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Application Note

Process Development and Scale-Up Strategy for the Mobius® Multi Column Capture System

Introduction

This document describes a methodology for developing and scaling up a process for a multi-column capture operation using the Mobius® Multi Column Capture. This system with a single-use flow path has been developed for the continuous processing of capture chromatography.

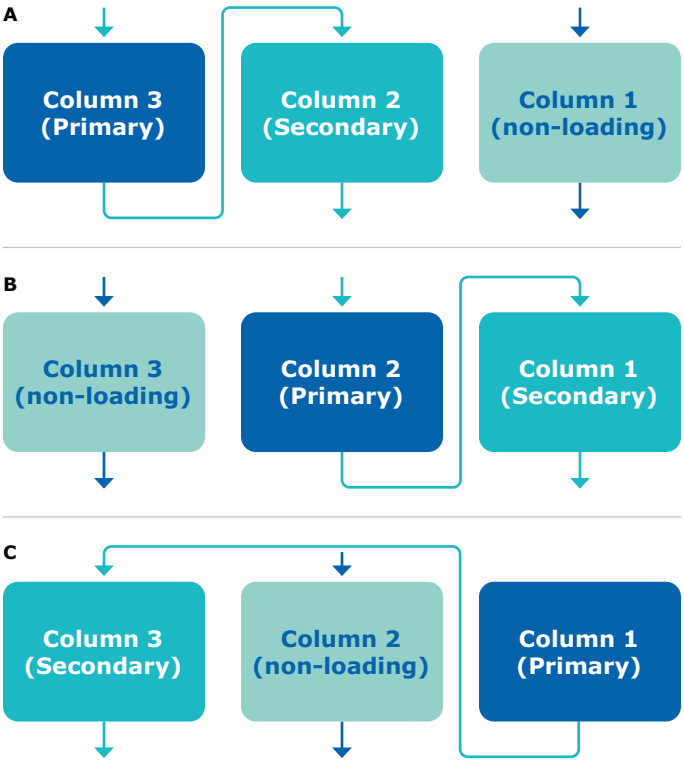

Figure 1.

(**A**) Column 3 (primary) is loaded, with breakthrough directed onto column 2 (secondary). Column 1 undergoes non-loading steps. (**B**) Column 2 (primary) is loaded, with breakthrough directed onto column 1 (secondary). Column 3 undergoes non-loading steps. (**C**) Column 1 (primary) is loaded, with breakthrough directed onto column 3. Column 2 (secondary) undergoes non-loading steps.

This system operates in periodic counter current mode using three columns as shown schematically in **Figure 1**. At any instant, two bind and elute columns are loaded in series with monoclonal antibody (mAb) feed, or other protein product, while the third column is washed, eluted, and regenerated. Loading two columns in series allows for overloading as the mAb breaking through the primary column is captured by the secondary column.

The ability to overload mAb results in an increase in resin media utilization and productivity. Once the primary column in series is loaded to a specified amount, loading shifts to the partially loaded column, which becomes the primary column in series and the regenerated column is now the secondary column in series. This sequence of events continues to enable an uninterrupted loading of the mAb feed enabling a continuous process. This document defines a “cycle” to be the loading, elution, and regeneration of one column. While process development methods for capture chromatography conducted in batch mode are well established, there are several additional considerations for process development of continuous capture. In this work, we demonstrate the use of a bench-scale chromatography system typically used for development of batch processes as a tool for process development and characterization for application with the Mobius® Multi Column Capture system.

The described approach is targeted to eliminate the need for a dedicated bench-scale system for multi-column capture process development resulting in significant CapEx savings. Specifically, we show that by selecting an appropriate bench-scale column size and generating a breakthrough curve on a single column in batch mode, all the parameters necessary for scale-up of multi-column capture process can be determined. Further work demonstrating process characterization at bench-scale with simple batch chromatography system modifications is included, though not necessary for scale-up.

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Materials and Methods

mAb Feed Preparation

All materials were obtained from Merck unless noted otherwise. The mAb feeds were produced using a CHOZN® GS Chinese Hamster Ovary (CHO) cell line in a 200 L Mobius® single-use bioreactor operated in fed-batch mode. Cell culture fluids containing the mAb were harvested using a two-stage depth filtration strategy that employs Millistak+® HC Pro filters with media grades D0SP and X0SP at an area ratio of 2:1. Clarified mAb feed was further filtered using Millipore Express® SHC filters (0.5/0.2 µm).

Chromatography Materials

Three bench-scale columns were packed with Eshmuno® A media. A 2.2 cm inner diameter (ID) Vantage™ column at 6.6 cm bed height (BH), a 1.6 cm ID Vantage™ column at 9.9 cm BH, and a 0.66 cm ID Omnifit® (Diba Industries) at a 7.0 cm BH were used, resulting in column volumes (CVs) of 25.0 mL, 20.0 and 2.40 mL, respectively.

All the bench-scale chromatography experiments were performed using an ÄKTA avant 25 or ÄKTA pure 25 (Cytiva) system. At both scales, the chromatography method included equilibration (5 CVs), wash (4 CVs), elution (4 CVs), and regeneration (5 CVs) phases in addition to loading mAb.

A Mobius® Multi Column Capture system equipped with three Quikscale® columns was used for process-scale experiments. Each column was of 10.0 cm ID and packed with Eshmuno® A media to a BH of 7.0 cm resulting in a CV of 550 mL.

Analytical Methods

Samples were taken from the load, each individual elution, and the wash after loading, known as the reserve. mAb titer was measured by Protein A-HPLC using a Poros A 20 µm Protein A Affinity Column (Thermo Scientific).

A CHO HCP ELISA, 3G (F550-1) (Cygnus Technologies) was used to quantify host cell protein (HCP). Size exclusion HPLC (SE-HPLC) using a TSKgel® G3000SWXL 5 µm column with a TSKgel® SWXL Type Guard Column 7 µm (Tosoh Bioscience) was used to measure high molecular weight (HMW) species, low molecular weight (LMW) species, and monomer.

Process Development and Scale-up Approach

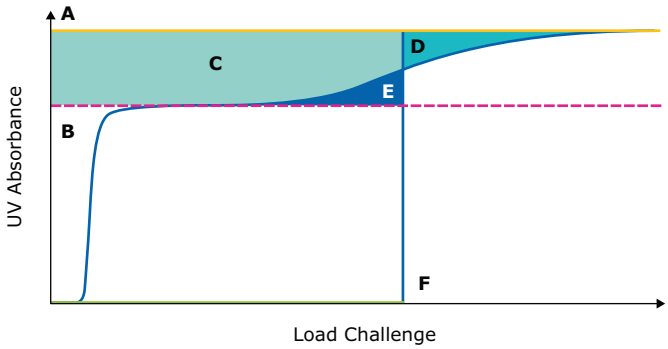


Figure 2.

Example breakthrough curve of a mAb and Protein A media combination. (**A**) UV absorbance of feed material. (**B**) Plateau at UV absorbance of impurity flowthrough. (**C**) The area above the breakthrough curve, representing mAb binding to the media at a given load challenge. (**D**) C+D = the total area above a breakthrough curve of a column loaded to 100% breakthrough. This is the maximum binding capacity. Utilization is calculated as the percent of bound area, "C", over the maximum binding capacity area, "C+D". (**E**) mAb breaking through being preloaded onto the next column in series. (**F**) The total mAb loaded onto the column up to a given breakpoint.

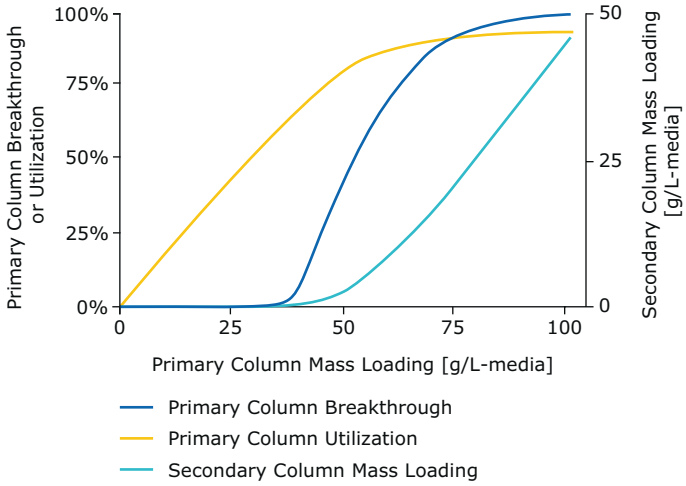


Figure 3.

A breakthrough curve to 100% breakthrough as shown in **Figure 2** is analyzed to generate curves for % breakthrough, utilization, and secondary column mass loading as a function of primary column mass loading.

The design of a multi-column capture process requires additional considerations as compared to that of a batch capture process. While batch capture processes are designed to maximize yield and purity for a specific mAb and protein A media combination, multi-column capture processes additionally require a design around maximizing media utilization and productivity while achieving continuous operation without pause in loading. These considerations were achieved by selecting a residence time (RT) with a given media and mAb combination. Breakthrough curves as shown in **Figure 2** were generated using a single bench-scale column to evaluate breakthrough profiles at different RT values. Such breakthrough curves can be generated with purified mAb or clarified harvest. The breakthrough curves were further analyzed using the trapezoidal rule of integration followed by calculating media utilization and mAb mass in the load flowthrough (which is loaded onto secondary column) at different breakthrough points. The utilization was calculated by dividing the mAb bound (C) by the maximum binding capacity (C+D), reported as a percent. The mAb mass in the flowthrough is shown by area E. As an example, the utilization and mAb mass in the flowthrough are plotted as a function of mass loading as shown in **Figure 3**.

When selecting a RT, the following considerations were made:

1. Maximizing utilization:

The two main constraints in maximizing the utilization are assurance of a continuous process and that there is no mAb breakthrough from secondary column. At a given target utilization, **Figure 3** was analyzed to determine the mass loading on the primary column shown on X-axis, which can be used in combination with the RT to calculate the load time. Similarly, at the same target utilization, mass loading on the secondary column was determined (**Figure 3**, right-hand Y-axis). Since it is assumed that the secondary column will follow the same breakthrough curve profile as the primary column, the primary column breakthrough curve is used to determine if the mAb would breakthrough at the secondary column's mass load level.

2. Maximizing productivity:

At a given utilization, productivity of the step was determined by dividing the mAb bound by the CV and the total time (load + non-load) to complete the cycle.

3. Continuous process without pause in loading:

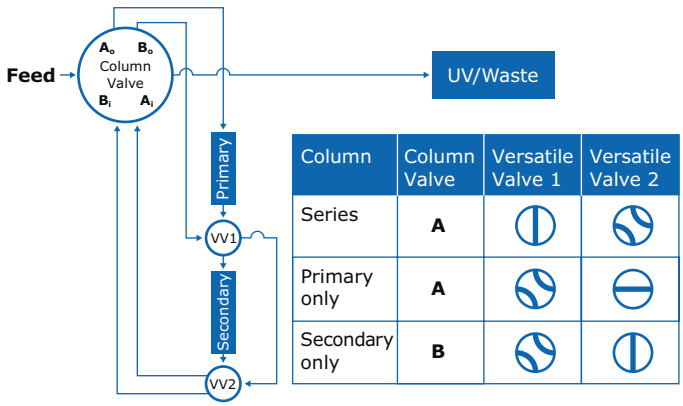
A continuous operation is ensured if the load time exceeds the time for all non-load steps. Total time for non-load steps was calculated by multiplying the total CVs of non-load steps with the RT of those steps. The mass loading shown in **Figure 3** allowed for calculation of load CVs, and subsequently the load time by multiplying CVs with the RT of the load step. The Mobius® Multi Column Capture system has additional build-in flexibility to automatically adjust RTs within a specified range for both load and non-load steps in case of process deviations.

Additionally, the Mobius® Multi Column Capture system has three methods of automatically triggering column switching to enable continuous loading: absolute UV value, % UV breakthrough, and area under the curve. This process development and scale-up approach utilizes the % UV breakthrough method. The maximum absorbance value was measured using the system's UV detectors prior to connecting the columns and was input once for the entirety of the process. For each cycle, the system detected the loading impurity plateau within a specified dead band and time range. The initial setpoint to begin detecting the plateau was input as derived by the ratio between the value A for maximum absorbance and value B for the absorbance of impurity. Alternately, the value E could also be used to control the process-scale system for the area under the curve method.

Process characterization of overloading onto the primary column and simultaneous preloading onto the secondary column was performed at bench-scale to evaluate the yield, product purity, and media lifetime.

This activity was not critical to identify parameters necessary for the scale-up but allow confirmation of similar process performance at the two scales. To perform the process characterization, simple modifications as shown in **Figure 4** were made to an ÄKTA avant 25 to operate two columns in series at bench-scale. In this setup, the first column was representative of the first cycle of the process-scale multi-column process, while the second column was representative of all cycles after the first at process-scale.

As shown in **Figure 4**, a flow path was created using two versatile valves (V9-V) (Cytiva), allowing the two columns to be operated both in series and individually. The primary column was loaded while preloading the secondary column, followed by loading of the secondary column, and finally the non-loading steps for each column are performed individually. The reserve and the elution pool were collected for each column individually and analyzed for yield and impurity levels.



Column	Column Valve	Versatile Valve 1	Versatile Valve 2
Series	A		
Primary only	A		
Secondary only	B		

Figure 4.

A schematic depicting the use of two versatile valves to operate two columns in series, and independently, on an ÄKTA avant.

Results

RT and % UV breakthrough selection, and the impact of column ID

In our study, bench-scale breakthrough curve data were analyzed to identify the appropriate load RT and UV breakthrough value for column switching as described above. Experiments were conducted at both 1 minute and 2 minute RTs and analyzed to generate curves as shown in **Figure 3**.

The experiments utilized Eshmuno® A media packed in either 1.6 cm or 2.2 cm ID columns, and mAb feed material which was either clarified harvest or Eshmuno® A purified eluate material.

Table 1.

Summary of breakthrough curve analyses at RTs of 1 and 2 minutes for different combinations of ID and feed types.

Process Development Objective	RT for Load Steps	1 min		2 min		
	Column ID [cm]	1.6		1.6	2.2	2.2
	Feed type	Purified		Purified		Clarified Harvest
Maximize Media Utilization	Utilization [%]	80	86	80		
	Primary column load challenge [g/L-media]	64.7	82.6	51.6	49.6	50.8
	Mass bound to primary column [g/L-media]	47.2	50.7	47.2	47.2	47.2
	% UV breakthrough	73%	86%	50%	40%	47%
	Secondary column load challenge [g/L-media]	17.5	31.9	4.4	2.4	3.6
	Secondary column breakthrough [g/L-media]	0.01	1.05	0.00	0.00	0.00
Maintain Continuous Process	Load time at steady state at 3.6 g/L feed concentration [min]	13.1	22.5	26.2	26.2	26.2
	Non load time @ RT = 1 min [min]	18	18	18	18	18
	Non load time @ RT = 2 min [min]	36	36	36	36	36

Table 1 summarizes the parameters necessary for scale-up at the target utilization of ≥80% at RTs of 1 and 2 minutes after analysis of breakthrough curves generated using both clarified mAb harvest and purified mAb, and column IDs of 1.6 cm and 2.2 cm. In our experiments, RTs lower than 1 minute were not explored due to pressure-flow considerations for Eshmuno® A media. As expected, at 80% utilization, the % breakthrough and mAb mass breaking through to the secondary column with a 1 minute load step RT was higher than that at a 2 minutes load step RT. Additionally, the load step was shorter than the non-load time at RTs of both 1 and 2 minutes.

In this case, to increase the load time, one would need to operate at higher than 80% utilization, which would consequently increase the mAb breakthrough from secondary column risking the yield loss.

At a load step RT of 2 minutes, the load step duration was also observed to be shorter as compared to the non-load steps duration when the non-load steps were performed at a RT of 2 minutes. However, the load duration was longer than duration of non-load steps when the non-load steps were performed at a RT of 1 minute. Additionally, at 80% utilization, the mAb breakthrough to second column was less than 5 g/L-media indicating that there is no risk of breakthrough from secondary column and resulting yield loss.

Based on these observations, a RT of 2 minutes was chosen for load steps and a range of RTs between 1 minute and 2 minutes was chosen for non-load steps, allowing the system to automatically adjust as needed to maintain process synchronization.

Dynamic Binding Capacity Curve Analysis for Scale-Up

It is expected that the mAb harvest used for process-scale runs is slightly different than the one used for process development. Therefore, prior to executing continuous capture process, a breakthrough curve was generated at bench-scale again at selected process conditions (RT and utilization) with the same lot of mAb harvest used for the process-scale operation. In our experiments, the titer of the feed was 3.64 g/L. The percent UV breakthrough target selected was 52%, with an estimated steady state load challenge of 49.7 g/L-media.

Scale-Up

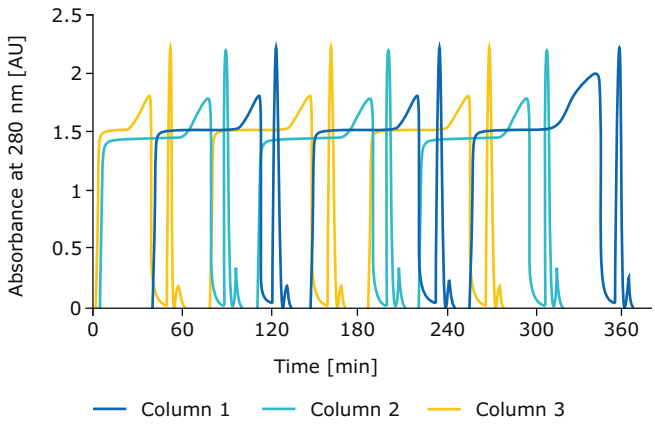


Figure 5.

This chromatogram shows 3 cycles performed on each of the three columns at a 52% UV breakthrough trigger (the final cycle, cycle 9, loaded to >90% UV BT). 340 g of mAb was processed with a 90% overall yield, in 6.25 hours.

The chromatogram of the process-scale operation is shown in **Figure 5** for three columns. A total of nine cycles were successfully executed without interruption in loading. Of those nine cycles, cycles 4 through 8 represent steady state operation, whereby all the columns were preloaded with the breakthrough mAb. A 9th cycle was performed at a UV breakthrough of approximately 90% to estimate the maximum media binding capacity of the 10 cm ID columns. This value was used to calculate the utilization achieved at process-scale for comparison with bench-scale data. Over the course of 6.25 hours, 340 grams of mAb was processed with a 90% yield which was consistent with small scale experiments. Optimization of process washes and elution were out of the scope of this study.

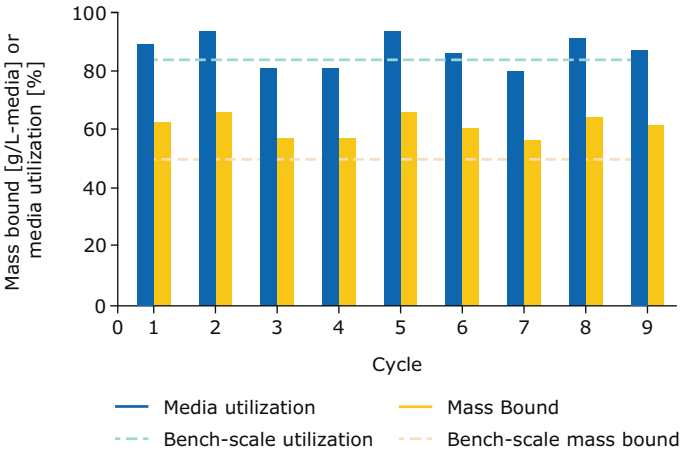


Figure 6.

The mass bound and utilization of each cycle is calculated and compared to data from the bench-scale breakthrough experiment using a 2.2 cm i.d. column.

mAb binding and utilization for each cycle compared to bench-scale is shown in **Figure 6**. The utilization for all cycles was observed to be similar to the bench-scale experiments confirming successful scale-up. It was observed that mAb bound was greater at process-scale than bench-scale. This higher mAb bound at the same % breakthrough at process-scale can be attributed to differences in breakthrough curves and binding at different scales. To compare the binding at different scales, breakthrough curves generated using different ID columns were compared.

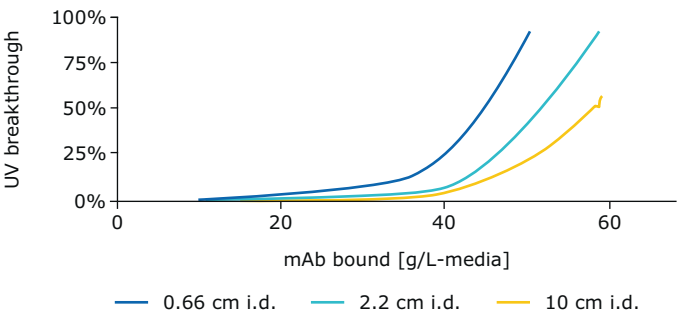


Figure 7.

Mass of mAb binding to columns increases at a given % breakthough value with increasing column ID.

Figure 7 shows the comparison of breakthrough curves as a function of column ID. It was found that when overloading columns, column ID had an impact on the binding capacity of the media at high breakthrough values. This can be due to differences in column aspect ratios and wall effects, compression factors, the quality of column packing, or other system contributions. The mass of mAb bound at bench-scale was more representative of process-scale with increasing column ID. As a result, a bench-scale column of at least 2.2 cm ID was found to demonstrate the most comparable mAb bound of sizes assessed to the process-scale columns.

Multi-column Process Characterization at Bench- and Process-Scale

Yield and impurity removal were evaluated at bench- and process-scale. A bench-scale experiment was performed to load two columns in series followed by loading of the secondary column. This preloaded secondary column was representative of all of the preloaded cycles of the process.

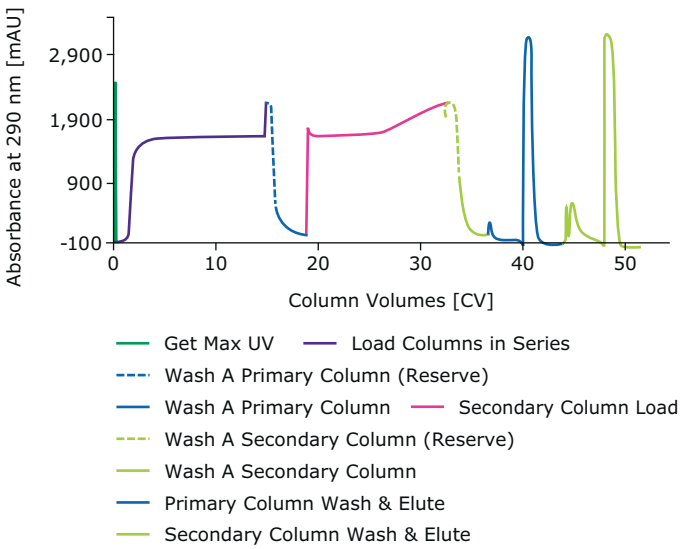


Figure 8.

Chromatogram of two columns loaded in series on an ÄKTA avant.

The chromatogram shown in **Figure 8** confirms that the bench-scale multi-column run operated as expected. With the column in bypass, the maximum UV of the feed was measured. The two columns were then loaded in series where all of the breakthrough from the primary column was directed onto the secondary column. When analyzing this part of the chromatogram, it is important to note that this system was equipped with a single UV detector, therefore only the flowthrough of the secondary column was detected when the columns are in series. After the secondary column was preloaded, it was loaded independently. The reserve, or first CV of Wash A after column loading was collected for each column, as well as each elution pool.

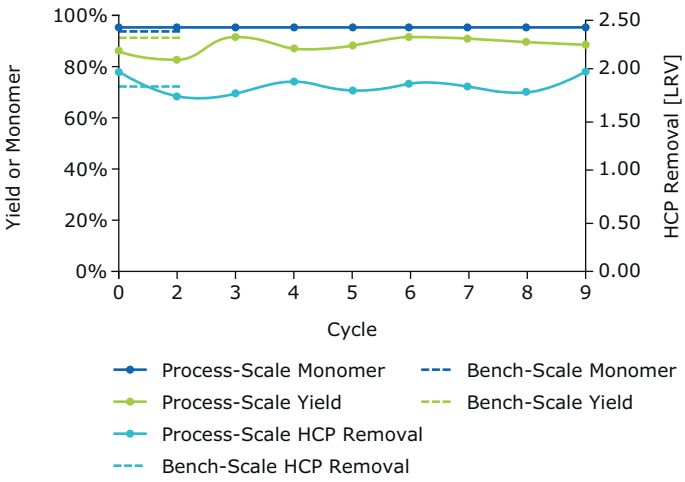


Figure 9.

Yield, % monomer, and HCP removal (LRV) are plotted for each cycle of the process-scale operation. Bench-scale data for each attribute from a pre-loaded column is plotted for scale-up comparison of process performance.

Process performance comparability between bench- and process-scale was quantified by HCP removal, monomer contents and process yield (**Figure 9**). Performance of each cycle was very consistent and comparable to the bench-scale data. These results indicate that minor modifications to ÄKTA allow development and optimization of the non-loading steps of the process to achieve yield and impurity removal targets (not within the scope of this work). This analysis also proves that the multi-column process does not compromise process performance. The reserve, or first column volume of wash after loading was collected at both bench- and process-scale. The mAb in the process-scale reserve was at a concentration of 2.1 g/L accounting for 3.1% of the loaded mass. The mAb in the bench-scale reserve was at a concentration of 1.1 g/L accounting for 2.1% of the loaded mass. The mass recovered in the reserve is in addition to the mass recovered in the elution.

Productivity was calculated using the loaded mass and total media volume of the system. The process-scale system's productivity was 32 g-mAb/L-media/hr which is comparable to the bench-scale system's productivity of 31 g-mAb/L-media/hr. This is a notable increase over a batch process of a 6 minute RT loaded to 30 g/L-media with a 160 minute cycle time, with a productivity of 11 g-mAb/L-media/hr.

Conclusion

While there are additional considerations when scaling up a process to a multi-column capture operation as compared to batch, this work demonstrates that existing process development tools can be used to enable next generation processing. An ÄKTA avant can successfully be used to determine scale-up parameters and to evaluate process performance of a multi-column process.

Furthermore, by analyzing a bench-scale breakthrough curve using a column of appropriate ID, the mAb mass loading and mAb bound can be determined. This allows the user to manage non-loading steps for time synchronization, plan the number of cycles and hold times involved for the feed material to be processed, and calculate buffer needs.

Comparison of the utilization and productivity of bench- versus process-scale column supports the success of the scale-up. Simple and inexpensive modifications made to an ÄKTA avant using versatile valves allows evaluation of yield and impurity removal for process development and characterization of the multi-column process.

Additionally, mAb yield and purity between the two scales was comparable. This confirms that process performance was similar when scaled up.

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