovered volume in the fraction bag was 82.6 L at a concentration of 11.7 g_{mAb}/L representing 966.4 g of product and 88.9 g recovery. The aggregate level in the final eluate pool was assessed at 2.1%. A volume corresponding to 39.6 L was sent to the reserve; the reserve concentration was monitored 1.95 g_{mAb}/L, representing 7.1% of the initial product amount.

The overall yield was 87% which, if the entire product in the reserve could be recaptured, could increase to 94.4%.

In summary, with 966.4 g of product recovered in 8 hours of processing time and 5.6 L of total resin volume, the productivity was 136 g_{mAb}/h, 21.4 $g_{mAb}/L_{resin}/h$.

Table 5. Buffers and operating parameters

Step	Details	Large scale		
		CV	Flow rate (mL/min)	Residence time
Equilibration	50 mM $NaHPO_4$ pH 7.1	5	≤1.21	≤1.65
Loading	HCCF filtered through 0.22 µm	16.5	0.925	2
Wash	50 mM $NaHPO_4$ pH 7.1	1.5	≤1.21	≤1.65
Wash 1	50 mM $NaHPO_4$ 1 M NaCl pH 7.1	4	≤1.21	≤1.65
Wash 2	50 mM $NaHPO_4$ pH 7.1	3	≤1.21	≤1.65
Elution	50 mM Sodium Acetate pH 3.5	5	≤1.21	≤1.65
Regeneration	0.1 M NaOH	3	≤1.21	≤1.65
Storage	20% (v/V) Ethanol or 0.1 M NaOH	3	≤1.21	≤1.65

Discussion and Perspectives

Process yield, HMW content, and outcomes were comparable between the lab-scale process on the ÄKTA Avant™ system and the larger-scale process Mobius® Multi Column Capture system (**Figure 10**).

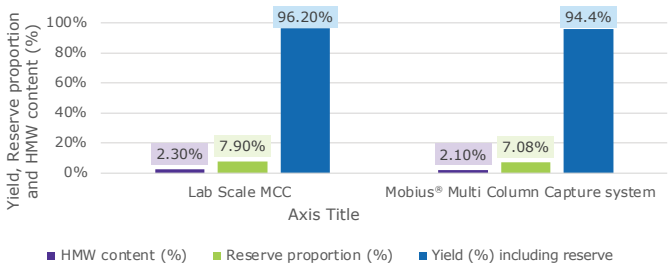


Figure 10. Comparison system between at-scale process using Mobius® Multi Column capture with lab-scale trials.

Representative scalability

The multi-column capture operation successfully scaled from the ÄKTA Avant™ system to the Mobius® Multi Column Capture system, highlighting the effective integration of the two systems (**Figure 10**). Furthermore, aggregate level in eluate pools were comparable in both lab and large-scale studies: 2.3% at lab-scale and 2.1% at large-scale.

Key benefits of Mobius® Multi Column Capture system

Reduced resin volume: The Mobius® Multi Column Capture system was developed to enhance the productivity of the protein A capture step and reduce the cost of consumables. With these studies, we have demonstrated that the Mobius® Multi Column Capture system enhances the productivity of the protein A capture step while reducing consumable costs. Specifically, only 5.6 L of resin was utilized, which is a significant reduction compared to the approximately 120 L required for the batch process and around 30 L needed for a split batch process.

Increased productivity: With the selected conditions, the time required to process 395 L was 8 hours (or 9 hours with reserve processing) and for a 2000 L bioreactor is estimated at 41 hours (or 46 hours with reserve processing). When compared to a batch process, the Mobius® Multi Column Capture system enables a productivity of 21.4 $g_{mAb}/L_{resin}/h$ against 8.6 $g_{mAb}/L_{resin}/h$ in classical batch process, assuming a 64 L column is re-used 4 times.

The reduced resin volume requirements and productivity gains are compelling reasons for manufacturers to shift towards intensified processing.

The Mobius® Multi Column Capture system is an automated, single-use solution designed to streamline operations and maximize the benefits of intensified capture of clarified harvest material from fed-batch or perfusion processes. This system helps manufacturers transition from traditional to intensified processing and realize the benefits of improved productivity with reduced costs.

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